Behavioral, electrophysiological, and cerebral correlates of vulnerability to sleep loss: The impact of sleep pressure, circadian phase, and a PER3 polymorphism.

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Micheline Célia Dina Maire

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auf Antrag von

Prof. Dr. Dominique de Quervain
Prof. Dr. Christian Cajochen

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Abstract

The impact of sleep loss is highly variable across individuals, and among other influences, genetic factors determine the vulnerability to its detrimental effects. The present thesis aimed to investigate sleep-loss related decrements in subjective and physiological sleepiness, sustained attention, and underlying cerebral correlates by taking into account related genetic vulnerability. Using a candidate-gene approach, we considered a variable-number-tandem-repeat polymorphism in the clock gene *PERIOD 3 (PER3)*, previously related to vulnerability to sleep loss. We compared 14 homozygous long allele carriers (*PER3*$^{5/5}$) with 14 homozygous short allele carriers (*PER3*$^{4/4}$). The former genotype was previously reported to be more vulnerable to sleep loss than the latter. In a within-subject design, the sleep homeostatic level was manipulated by two conditions, a 40-h sleep deprivation (SD, high sleep pressure condition) protocol and a 40-h multiple nap protocol (NP, low sleep-pressure condition). We used questionnaires, electrophysiology, hormonal assays, cognitive tasks, and functional magnetic resonance imaging to assess the impact of sleep pressure levels and circadian phase under stringently controlled laboratory conditions.

Our data indicate that sleep loss had more detrimental effects in *PER3*$^{5/5}$carriers. They were subjectively sleepier and had a greater amount of unintentional sleep episodes. Sustained attention performance was likewise more affected by sleep loss in *PER3*$^{5/5}$carriers, as evidenced by more intermittent lapses and increasing performance variability with time-on-task during SD. Moreover, nap sleep efficiency tended to be higher in the vulnerable genotype throughout the circadian cycle, which in turn correlated positively with attentional lapses in SD. During the biological night, when performance is usually most compromised due to strongest circadian sleep promotion, higher vulnerability was mirrored in cortical and subcortical deactivation patterns during sustained attention performance under SD. In contrary, resilient participants (*PER3*$^{4/4}$ carriers) showed increases in task-related brain activity. Likewise, thalamic structures were progressively less recruited with time-on-task and functionally less connected to other arousal-promoting and task-related brain areas in the vulnerable group. Finally, our data suggest that more vulnerable participants are prone to shift into a task-inactive brain default-mode network during the absence of task-relevant stimuli, likely causing the greater “state instability” we observed behaviorally.

In sum, our data show that genetic vulnerability to sleep loss is consistently detectable at the subjective, electrophysiological, and cerebral level. Investigating individual differences in response to sleep loss in combination with basic processes implicated in sleep-wake regulation therefore seems a promising approach to identify phenotypes that allow the prediction of sleep-loss related decrements. A challenging long-term goal is to translate our findings from a controlled laboratory to a field setting with shift workers who face adverse sleep-wake timing and sleep loss on a daily basis.

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Abbreviations

ACh: acetylcholine

ADA: adenosine deaminase

BDNF: brain-dervived neurotrophic factor

BOLD: blood oxygen level-dependent

CRY: cryptochrome

DMH: dorsomedial nucleus of the hypothalamus

DMN: default-mode network

DSPD: delayed sleep phase disorder

EEG: electroencephalogram

FD: forced desynchrony

fMRI: functional magnetic resonance imaging

GABA: gamma-amminobutyric-acid

ISI: inter-stimulus interval

KSS: Karolinska Sleepiness Scale

LC: locus coeruleus

LSEQ: Leeds Sleep Evaluation Questionnaire

mRNA: messenger ribonucleic acid

NP: nap protocol

PER: PERIOD

PVT: Psychomotor Vigilance Task

RT: reaction time

SCN: suprachiasmatic nuclei

SD: sleep deprivation

SEM: slow eye movement

SWA: slow wave activity

SWS: slow wave sleep

THA: thalamus

VLPO: ventrolateral preoptic area

VNTR: variable-number-tandem-repeat

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1. Introduction

Due to professional demands or social reasons, we often curtail our sleep, or we sleep at non-optimal times throughout the 24h-cycle, which can majorly impact on well-being and cognitive performance (reviewed in Chee & Chuah, 2008; Killgore, 2010). Particularly, professions in health care or aviation where failures in attention can be fatal often involve night or rotating shift work. Some individuals are more capable to cope with such adverse conditions than others, either on the subjective and behavioral level as well as on the physiological level (Van Dongen, Baynard, Maislin, & Dinges, 2004). Despite a call for more research investigating these individual differences in vulnerability to sleep loss (Van Dongen, Vitellaro, & Dinges, 2005), and a growing number of studies dealing with this topic, several essential questions remain unexplored.

Hence, the objective of this work was to investigate the impact of differential vulnerability to sleep loss on subjective and physiological sleepiness, sustained attention, and its underlying cerebral correlates. According to previous reports (Groeger et al., 2008; Viola et al., 2007), a variable-number-tandem-repeat (VNTR) polymorphism in the clock gene *PERIOD3 (PER3)* contributes to vulnerability to sleep loss on the behavioral and physiological level. It was hypothesized that this polymorphism acts mainly on the sleep homeostatic process (Dijk & Archer, 2009). Here we took advantage of these findings, and selected participants with genotypes that were reported to be either more resilient (*PER3*$^{4/4}$ carriers) or more vulnerable (*PER3*$^{5/5}$ carriers) to sleep loss to investigate vulnerability in a multi-methodological approach.

We aimed at answering the following open questions. First, to what extent is genetic vulnerability to sleep loss modulated by sleep pressure, and/or circadian phase, or the interaction thereof? To test whether this polymorphism phenotypically impacts mainly on sleep homeostatic regulation as previously postulated (Dijk & Archer, 2010), we applied an elaborated research protocol targeting both circadian and homeostatic aspects of human sleep regulation. In order to do so, the two main determinants of our sleep-wake cycle - the circadian process (internal clock) and the homeostatic process (an hourglass-process tracking sleep-wake-history) - must be separated. By combining a 40-h sleep deprivation (SD) protocol (high sleep pressure condition) with 40-h a multiple nap protocol (NP, alternating cycles of 160 min of wakefulness with 80 min naps, low sleep pressure condition), we were able to disentangle the contribution of these two processes, yet are still able to investigate their interaction. We characterized the two genotypes extensively in terms of their subjective, neurobehavioral and physiological reaction to high and low sleep pressure conditions, looked at nap sleep over the course of 40h, and linked sleep efficiency to neurobehavioral performance. The results and discussion of this extensive phenotypic characterization are addressed

in the first publication **"Sleep ability mediates inter-individual differences in the vulnerability to sleep loss: Evidence from a *PER3* polymorphism"**.

Second, how is vulnerability to sleep loss related to attentional failures, and what is the impact of time-on-task and motivation? Previous reports investigating *PER3*-modulated vulnerability to total SD focused on the investigation of deterioration in working memory performance (Groeger et al., 2008; Vandewalle, Archer, et al., 2009). Apart from recent publications (Kuna et al., 2012; Lo et al., 2012), these studies did not investigate sustained attention in detail. This basic cognitive process describes the ability to attend a task with few and unpredictable stimuli during minutes or hours, and was often described as a prerequisite for higher order cognitive functions (Sarter, Givens, & Bruno, 2001). Sustained attention is a major demand of occupations particularly in industrial settings where shift and night work is common. An often neglected aspect within the assessment of sustained attention in sleep and chronobiology research is the duration of the task, although time-on-task decrement might be one of the most compelling consequences of sleep loss (Doran, Van Dongen, & Dinges, 2001). With the second publication entitled **"Time-on-task decrement in vigilance is modulated by inter-individual vulnerability to homeostatic sleep pressure manipulation",** we aimed to cover these two aspects.

Third, we tackled the following question: What are the cerebral underpinnings of genetic vulnerability to sleep loss-related attentional decrements at the most adverse circadian time? By answering this question, we aimed to fill a major gap in the current literature. Although the cerebral basis of sustained attention has been investigated under well-rested conditions (reviewed in Sarter et al., 2001; Coull, 1998) and after SD during the biological day (e.g., Drummond, Bischoff-Grethe, et al., 2005), it was never explored at night time, when alertness levels are lowest and staying awake is most difficult, because the circadian timing system maximally promotes sleep. Moreover, even if differential vulnerability to sleep loss has been taken into account in a small number of functional neuroimaging studies (e.g., Chee & Tan, 2010), it is not yet known how it manifests on the cerebral level during the night. In our third publication **"How the brain copes to sustain attention at night-time: Impact of sleep pressure and genetic vulnerability to sleep loss."**, we present functional magnetic resonance imaging (fMRI) data on brain correlates of sustained attention during the biological night under high and low sleep pressure, taking into account differential vulnerability to sleep loss based on the *PER3* polymorphism.

In these three publications, we provide insight into behavioral, physiological, and cerebral underpinnings of vulnerability to sleep loss under stringently controlled laboratory conditions. We applied a multi-method approach by integrating subjective questionnaires, electrophysiology, neurobehavioral performance, and fMRI data. Our results suggest that study volunteers with a greater genetic vulnerability to sleep loss experience higher sleep propensity, already after being

awake for a relatively short time. Furthermore, greater wake-state-instability, as indexed by more unintentional sleep and attentional failures during wakefulness, was observed in the more "sleep-loss-vulnerable" genotype, potentially reflecting a lower arousal-promotion. This group was also more prone to switching into task-inactive cerebral networks in the absence of behaviorally relevant stimuli compared to the resilient group. In sum, vulnerable participants were less capable of maintaining stable attentional levels, which was demonstrated both in terms of cerebral activity and on the behavioral level. Thus, our data suggest a less efficient cerebral coping mechanism to counteract sleep loss-related attentional decrements in more vulnerable study volunteers.

2. Theoretical Background

2.1 Regulation of sleep and wakefulness

2.1.1 Circadian and homeostatic regulation of sleep & wakefulness

The alternating pattern of wakefulness and sleep in humans has been pinned down to two forces, the sleep homeostatic and the circadian process. These act either in synchrony or opposition to each other (see Figure 1, Borbély, 1982; Daan, Beersma, & Borbély, 1984). The sleep homeostatic process (also referred to as process S) reflects our sleep wake history and represents an hourglass process, with a steady build-up of sleep propensity or sleep pressure with increasing time awake, and an exponential decline during sleep. Typical electrophysiological markers for the dynamics of this process are electroencephalographic (EEG) slow wave activity (SWA, 0.5-4 Hz) during sleep (Borbély & Achermann, 2005), and alpha (7.5-11.5 Hz) and theta (4.5-7 Hz) activity during wakefulness (Cajochen, Brunner, Kräuchi, Graw, & Wirz-Justice, 1995; Finelli, Baumann, Borbély, & Achermann,

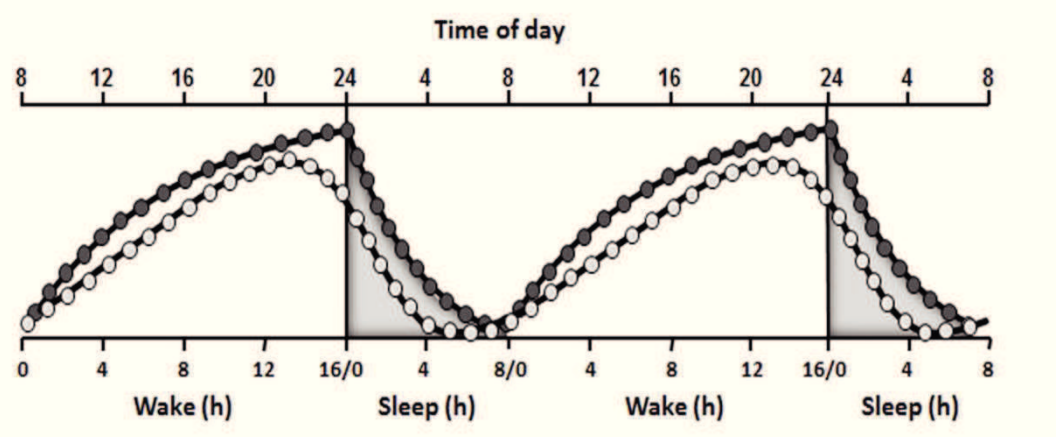

Figure 1. Schematic illustration of the two-process model of sleep-wake regulation (modified from Daan et al., 1984). The circadian process C (light grey) oscillates with a phase of nearly 24 h independent of the prior sleep-wake history. Mainly reset by the light-dark-cycle, it promotes wakefulness and sleep under entrained conditions according to time of day. In contrast, the homeostatic process S (dark grey) increases with enduring wakefulness and declines during sleep relatively unaffected by the 24-hour cycle. In the end, the interaction of both processes determines the timing, the duration and the quality of sleep and wakefulness. *Figure and legend from Maire, Reichert & Schmidt (2013), p. 135.*

2000). The circadian process (process C), represents a clock-like process with a pace of nearly 24 h regarding the propensity for sleep and wakefulness, which is set by our "master clock" located in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus (Edgar, Dement, & Fuller, 1993; Mistlberger, 2005). The light-dark-cycle is the strongest synchronizing agent for this process (for a review, see Münch & Bromundt, 2012), and its best endogenous marker is melatonin, a hormone secreted in the pineal gland with peak levels during the biological night (reviewed in Cajochen,

Kräuchi, & Wirz-Justice, 2003). The interplay of the circadian and homeostatic processes leads to consolidated states of sleep and wakefulness, determining its quantity, quality and timing (Dijk & Czeisler, 1995; Dijk & Franken, 2005). When homeostatic sleep pressure reaches high levels towards the end of the day, the circadian arousal signal opposes this increasing sleep propensity and leads to the maintenance of wakefulness until bedtime (Dijk & Czeisler, 1994). On the other hand, due to the circadian sleep-promoting signal in the early morning hours, sleep remains consolidated in spite of homeostatic sleep pressure dissipation over the course of the night (Dijk & Czeisler, 1994; Edgar et al., 1993). Wake up occurs in the morning when sleep pressure has dissipated and the circadian signal no longer promotes sleep.

Importantly, the interaction of these two processes influences cognitive and affective processes, ranging from more basic vigilance processes to higher-order cognitive function, well-being, mood and emotion processing (Wright, Lowry, & Lebourgeois, 2012). As long as these two processes are properly aligned (i.e., under entrained conditions), rather stable vigilance and performance levels are observed throughout a 16-hour waking day (Maire, Reichert, & Schmidt, 2013). If wakefulness is extended into the biological night, a steep decrease in neurobehavioral performance is observed, resulting in most detrimental levels at the end of the biological night in the early morning hours (Maire et al., 2013). A further extension of wakefulness into the next biological day results in a relative stabilization of neurobehavioral performance levels, as the circadian process again promotes wakefulness. This typical time course is visible below in Figure 2 (left upper panel), where attentional lapses during a repeatedly administered sustained attention task (Psychomotor Vigilance Task, PVT; Dinges & Powell, 1985) are depicted over the course of 40 h SD.

2.1.2 Experimental approaches to investigate the sleep-wake cycle

In the field of human chronobiology, several standard protocols have been developed to allow a fine-grained exploration of the sleep-wake cycle and its underlying processes (cf. Maire et al., 2013). The forced desynchrony (FD) paradigm can be considered as the gold standard (first described by Kleitman, 1963). In this protocol, participants undergo a sleep-wake cycle significantly longer or shorter than 24 h (e.g., 28 instead of 24 h). This leads to a desynchronisation between circadian rhythmicity and the sleep-wake cycle, and therefore allows the investigation of sleep and waking performance at virtually all circadian phases. The protocol is very time-consuming and requires temporal isolation units in which volunteers have to stay for several weeks (generally two to eight weeks, studies in which such a protocol was used were e.g., Cajochen, Wyatt, Czeisler, & Dijk, 2002; Wyatt, Ritz-De Cecco, Czeisler, & Dijk, 1999). A more feasible approach is the constant routine protocol: it allows the evaluation of circadian phase and amplitude during a relatively short stay in the laboratory, usually under SD (most often about 40 h), while body posture, nutrition and light influence are kept constant (Mills, Minors, & Waterhouse, 1978). One disadvantage of this protocol is

that the gradually increasing level of homeostatic sleep pressure may also affect circadian phase position (Cajochen, Jewett, & Dijk, 2003). As an alternative, the circadian effects may be more clearly highlighted when study volunteers are exposed to ultra-short sleep-wake cycles, such that episodes of sleep are equally distributed over the circadian cycle in order to keep the sleep homeostat at rather constant levels (Carskadon & Dement, 1975; Lavie, 1986). With this approach, the time course of endogenous circadian sleep-wake propensity can be investigated conveniently. However, an analysis of sleep structure during an entire night is not possible with this paradigm. Cajochen et al. (Cajochen, Knoblauch, Kräuchi, Renz, & Wirz-Justice, 2001) combined such a "nap protocol" (NP), respecting the habitual ratio between sleep and wakefulness (2:1), with SD under constant conditions in a within-subject design. With the combination of the two protocols, the effect of low and high homeostatic sleep pressure levels at many circadian phases can be compared. In the present thesis, this same approach was adopted. Illustrating the resulting time course for each protocol, Figure 2 depicts neurobehavioral performance (i.e., attentional lapses), wake-EEG activity, and subjective sleepiness values derived from the present dataset. Comparing the three variables in

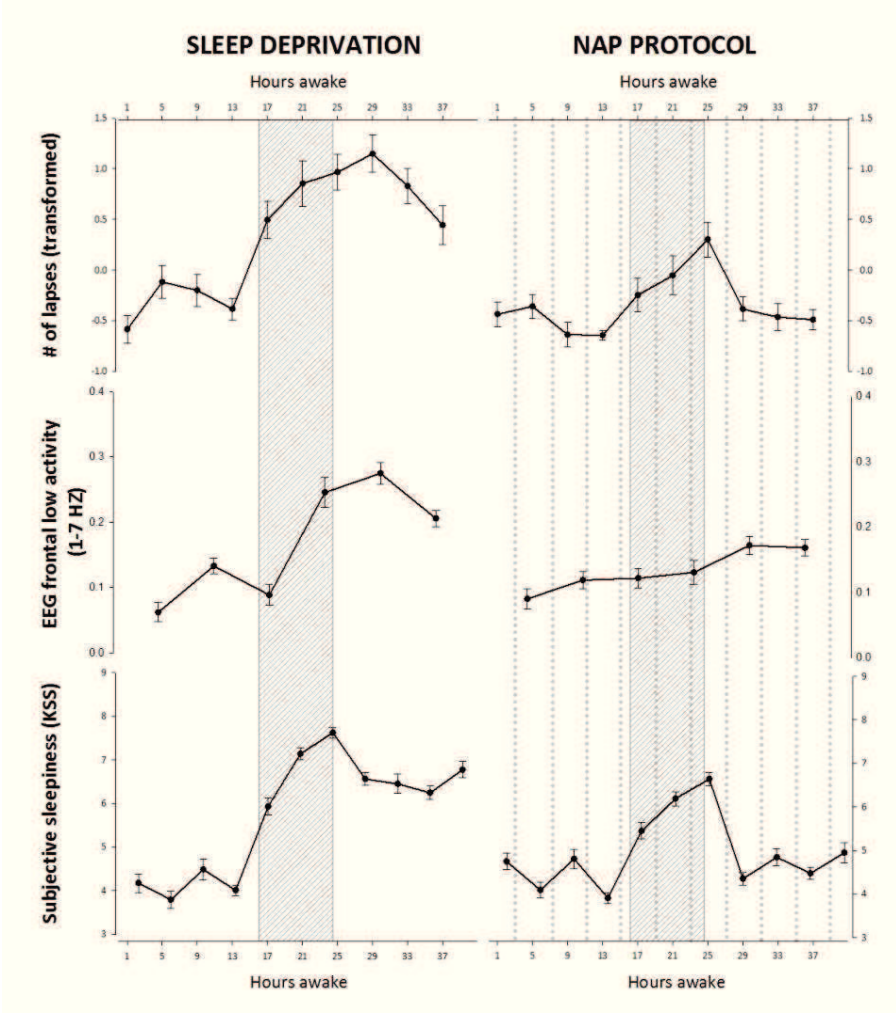

Figure 2. Time course of attentional lapses, EEG frontal low activity, and subjective sleepiness during a 40-h sleep deprivation and a 40-h nap protocol (alternating cycle of 160 min wakefulness and 80 minutes nap).
Upper panel: lapses (reaction times > 500 ms) during the Psychomotor Vigilance Task. *Middle panel:* EEG frontal low activity (1-7 Hz; log-transformed). *Lower panel:* Subjective sleepiness (assessed with the Karolinska Sleepiness Scale, KSS). Dashed grey area frames the biological night; dashed lines in the left panels schematically indicate the timing of the nap episodes. N = 29,

Figure 2, it is evident that EEG frontal low activity (middle panel) is increasing under sleep-wake homeostatic control, but shows little circadian variation (compatible with previous reports, e.g.,

Cajochen et al., 2001). In contrast, neurobehavioral performance and subjective sleepiness depict a homeostatic-dependent as well as a circadian modulation (cf. Cajochen et al., 2001; Graw, Kräuchi, Knoblauch, Wirz-Justice, & Cajochen, 2004). Thus, with the present data we again confirmed that this combined NP/SD approach seems suitable to disentangle the circadian and homeostatic influences on different outcomes waiving the more effortful FD protocol.

2.1.3 Cerebral basis of sleep-wake regulation

WAKEFULNESS. Multiple brain structures and pathways are implicated in the sleep-wake control. Wakefulness mainly relies on the ascending arousal system which was initially described by Moruzzi and Magoun (1949). Two main pathways arising from the brainstem reticular formation form this system: one innervates the thalamus, and the second one extends into the posterior hypothalamus and forebrain (Jones, 2005). Glutamate is the main neurotransmitter released in the brainstem to excite the projecting neurons within these two pathways to stimulate cortical activation (Jones, 2003). However, several other neurochemicals are implicated in this brain circuitry. Table 1 provides an overview of the most important involved neurotransmitters or neuromodulators, brain structures and the respective mechanisms that all together regulate wakefulness.

Table 1. Neurochemicals and brain structures involved in arousal promotion

Neurotransmitter/ Neuropeptide	Brain structures	Projections	Mechanism	
Acetylcholine	Reticular formation, pedunculopontine / laterodorsal tegmental nuclei	THA, basal forebrain	Excitation of thalamocortical cells	McCormick & Bal, 1997, Jones, 2008
	Basal forebrain	Cortex, midbrain	Promotion of behavioral and cortical arousal	Steriade, 1992
Dopamine	Substantia nigra, ventral tegmental area	Striatum, frontal cortex, nucleus raphe, LC, THA, limbic structures	Behavioral arousal, motivation, movement	Monti & Monti, 2007
Glutamate	Ascending reticular activating system (large brainstem reticular core)	Cerebral cortex, forebrain, THA, brainstem	Largest amounts released during wakefulness, antagonists induce sleep	Jones, 2003, 2005; Lin, Anaclet, Sergeeva, & Haas, 2011
Histamine	Posterior hypothalamus (tuberomammilary nuclei)	Lateral hypothalamus	Exitatory input to the cerebral cortex, inactive during sleep	John, Wu, Boehmer, & Siegel, 2004; Jones, 2005
Norepinephrine (Noradrenaline)	LC, medullary reticular formation	Forebrain, all areas of the cerebral cortex	Stimulation and maintenance of cortical activation, inhibition of sleep-promoting areas (VLPO)	Jones, 2005; Saper, Lu, Chou, & Gooley, 2005
Orexin (Hypocretin)	Lateral hypothalamus	Brainstem, thalamus, hypothalamus, cerebral cortex	Cortical activation, highest firing rates during waking	Jones, 2005; Wright et al., 2012
Serotonin	Dorsal and median raphe nuclei	Cortex, spinal cord	Inactive during sleep, promotion of cortical and behavioral arousal, inhibition of sleep-promoting areas (VLPO)	Jones, 2005; Saper, Lu, et al., 2005

Abbreviations: LC = locus coeruleus, THA = thalamus, VLPO = ventro lateral preoptic area.

SLEEP. The transition from wakefulness to sleep is thought to be induced by neurons in the ventrolateral preoptic area (VLPO) and the median preoptic nucleus (Lu, Greco, Shiromani, & Saper, 2000; McGinty & Szymusiak, 2001; Saper, Scammell, & Lu, 2005). These VLPO neurons contain the inhibitory gamma-amminobutyric-acid (GABA) and galanin, and innervate all components of the arousal system. By firing, they inhibit the latter and promote sleep, whereas during wakefulness, they remain silent (Saper, Chou, & Scammell, 2001). Especially the monoaminergic arousal systems project back to the VLPO and actively inhibit this structure during wakefulness (Chou et al., 2002). The interplay between sleep- and wake-promoting brain areas is thus mutually inhibitory, and was described as a flip-flop system (Saper et al., 2001). In other words, the major arousal systems and the VLPO each strongly inhibit the other, resulting in a feedback loop where two stable patterns are possible (Saper et al., 2001). Orexin/hypocretin seems to play a central role in this flip-flop system, potentially acting as the "finger" pressing the switch to stay awake, and preventing the flip-flop to switch to sleep (Saper et al., 2001). This concept also accounts for the relatively abrupt and short wake-sleep transitions which are normally experienced (Saper et al., 2001). Figure 3 depicts the most important structures implicated in the arousal and sleep promotion.

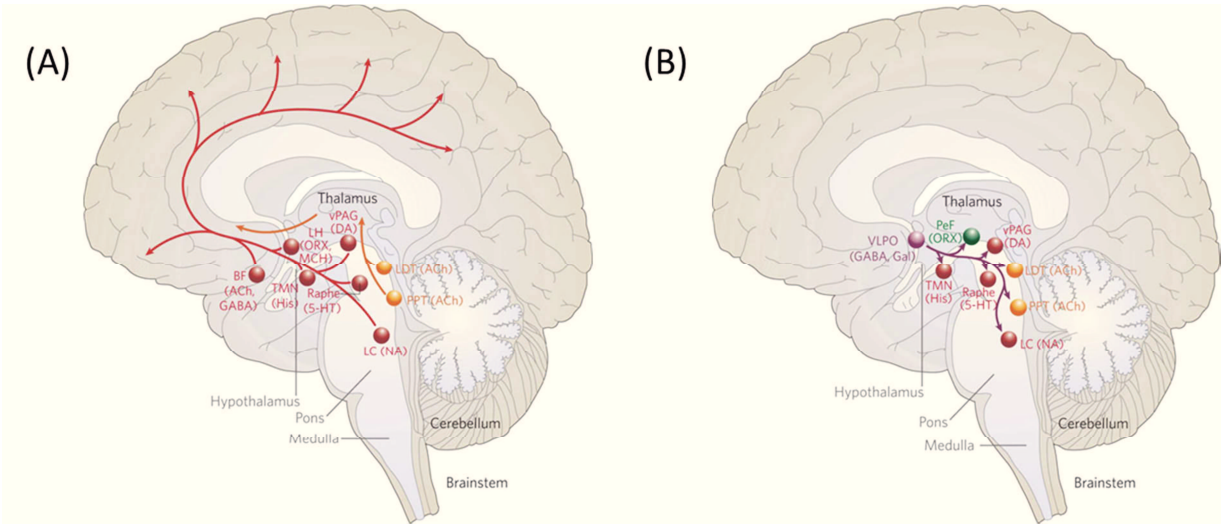

Figure 3. Schematic drawing depicting **(A)** the key components of the ascending arousal system and **(B)** the projections of the VLPO to these key components. *From Saper et al., 2005.*

CIRCADIAN PROCESS. In the perspective of the two process model of sleep regulation (Borbély, 1982), attempts have been made to separate the cerebral basis of the homeostatic and circadian process. With the SCN incorporating the master clock, a main circadian brain correlate was clearly identified: lesions of this structure lead to a disruption of the sleep wake rhythm (Mistlberger, Bergmann, Waldenar, & Rechtschaffen, 1983). On the molecular level, a transcriptional-translational loop results in a rhythmic 24 h-firing-pattern in the SCN neurons (Reppert & Weaver, 2002; Saper, Scammell, et al., 2005). Projections to sleep-promoting areas such as the VLPO and wake-promoting

orexin neurons are present, but are mostly indirect (Deurveilher & Semba, 2005). It was suggested that norepinephrine neurons in the LC play a role in the circadian regulation of wakefulness, with an indirect input from the SCN to the LC with the dorsomedial nucleus of the hypothalamus (DMH) as a relay-structure (Aston-Jones, 2005; Aston-Jones, Chen, Zhu, & Oshinsky, 2001). The DMH is thought to influence the circadian rhythm of sleep and waking, locomotor activity, feeding and corticosteroid production (Saper, Lu, et al., 2005). With its projections to the LC, which is an arousal promoting area with multiple projections throughout the brain, a possible pathway for the circadian regulation of arousal and higher cognitive functions was proposed. Insights from functional imaging studies to the cerebral bases of the circadian system, more precisely the circadian arousal signal, mostly involved the investigation of light exposure on task-related brain responses (Perrin et al., 2004; Vandewalle et al., 2006; Vandewalle et al., 2007). These studies showed that light - as the most important zeitgeber[1] for the circadian system - is implicated in the regulation of both cortical and subcortical brain functions and is potent to modulate cognition (Vandewalle, Maquet, & Dijk, 2009).

HOMEOSTATIC PROCESS. It is somewhat harder to assign specific brain correlates to the sleep homeostatic process, although it was shown to be functionally and anatomically independent of the SCN (Trachsel, Edgar, Seidel, Heller, & Dement, 1992). According to Tononi and Cirelli (2006), sleep homeostasis is related to synaptic plasticity processes: daily learning and experience during wakefulness result in a net increase in synaptic strength in several brain circuits. During sleep, the synaptic strength is downscaled to baseline levels to reach an energetically sustainable synaptic weight (Tononi & Cirelli, 2006). Kattler et al. (Kattler, Dijk, & Borbély, 1994) observed that somatosensory brain areas which were selectively activated in humans before sleep, showed an increase in delta activity during subsequent sleep, suggesting that sleep homeostasis is local and use-dependent. Huber and colleagues later confirmed this with a learning paradigm in rats (Huber, Ghilardi, Massimini, & Tononi, 2004). The sleep homeostatic process might thus be rather represented by neurochemical factors instead of specific anatomical correlates. Accordingly, it was suggested that an accumulating sleep-promoting substance could enhance the activity of sleep-promoting cells and inhibit wake-promoting neurons (Saper, Scammell, et al., 2005). Adenosine was proposed to serve as such a substance, since its concentrations in the basal forebrain seem to reflect homeostatic sleep drive (Basheer, Porkka-Heiskanen, Stenberg, & McCarley, 1999; Porkka-Heiskanen et al., 1997). That is, it rises with increasing time awake, and dissipates during sleep (reviewed in Landolt, 2008). Adenosine additionally disinhibits the GABAergic inputs to the VLPO (Saper, Scammell, et al., 2005). Correspondingly, it was shown in animals that VLPO neurons fire twice as much during recovery after SD as during normal sleep (Lu et al., 2002). In the same line, a

[1] *Zeitgeber* refers to an external or environmental cue; this expression was first used by Jürgen Aschoff, a German pioneer in chronobiology research, and subsequently became adopted by the English language.

polymorphism in the adenosine deaminase (ADA) gene was recently shown to modulate sleep homeostatic markers in humans (Bachmann et al., 2012). Further, brain-derived neurotrophic factor (BDNF), which is involved in synaptic potentiation, has been associated with sleep need: injections of BDNF in rat cortices resulted in an increase in SWA (Faraguna, Vyazovskiy, Nelson, Tononi, & Cirelli, 2008; Hanlon, Vyazovskiy, Faraguna, Tononi, & Cirelli, 2011). Interestingly, BDNF requires endogenous adenosine for long-term potentiation (Fontinha, Diogenes, Ribeiro, & Sebastiao, 2008), suggesting that these two systems interact (Bachmann et al., 2012). A recent study by Holst et al. (2014) further proposed that striatal dopaminergic neurotransmission plays a role in sleep homeostasis. The authors showed that electrophysiological markers of sleep homeostasis (SWS, SWA) after SD were enhanced in humans carrying a polymorphism previously associated with less striatal dopamine transporter protein expression. Despite the growing interest in this topic, the cerebral basis of the sleep homeostatic processes remains hardly understood. A growing body of neuroimaging experiments attempted to gain insight into cerebral correlates of sleep homeostasis, mostly by assessing task-related brain activity after SD compared to rested wakefulness. Although it is difficult to directly link findings of such studies to the cerebral basis of sleep homeostasis (i.e., because activity per se is most often elicited by performance of a certain task), this approach is promising to better understand sleep homeostasis and to generate novel research questions. Further information on these studies is provided below.

INTERACTION OF CIRCADIAN & HOMEOSTATIC PROCESS. Derived conceptually from the two-process model (Borbély, 1982), the circadian and homeostatic process interact to regulate the sleep-wake cycle. Thus, one would expect certain brain areas to serve as an interface for this interaction. Despite an ongoing debate about the exact nature of the interaction between these two processes (see e.g., Franken & Dijk, 2009), several findings point towards a non-additive, bidirectional interaction at least at the molecular level (Dijk & Franken, 2005). Most strikingly, Deboer and colleagues showed altered electric activity in the SCN after sleep deprivation. Their finding further supported the assumption that the sleep state influences the circadian clock (Deboer, Detari, & Meijer, 2007; Deboer, Vansteensel, Detari, & Meijer, 2003). Conversely, it was shown in all species investigated that an ablation of the SCN did not result in an alteration of the sleep homeostatic process, which points to a separation of the neuroanatomical correlates (Dijk & Franken, 2005). Clock genes generate circadian rhythms through a network of transcriptional regulators in the SCN (for a review, see Takahashi, Hong, Ko, & McDearmon, 2008), but are also expressed in other brain tissues. Interestingly, it seems that clock gene expression particularly in the cerebral cortex depends on prior wake history (Franken & Dijk, 2009), whereas the expression in the SCN per se does not. Additionally, the amplitude of the circadian variation in alertness, performance and other sleep variables was shown to depend on the status of the sleep homeostat (Cajochen, Jewett, et al., 2003; Dijk &

Czeisler, 1995; Dijk, Duffy, & Czeisler, 1992; Franken & Dijk, 2009). However, it remains hardly explored how much the findings at the molecular level extend to the brain circuitry involved. It was shown in rats that the SCN receives multiple inputs from hypothalamic, thalamic and brainstem nuclei (Krout, Kawano, Mettenleiter, & Loewy, 2002), which might modulate the interaction between the circadian and homeostatic process. An important neuromodulator involved in the interaction between sleep homeostat and the circadian pacemaker is the neuropeptide hypocretin (see also Table 1). Zeitzer et al. (2003) showed that it works reactive to increasing sleep need, but its time course is also regulated by the circadian pacemaker. Finally, Schmidt et al. (2009) found that regional blood flow in the LC and in the anterior hypothalamus (suprachiasmatic area) during a sustained attention task was higher in participants with lower sleep pressure accumulation. Moreover, lower activity in this latter region was related to higher SWA levels during subsequent sleep (Schmidt et al., 2009). Although these data support that the interaction between the two processes are detectable on the cerebral level, a clear picture about specific structures and pathways involved is still missing.

2.1.4 Molecular basis of sleep-wake regulation

CIRCADIAN RHYTHM. The circadian system in mammals is orchestrated by a hierarchy of oscillators, with the SCN at the top of this hierarchy. It receives direct light input via a retinohypothalamic tract to entrain the clock to the 24-h day (Reppert & Weaver, 2002). Within the neuron of the SCN, a feedback loop involving activator elements (the genes *CLOCK, BMAL1*), and repressor elements (the genes *PER1*, *2*, and *3*, *Cryptochrome* [*CRY*] *1* and *2*) generates rhythmicity (Takahashi, Hong, et al., 2008). CLOCK interacts with BMAL1 to activate transcription of the *PER* and *CRY* genes during the day (Takahashi, Shimomura, & Kumar, 2008). The *PER* and *CRY* messenger RNAs are translocated to the cytoplasm for translation to the proteins PER1, PER2, PER3 and CRY1 and CRY2. The resulting PER and CRY proteins heterodimerize (i.e., form a molecule consisting of two subunits) and translocate back to the nucleus to inhibit their own transcription via the inhibition of the complex CLOCK/BMAL1. When the transcription of *PER* and *CRY* decreases, their repressor effect stops and the complex CLOCK/BMAL1 can activate a new cycle of transcription. This circuit is also modulated by another loop in which the CLOCK/BMAL1 complex activates the transcription of *REV-ERBα* and *RORα* genes. The two arising proteins, REV-ERBα and RORα, regulate - the first negatively and the latter positively - the transcription of the gene *BMAL1* (Preitner et al., 2002; Sato et al., 2004). One cycle of this feedback loop lasts about 24h and induces the rhythmic expression of the targeted genes, resulting in a rhythmic neuronal activity and thus constituting the circadian rhythm (Ko & Takahashi, 2006). Figure 4 from Ko and Takahashi (2006) displays this feedback loop. The loops are also influenced by post-translational modifications (such as the phosphorylation or the ubiquitination of their components), constituting another level of modulation of the clock period (Harms, Kivimae, Young, & Saez, 2004).

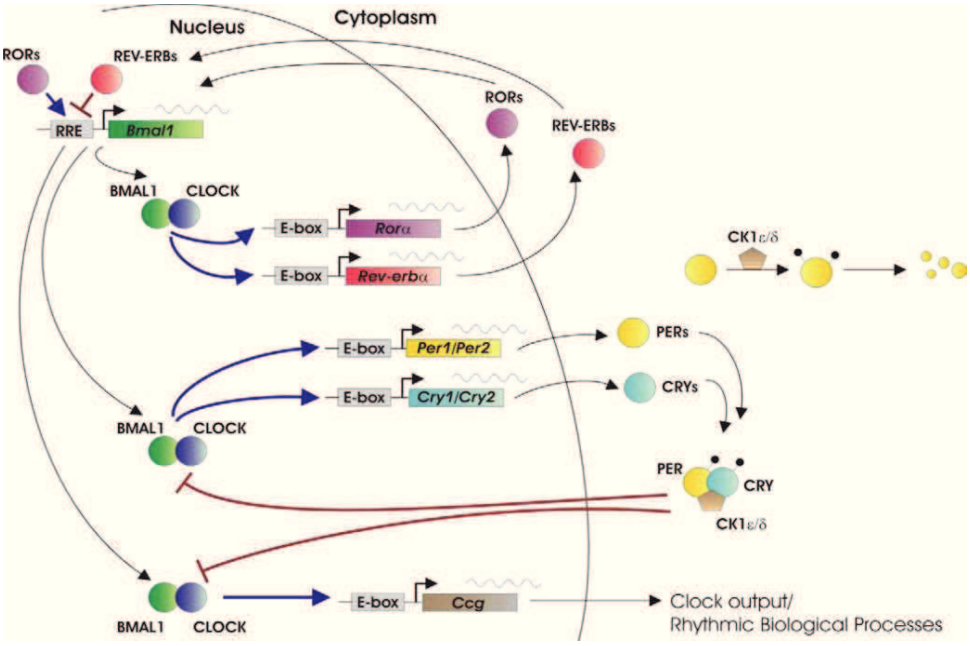

Figure 4. Molecular feedback loop of mammalian circadian rhythmicity. From *Ko & Takahashi, 2006.*

The SCN also coordinates the timing of "slave oscillators" throughout the body (Reppert & Weaver, 2002): The same genes involved in the SCN clock mechanism are also expressed in other brain areas (e.g., the cortex) and in peripheral organs (Yagita, Tamanini, van Der Horst, & Okamura, 2001; Yamamoto et al., 2004), such as the liver and kidney. The peripheral oscillators are independent, multi-oscillatory entities and can integrate their own messages, but depend on the input of the SCN for the synchronization of their oscillations (Abe et al., 2002; Guo, Brewer, Lehman, & Bittman, 2006), which regulate local rhythms in behavior and physiology (Reppert & Weaver, 2002). The importance of the clock genes for circadian rhythmicity has been shown by targeted disruption of one or a combination of these genes in mice (Franken & Dijk, 2009). When *BMAL1*, *CLOCK*, *NPAS2*, *CRY1* and *2*, *PER1* and *2* are lacking, circadian organization of behavior is disrupted under constant dark conditions (Lowrey & Takahashi, 2004).

HOMEOSTATIC PROCESS. The molecular mechanisms of sleep homeostasis remains less well determined. The dynamics of the sleep homeostatic process, namely SWS accumulation and EEG delta power, are genetically determined as shown in mice (Franken, Chollet, & Tafti, 2001). This indicates that this process has a molecular substrate (Franken, 2013). In humans, a functional genetic variation in the adenosine-metabolizing enzyme ADA was shown to have an impact on non-REM (NREM) SWS and SWA (Retey et al., 2005). Further, Homer1a was determined as a core brain molecular correlate of sleep loss, as this gene is overexpressed after SD in mice (Maret et al., 2007), and its transcripts are up-regulated after SD in rats (Cirelli, Gutierrez, & Tononi, 2004).

Besides these results, the most striking evidence to uncover molecular sleep homeostatic processes stems from findings suggesting a non-circadian role for clock genes in sleep-wake regulation. Indeed, disruption of clock genes do not only alter circadian rhythmicity, but also has an impact on variables that are considered typical markers of the sleep homeostatic process, such as sleep duration, sleep structure, and EEG delta power (reviewed in Franken & Dijk, 2009). For instance, besides showing arrhythmic behavior, mice lacking *CRY1* and *CRY2* spent more time in NREM sleep, and had more EEG delta power (Wisor et al., 2002). Mice with homozygous *BMAL1* deletion showed increases in total sleep time, sleep fragmentation and EEG delta power (Laposky et al., 2005). Moreover, Franken et al. showed that in mice, brain *PER1* and *PER2* expression was dependent on the sleep-wake history: it increased linearly with time awake in the forebrain (Franken, Thomason, Heller, & O'Hara, 2007), also depending on the strain (i.e., genetic background) of the mice. Several other corroborating findings from animal research are reviewed in Franken and Dijk (2009). Additional evidence from human studies points in a similar direction: a VNTR polymorphism in the human clock gene *PER3* was associated with increased homeostatic sleep pressure, as evidenced by more SWS and higher SWA during baseline and after SD (Viola et al., 2007).

In sum, growing evidence seems to confirm the suggested coupling of circadian and homeostatic processes at the molecular level. Hence, it was proposed that the conceptual separation of the two components stated by Borbély and colleagues (Borbély, 1982; Daan et al., 1984) does not extend to the molecular level (Franken & Dijk, 2009).

2.2 Sustained attention

2.2.1 Definition and differentiation: Sustained attention, vigilance, alertness and arousal.

In most professions, in social situations, or even in our everyday life, we are frequently required to direct attention towards a task over a certain time, that is, to sustain attention. As a psychological construct, sustained attention describes a fundamental component of attentional processes, determinant for more complex aspects of attention and general cognitive capacity (Sarter et al., 2001). It denotes the readiness to react to stimuli that occur unpredictably and relatively rarely over a prolonged period of time (Sarter et al., 2001). It is conceptually (Kahneman, 1973; Langner & Eickhoff, 2013; Parasuraman, Warm, & See, 1998) and neuroanatomically distinguished from selective, shifted, or divided attention (Coull, 1998).

"Vigilance" or "vigilant attention" are two terms that are used interchangeably with sustained attention (Langner & Eickhoff, 2013; Oken, Salinsky, & Elsas, 2006), and we will do so in the present work. "Alertness", another commonly encountered term, is further classified into a phasic and a tonic component (Oken et al., 2006; Posner & Petersen, 1990). The definition of the tonic component overlaps with the concept of sustained attention, whereas the phasic component is

normally associated with the orienting response after a warning signal (Cohen & O'Donnell, 1993).[2] In sleep and chronobiology research, alertness is often used to describe a general subjectively or objectively assessed state (i.e., the contrary of sleepiness as drawn from self-rating scales or EEG), but is not necessarily derived from performance on a task. Importantly, we distinguish all before mentioned concepts from "arousal", which refers to a more physiological concept (i.e., the basic ability of the organism to react to sensory inputs). Its degree would typically be derived from neurophysiological data such as EEG (Sarter et al., 2001), and a certain level is a prerequisite for the wake state and thus attentional process.

Researchers concerned with attentional processes adopted the distinction of "top-down" and "bottom-up" processes otherwise commonly used in perceptual psychology. Thus, a short reference to this often-encountered concept seems helpful. The top-down process is goal-directed; driven by knowledge-based mechanisms (concepts) enhancing the contrast between signal and noise, and producing biases to stimulus features that are relevant. Bottom-up describes an attentional process driven by stimulus features (such as sensory salience) and its sensory context (Egeth & Yantis, 1997). Importantly, to reach optimal attentional performance, top-down and bottom-up processes interact (comprehensively reviewed in Egeth & Yantis, 1997; Sarter et al., 2001). Importantly, top-down and bottom-up processes are conceptual principles, and do not refer to anatomical ascending and descending neuronal projections (Sarter et al., 2001). Roughly, top-down processes are assigned to frontal brain areas involved in cognitive control, whereas bottom-up processes are mainly governed by primary sensory brain regions (Sarter et al., 2001). Even if a clear distinction concerning the neuroanatomical level is difficult to make, the concept is helpful to understanding processes that might be involved in successful or unsuccessful sustained attention (Lim & Dinges, 2008).

2.2.2 Measuring sustained attention

The range of tasks which are used to experimentally assess sustained attention and underlying processes is broad. However, certain characteristics are common to all, that is, they all require participants to monitor their environment for a pre-specified target (Langner & Eickhoff, 2013) of a given modality, and per definition have a duration of at least several minutes. Langner and Eickhoff (2013) describe three broad task categories: continuous stimulus detection (simple reaction time [RT] tasks, all cues are targets), continuous stimulus discrimination (go/no-go tasks), and sustained silent target counting. In sleep and chronobiology research, the PVT, initially presented by Dinges and Powell (1985) is most commonly used and belongs to the first of these three categories.

[2] Detached from this classical definition, in the present thesis, we use the terms tonic and phasic to more specifically differentiate processes during a sustained attention task despite the absence of a warning signal before the stimuli. That is, we consider the waiting period during the inter-stimulus interval as tonic attentional process, whereas the phasic component is activated as soon as the stimulus is visually perceived.

In the classical version of the task, a visual cue (i.e., a millisecond counter) occurs after random inter-stimulus intervals (ISI) ranging from 2 to 10 sec, with a standard duration of 10 minutes. Subjects are instructed to fixate the screen and to react as fast as possible with a simple key press as soon as the stimulus appears. Feedback is given by displaying the RT in ms after each trial for one second. Various outcome measures, all based on RTs, can be derived from this simple task (see Basner & Dinges, 2011 for a discussion). What makes the task attractive for sleep and chronobiology research is the absence of a learning curve, meaning it can be administered repeatedly over time (Lim & Dinges, 2008). Likewise, it is very sensitive to SD, and both homeostatic and circadian variation are observed (Blatter et al., 2006; Graw et al., 2004). Finally, the task has also proven suitable for neuroimaging studies (Drummond, Bischoff-Grethe, et al., 2005; Schmidt et al., 2009).

2.2.3 Neural underpinnings of sustained attention

To be attentive and responsive to a task, obviously it is required to be awake. Brain areas involved in the regulation of the sleep-wake state are thus a central component in the regulation of sustained attention. An adequate arousal level is ensured by an ascending activation of the brain stem, basal forebrain, and hypothalamus to the cortex and the midbrain (reviewed in Wright et al., 2012). Wakefulness-promoting neurotransmitters include acetylcholine (ACh), dopamine, glutamate, histamine, norepinephrine, orexin, and serotonin (see also Chapter 2.1, Table 1). Evidence from brain damage and neuronal degeneration studies broadly suggests that lesions in the frontal cortex as well as the inferior parietal lobe functioning are accompanied by sustained attention impairment (Sarter et al., 2001). More specific evidence however stems from functional imaging studies with healthy volunteers: A recent comprehensive meta-analytic review by Langner and Eickhoff (2013) identified dorsomedial (motor preparation and inhibition, motivation), mid- and ventrolateral prefrontal cortex (monitoring, link stimulus - motor response), the anterior insula (re-/activation of relevant task schema), parietal areas including the intraparietal sulcus and temporo-parietal junction (target detection, interface between top-down and bottom-up processing, stimulus-driven re-orienting), and subcortically the midbrain and thalamus (maintenance of attention, modulation of cortical arousal), ceberellum (anticipatory timing of motor output) and putamen, to be most consistently activated in sustained attention task of any modality (Langner & Eickhoff, 2013).

Neurochemically, ACh has been suggested to be majorly implicated in the neuronal circuits mediating sustained attention performance. This hypothesis was mainly posited and investigated by Sarter and colleagues (Himmelheber, Bruno, & Sarter, 2000; Sarter, Bruno, et al., 2006; Sarter, Gehring, & Kozak, 2006; Sarter et al., 2001). According to these authors, the basal forebrain cholinergic system is necessary to activate the cortical cholinergic input system. Increases in prefrontal cholinergic activity might contribute to the activation of the anterior attention system (a term introduced by Posner, e.g., Posner & Petersen, 1990) involved in top-down optimization of

input processing in sensory regions (Sarter, Gehring, et al., 2006). Cholinergic increases in sensory and other posterior cortical regions are thought to mediate these top-down effects. Likewise, cholinergic inputs also mediate the facilitation of bottom-up-sensory information processing, mainly via the noradrenergic projections originating in the LC which terminate in the thalamus and the basal forebrain (Sarter et al., 2001). The involvement of the LC in attentional processes was also described by (Aston-Jones, Rajkowski, & Cohen, 2000). The authors showed that the LC exhibits tonic or phasic activation, depending on task performance. The tonic mode was associated with behavioural flexibility or scanning attentiveness, whereas the phasic mode was related to selective, focused attention (Aston-Jones, 2005). Importantly, the LC also receives strong excitatory input from the prefrontal cortex (Jodo, Chiang, & Aston-Jones, 1998); suggesting a potential top-down modulation of this structure, for instance in response to the detection of performance decline (Sarter, Gehring, et al., 2006). Notably, the LC also might represent a key structure involved in the effects of sleep loss on attention and other cognitive processes (Aston-Jones, 2005). In the next chapter, the effects of SD will be discussed in detail.

2.3 Consequences of sleep loss

Sleep deprivation or sleep loss elicits numerous effects on many physiological and psychological processes such as attention, executive functions, learning and memory, decision making, emotions, mood, and other behaviour such as food intake and physical activity. Further, sleep loss was suggested to be a risk factor for psychiatric and somatic disorders. As a research topic, it has gained more and more attention especially over the last two decades: when searching for “sleep deprivation” or “sleep loss”, PubMed (http://www.ncbi.nlm.nih.gov/pubmed) lists roughly 15’000 articles starting in the year 1949 (see Figure 5).

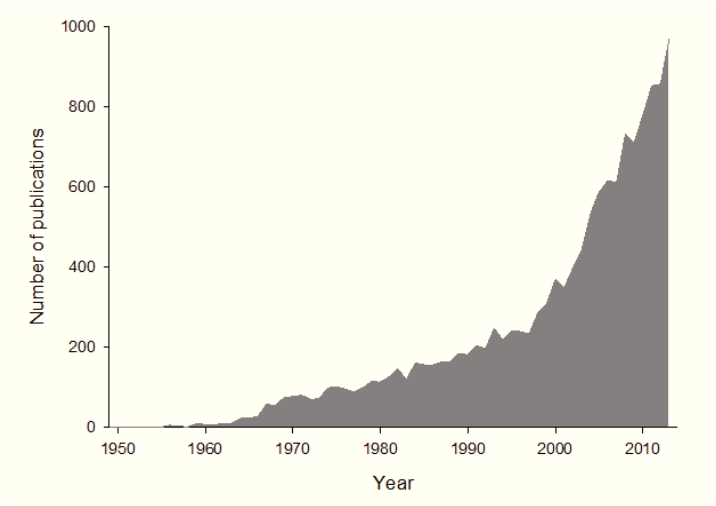

Figure 5. Number of publications per year for the search term "sleep deprivation" or “sleep loss” derived from the PubMed database, August 2014. Indicated years : 1949-2013.

Here, we focus on the impact on neurophysiology and neurobehavioral performance, specifically, sustained attention. We consider the effects of acute total SD (i.e., experimentally induced sleep loss). Last, the influence of individual variability in response to SD will be discussed, and the impact of *PER3* as a candidate gene will be highlighted.

2.3.1 Impact of sleep loss on neurophysiology during waking and sleep

Consequences of SD on neurophysiology can be reliably detected and assessed with electrophysiological methods, both during waking or subsequent recovery sleep. Waking EEG power density analysis provides insight to arousal states (Santamaria & Chiappa, 1987): Low frequencies, namely delta (1-4 Hz) theta (4-8 Hz) and low alpha (8-9 Hz), increase with time awake with a frontal predominance (Cajochen et al., 1995; Cajochen, Khalsa, Wyatt, Czeisler, & Dijk, 1999), and are validated markers of the sleep homeostatic process (Finelli et al., 2000). Slow rolling eye movements (SEM) occur more frequently with increasing sleepiness and thus mirror low arousal and drowsiness (Cajochen, Khalsa, et al., 1999).

During sleep after a prolonged waking period, a shortening of sleep latency, fewer awakenings, less stage 1 (light sleep) after sleep onset, as well as an increase in total sleep time are commonly observed. Likewise, a relative increase in the amount of deep sleep (SWS, stages 3 and 4) and SWA as well as theta compared to baseline conditions occurs (Borbély, Baumann, Brandeis, Strauch, & Lehmann, 1981; Cajochen, Foy, & Dijk, 1999; Finelli et al., 2000), mostly in the first part of the recovery sleep episode (Feinberg, Floyd, & March, 1987). On the other hand, activity in the sleep spindle range (12-15 Hz) is reduced (Dijk, Hayes, & Czeisler, 1993; Knoblauch, Martens, Wirz-Justice, & Cajochen, 2003). Importantly, many studies reported correlations of these physiological consequences with neurobehavioral performance decrements after sleep loss (see e.g., Cajochen, Khalsa, et al., 1999). By consequence, physiological parameters potentially serve as a predictor for the amount of decrement.

2.3.2 Impact of sleep loss on sustained attention

BEHAVIORAL CONSEQUENCES. The impact of sleep loss on human cognitive performance depends on several factors, such as prior wake duration (Van Dongen, Maislin, Mullington, & Dinges, 2003), the time of day when testing occurs (Schmidt, Collette, Cajochen, & Peigneux, 2007; Wright et al., 2012), the investigated cognitive domain (e.g., Lim & Dinges, 2010; Lo et al., 2012), but also participants' characteristics such as age and gender (Blatter et al., 2006). Here, we will focus on the general effects of sleep loss on sustained attention as defined above.

Primarily, the duration of the prior wake episode plays a central role. Accordingly, the amount of attentional lapses in sustained attention displays a near-linear relation to the cumulative duration of wakefulness after the regular approximate 16h-waking period (Van Dongen et al., 2003).

Compared to other cognitive domains, performance on sustained attention tasks often seems to be most affected by SD (Lim & Dinges, 2008, 2010). A general slowing of RTs, a higher frequency and longer duration of lapses in attention (i.e., errors of omission, stimuli are entirely missed; or a response is given considerably later than average), and random or anticipatory button presses (errors of commission) become more frequent (Lim & Dinges, 2008). Task characteristics such as frequency and temporal irregularity of stimuli are important determinants for the extent of performance decrement (Cochran, Thorne, Penetar, & Newhouse, 1992; Warm, Parasuraman, & Matthews, 2008). Indeed, task duration or "time-on-task", seems to be one of the main factors which modulate how sleep loss impinges on performance (Doran et al., 2001). In this regard, the most general, task-type-independent effect of SD on performance was conceptualized as "state instability" (Doran et al., 2001; Durmer & Dinges, 2005). According to this hypothesis, sleep initiating mechanisms increasingly interfere with wakefulness, which in turn leads to an increasing performance variability including task disengagement, and a dependency on compensatory mechanisms (Doran et al., 2001; Dorrian, Rogers, & Dinges, 2005; Rogers, Dorrian, & Dinges, 2003). These increasing attentional failures are strongly dependent on task duration - the longer the task, the more likely the growing variability will be detected, as compensatory mechanisms are more likely to fail after a certain time (Doran et al., 2001). Lapses in attention will thus likely progress into uncontrolled sleep attacks due to increasing homeostatic sleep pressure (Doran et al., 2001). Accordingly, Figure 6A displays RT standard deviations as a measure for performance variability from the present data set during high and low sleep pressure conditions. After the regular 16h waking hours, variability starts to increase under high sleep pressure, but remains relatively little with low sleep pressure. Figure 6B shows the interaction of the number of lapses on the PVT with hours awake and minutes on the task during SD, speaking in favour of such a state instability as specified by Doran et al. (2001).

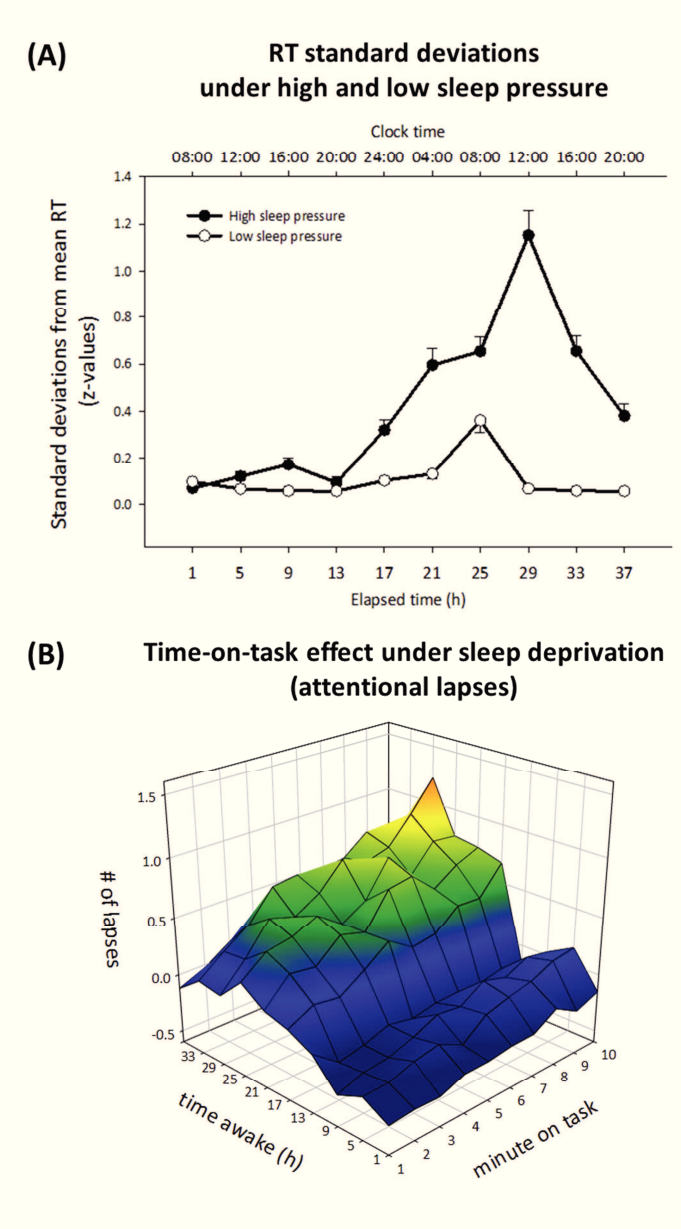

Figure 6. Indications for "state instability".
(A) Mean standard deviations derived from z-transformed RT as a measure for performance fluctuations under low (multiple nap protocol, open circles) and high sleep pressure (sleep deprivation, filled circles). Under high sleep pressure, variability increases during the course of the biological night and stabilizes on the second day with the reoccurring circadian arousal signal towards the biological evening. Under low sleep pressure, relatively little variability occurs. **(B)** Interaction between hours of wakefulness (time awake) during sleep deprivation (y-axis) and time-on-task (minutes on task, x-axis) number of lapses on the modulation of the Psychomotor Vigilance Task (PVT, z-axis). Higher values indicate higher levels of impairment. (B) is adapted from Maire et al., 2014b. N = 29.

Finally, motivational aspects seem to play a role in modulating the effects of SD on performance. Feedback and incentives were shown to counteract general and time-on-task-related vigilance decrement (Horne & Pettitt, 1985; Steyvers & Gaillard, 1993). Researchers thus concluded that the decrement must mainly result from boredom and thus a lack of motivation to perform monotonous tasks (Horne, Anderson, & Wilkinson, 1983). However, indicated by the presence of normal or even fast RT interspersed with long RT or lapses, other researchers concluded that motivation rather masks detrimental effects of SD (Doran et al., 2001; Kahneman, 1973). Accordingly, Sarter et al. (2006) recently proposed that increased attentional effort per se is a cognitive incentive and is able to counteract detrimental effects such as sleepiness or time-on-task. In any case, the assessment of subjective motivation along with performance on a task especially in SD studies seems helpful.

CEREBRAL CONSEQUENCES. Most insight into the detrimental effects of SD on the cerebral correlates of vigilance stems from neuroimaging studies. Although some of the first neuroimaging studies conducted after SD involved sustained attention tasks (Portas et al., 1998; Thomas et al., 2000; Wu et al., 1991), much more work was done regarding other cognitive domains such as working memory or selective attention over the last years. Again, the investigated cognitive domain and task difficulty modulate observed brain activations, but a common finding is a decrease in higher sensory cortices (according to the modality of the task at hand) and fronto-parietal activation when performance levels declined after SD, yet relatively preserved activation when performance was maintained (Chee & Thomas, 2013). These findings were interpreted as "compensatory" mechanism or, contrarily, inefficient processing on the cerebral level (Chee & Thomas, 2013), see also (Drummond, Meloy, Yanagi, Orff, & Brown, 2005) for a discussion of this issue).

Deficits specific for failures in sustained attention could be the consequence of an insufficient arousal level (Portas et al., 1998; Thomas et al., 2000). Chee and colleagues advocate the hypothesis that during SD, top-down attentional control, which is mediated by fronto-parietal areas, fails and thus leads to a loss of selective focus necessary to attend to a task (e.g. Chee & Tan, 2010; Chee et al., 2008; Chuah & Chee, 2008). Alternatively, it was proposed that bottom-up processes fail due to local sleep-like states (Van Dongen, Belenky, & Krueger, 2011), or a combination of both (Chee et al., 2011). Drummond et al. (2005) were the first to describe the neural basis underlying PVT performance, comparing the well-rested and sleep deprived state. The authors detected a higher recruitment of attention-related cortical and subcortical structures after SD for the slowest RT domain (i.e., non-optimal performance), whereas the activation pattern during optimal performance (fastest RTs) was not altered after SD. Others (Chee et al., 2008) reported that lapses in attention were associated with reductions in visual sensory cortices and thalamic activation, and showed that SD reduced the ability to raise activation in response to lapses compared to the well-rested state. Crucially, vulnerability to SD alters the cerebral response to SD considerably, resulting in differential activation-deactivation patterns (reviewed in Chee & van Dongen, 2013). Notably, most imaging studies investigating vulnerability to SD grouped their participants according to performance decrement (e.g. by median split; Bell-McGinty et al., 2004; Chee & Tan, 2010; Mu et al., 2005); for an exception, see Vandewalle, Archer, et al. (2009). This presents a possible confound because separation of performance-induced brain activity alterations from mere vulnerability correlates are difficult.

2.3.3 Inter-individual variability in response to sleep loss

A considerable inter-individual variability in sleep-wake regulation exists already under baseline conditions and applies to sleep duration, sleep timing, sleep architecture, waking and sleep EEG-profiles, and preferred timing of sleep and wakefulness (chronotype) (reviewed e.g., in Landolt, 2011;

Van Dongen et al., 2005). When it comes to the effects of SD, systematic inter-individual differences in the responses on the cognitive, electrophysiological, and even cerebral level become evident. Even under the experimental control of other influential factors such as age, gender, duration of prior wakefulness, time of day, cognitive domain, task duration, chronotype, posture, and light influence, variability between subjects is ample (Chee & van Dongen, 2013). Indeed, this sleep-loss related vulnerability seems to be trait-like (Frey, Badia, & Wright, 2004; Leproult et al., 2003; Van Dongen et al., 2004; Van Dongen et al., 2005). Under SD, 60-92 % of the variance in neurobehavioral performance can be explained by systematic inter-individual variability (Van Dongen et al., 2004). Notably, the impact of this variability on homeostatic regulation is almost twice as much as on the circadian process (Van Dongen, Bender, & Dinges, 2012). These observations speak in favor of a considerable genetic influence (Kuna et al., 2012), and fostered the search for specific genes which are implicated in this trait-like vulnerability. Several candidate genes have been considered, among these a number of genes involved in the generation of circadian rhythmicity, so called clock genes. The *PER* family belongs to the most prominent of these. Basically, these genes are involved in a complex molecular feedback loop setting the period of the oscillator (see section 2.1.3). Genetic variations in these clock genes have been linked to sleep loss related vulnerability in humans (e.g., Groeger et al., 2008; Lo et al., 2012; Viola et al., 2007), but also to circadian rhythm disorders (Archer et al., 2003; Ebisawa et al., 2001; Pereira et al., 2005; Toh et al., 2001). A VNTR polymorphism in *PER3* was rather consistently associated with differential vulnerability to SD both on the neurobehavioral as well as the physiological level (Groeger et al., 2008; Lo et al., 2012; Vandewalle, Archer, et al., 2009; Viola et al., 2007). Homozygous carriers of the short repeat allele ($PER3^{4/4}$) have been reported to be more resilient to the detrimental effects of SD than homozygous carriers of the long repeat allele ($PER3^{5/5}$). Within the next section, the contribution of this polymorphism to phenotypic sleep-wake regulation and response to sleep loss will be reviewed in detail.

2.3.4 The *PER3* VNTR polymorphism and vulnerability to sleep loss

In *PER3*, a primate-specific (Jenkins, Archer, & von Schantz, 2005) VNTR polymorphism contains a 54-nucleotide unit, which in humans is repeated 4 ($PER3^4$ allele) or 5 ($PER3^5$ allele) times. This polymorphism was first described by Ebisawa and colleagues (Ebisawa et al., 2001) in humans by screening for polymorphisms related to delayed sleep phase disorder (DSPD), a circadian rhythm disorder. Later, Archer et al. (Archer et al., 2003) showed the association of the short allele *($PER3^4$)* to DSPD and extreme evening preference (i.e., late chronotype), and related the long allele *($PER3^5$)* to morning preference (i.e., early chronotype). This association was reinvestigated by numerous studies, but not consistently replicated (among others; Ellis, von Schantz, Jones, & Archer, 2009; K. H. Jones et al., 2007; Osland, Bjorvatn, Steen, & Pallesen, 2011; Perea et al., 2014; Pereira et al., 2005). Viola et al. (2007) then conducted a prospective study, where young healthy participants were

selected solely based on their genotype, being either *PER3*$^{5/5}$ or *PER3*$^{4/4}$. Under baseline conditions (after a normal waking day), *PER3*$^{5/5}$ carriers showed shorter sleep latencies, an enhancement of slow wave sleep (SWS) and SWA in NREM sleep, as well as a steeper SWA decrease during sleep episodes compared to *PER3*$^{4/4}$ individuals; all being markers of sleep homeostasis. Furthermore, greater theta and alpha activity was found during wakefulness in *PER3*$^{5/5}$ carriers when sleep deprived (Viola et al., 2007). During extended wakefulness, cognitive performance deteriorated faster in homozygous long allele carriers, specifically during late night and early morning hours (Viola et al., 2007). Higher vulnerability of the long allele carriers seems to be especially pronounced for cognitive tasks challenging executive functioning (Groeger et al., 2008). However, a study investigating chronic partial SD (4 h/night over five consecutive days) following two nights of sleep extension revealed no differences in cognitive performance between genotypes, although differences for a marker of sleep homeostasis (more SWA in *PER3*$^{5/5}$ carriers) were detected after sleep restriction (Goel, Banks, Mignot, & Dinges, 2009). Notably, performance in this latter study was assessed during the course of the biological day after sleep restriction. This might explain the lack of difference, considering that detrimental effects on performance in the earlier studies were most pronounced during the night (Groeger et al., 2008; Viola et al., 2007). A recent study by Lo et al., exploring the effect of partial sleep restriction (6h/night) as well as subsequent acute total SD showed that subjective alertness was most impaired in *PER3*$^{5/5}$ carriers during sleep restriction days compared to the other genotypes (Lo et al., 2012). Likewise, acute total 40-h SD implemented after the partial sleep restriction days impacted most on working memory performance of *PER3*$^{5/5}$ carriers (Lo et al., 2012). A study by Vandewalle et al. (2009) revealed that the *PER3* polymorphism may also partially account for vulnerability correlates at the cerebral level. The authors acquired fMRI data during a working memory task in the morning and in the evening after a regular nights' sleep as well as in the morning after one night of SD. They showed differential activation patterns depending on the *PER3* genotype: *PER3*$^{5/5}$ carriers showed decreased cortical activations in the posterior dorsolateral prefrontal cortex already after a normal waking day (i.e., after 16h awake, morning vs. evening session), whereas *PER3*$^{4/4}$ showed no difference in activation profiles. Comparing the well-rested morning session with the morning session after SD, *PER3*$^{5/5}$ carriers showed widespread decreased cortical activations, while *PER3*$^{4/4}$ showed no decreased activations, but were able to recruit supplemental cortical regions in response to the sleep homeostatic challenge (Vandewalle, Archer, et al., 2009).

As markers of circadian rhythmicity, melatonin, cortisol, and clock gene mRNA levels have been investigated in this polymorphism. For melatonin secretion, no differences were found between genotypes (Lo et al., 2012; Vandewalle, Archer, et al., 2009; Viola et al., 2007), and neither for cortisol levels (Viola et al., 2007). *PER3* mRNA levels investigated in Archer et al. (2008) depicted no difference either, however, for *PER3*$^{5/5}$ carriers, a more robust association of mRNA levels with

sleep-wake timing was found, proposing a more rigid circadian control in this group. Additionally, our group showed a greater susceptibility to the effects of a blue light exposure in *PER3*$^{5/5}$ carriers on alertness and melatonin (Chellappa et al., 2012).

This body of evidence suggests that the *PER3* polymorphism mainly seems to influence variables which are regulated by the sleep homeostat (see Kuna et al., 2012 for a different point of view). Figure 7 depicts a conceptual model proposed by Dijk and Archer (2009, 2010) for the differential homeostatic-build up, its interaction with the circadian process, and the subsequent impact on performance. Another very impressive finding recently confirmed this assumption: Hasan et al. (2014) introduced the human *PER3* VNTR polymorphism into mice and assessed several

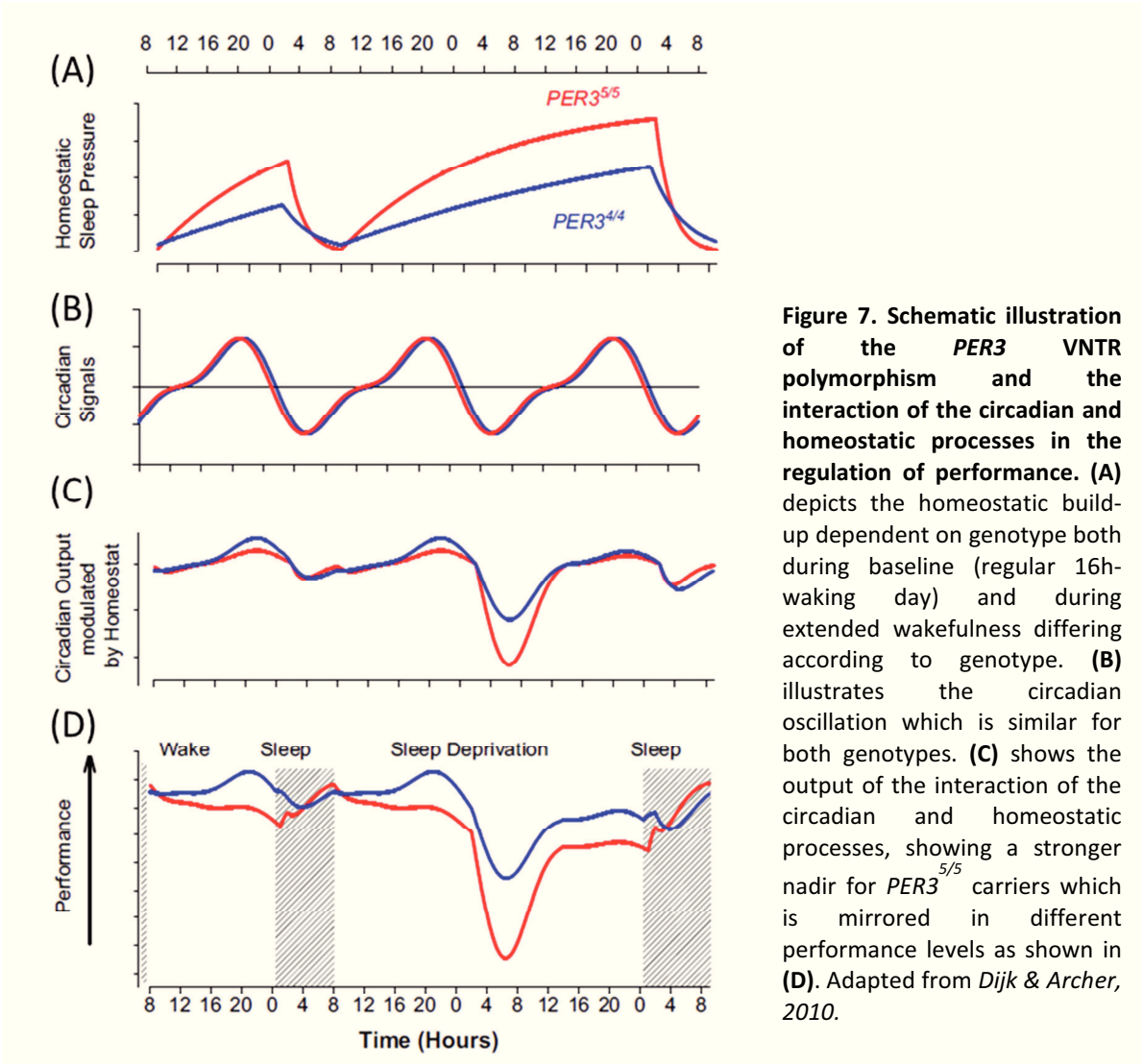

Figure 7. Schematic illustration of the *PER3* VNTR polymorphism and the interaction of the circadian and homeostatic processes in the regulation of performance. (A) depicts the homeostatic build-up dependent on genotype both during baseline (regular 16h-waking day) and during extended wakefulness differing according to genotype. **(B)** illustrates the circadian oscillation which is similar for both genotypes. **(C)** shows the output of the interaction of the circadian and homeostatic processes, showing a stronger nadir for *PER3*$^{5/5}$ carriers which is mirrored in different performance levels as shown in **(D)**. Adapted from *Dijk & Archer, 2010.*

circadian and sleep parameters under baseline and after 12h of SD. The authors showed that EEG theta power during wakefulness and delta power during sleep (baseline and recovery after SD) were greater for *PER3*$^{5/5}$ mice. Interestingly, clock gene expression in the hypothalamus and cortex was not altered in mice carrying either of the two human genotypes, but the expression of genes related to sleep pressure, including Homer1, was more upregulated in the *PER3*$^{5/5}$ mice (Hasan et al., 2014).

Interestingly, some genes which were differentially expressed in *PER3*$^{5/5}$ mice compared to wild-type mice are associated with neuronal development and signaling, indicating a potential mechanism involved in the differential cognitive response to sleep loss (Hasan et al., 2014).

3. Objectives and approach of the thesis

In this work, we aimed to investigate the influence of genetic vulnerability in response to sleep loss on electrophysiology, subjective sleepiness, and the cerebral correlates of vigilance. We combined a 40-hour SD and a 40-hour short sleep-wake cycle protocol to specifically investigate the interaction of circadian and homeostatic processes at very regular time intervals with respect to the *PER3* polymorphism. The combination of the two protocols in a within-subjects design allowed a bidirectional manipulation of the sleep homeostat, either by an increase (high sleep pressure; SD) or a decrease (low sleep pressure, naps) in prior wakefulness while assessing circadian sleep-wake propensity over the entire 24 h cycle. We were thus able to compare different *states* (i.e., sleep pressure level and time into the 24 h our cycle) in the same group of subjects, presenting heterogeneous *traits* in response to sleep loss *(PER3^{55} vs. PER3^{44}).* Figure 8 schematically displays the study design (A & B) as well as the expected resulting sleep homeostatic levels (C).

In this thesis, we aimed to answer following main questions:

- To what extent is genetic vulnerability to sleep loss modulated by sleep pressure or circadian phase, or the interaction of both?
- How is vulnerability to sleep loss related to attentional failures, and what is the impact of time-on-task and motivation?
- What are the cerebral underpinnings of genetic vulnerability to sleep loss-related attentional decrements at the most adverse circadian time?

The first publication deals with the general impact of sleep pressure (high vs. low) on subjective sleepiness, electrophysiological correlates of sleepiness, and sustained attention performance. The second publication presents data on whether time-on-task decrement, a consistently observed, but often neglected phenomenon mirroring a certain wake state instability, is greater in more vulnerable participants and how it depends on sleep pressure levels. The third publication addresses the cerebral correlates of vigilance and the respective impact of vulnerability based on the *PER3* polymorphism. Our main focus of interest was set on data acquired during the biological night. During this time window, the circadian timing system maximally promotes sleep. We thus attempted to probe the influence of sleep pressure and the *PER3* polymorphism-related vulnerability on task-related BOLD activity at most adverse time.

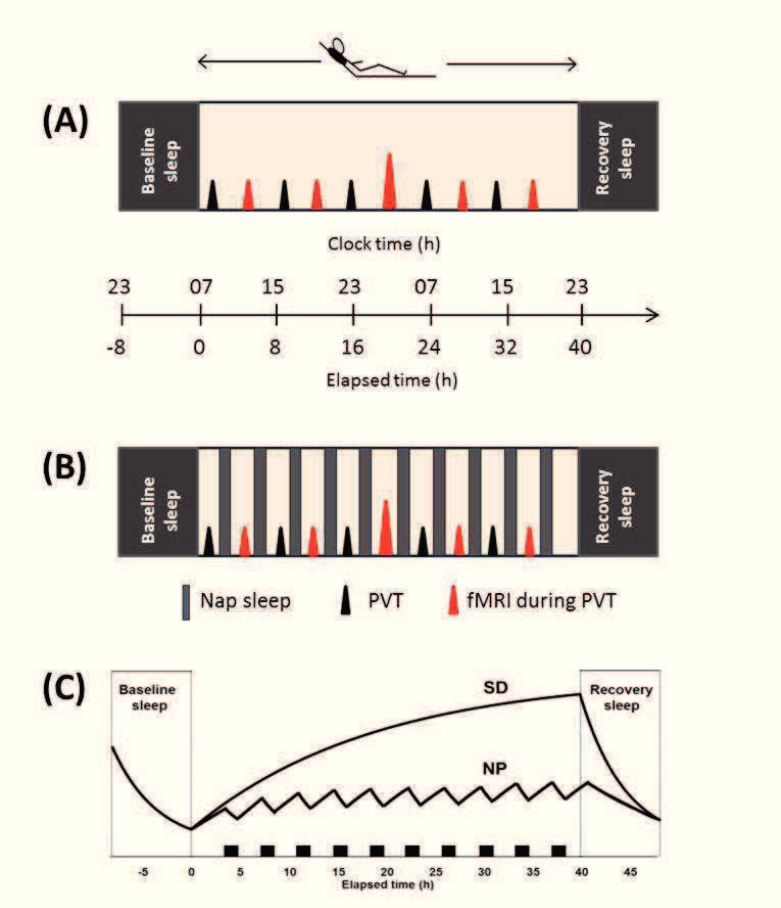

Figure 8. Overview of the experimental design. After baseline sleep (8 h), either a 40-h sleep deprivation **(A)** or a 40-h multiple nap paradigm (**B**, alternating cycle of 80/160-min sleep/wake) under controlled posture and light (< 8 lux) conditions was carried out in a within-subject design, followed by recovery sleep (8 h). Grey bars in **(B)** indicate scheduled nap sleep episodes (0 lux). During both protocols, subjective sleepiness was assessed with the Karolinska Sleepiness Scale and salivary melatonin assays were collected at regular intervals. After each nap, subjective sleep quality measures were assessed with the Leeds Sleep Evaluation Questionnaire (LSEQ). The Psychomotor Vigilance Task (PVT) and a working memory task (N-Back) were performed every 4 h starting 1 h after wake up, and every second time the assessment took place in the functional magnetic resonance imaging (fMRI) scanner. In the present thesis, we focus on PVT results, and on fMRI data collected in the biological night (large red triangle). Elapsed time indication is relative to 07:00 wake up time. **(C)** schematically depicts the differential build-up of homeostatic sleep pressure for sleep deprivation (SD) and the nap protocol (NP).

4. Research Publications

My contribution to the following three publications consisted in the elaboration of the experimental procedure, study preparation and organization, recruitment of volunteers, genotyping, study conduction and data acquisition, data processing and statistical analyses, and manuscript writing. All steps were carried out at least partly in collaboration with or with support of the listed co-authors or persons acknowledged within the research papers.

1. **Sleep ability mediates inter-individual differences in the vulnerability to sleep loss: Evidence from a *PER3* polymorphism.**
   Maire M, Reichert CF, Gabel V, Viola AU, Strobel W, Krebs J, Landolt HP, Bachmann V, Cajochen C, Schmidt C. *Cortex, 2014*

2. **Time-on-task decrement in vigilance is modulated by inter-individual vulnerability to homeostatic sleep pressure manipulation.**
   Maire M, Reichert CF, Gabel V, Viola AU, Strobel W, Krebs J, Landolt HP, Bachmann V, Cajochen C, Schmidt C. *Frontiers in Behavioral Neuroscience, 2014.*

3. **How the brain copes to sustain attention at night-time: Impact of sleep pressure and genetic vulnerability to sleep loss.**
   Maire M, Reichert CF, Gabel V, Viola AU, Phillips C, Krebs J, Scheffler K, Klarhöfer M, Strobel W, Cajochen C, Schmidt C. *Submitted.*

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Research report

Sleep ability mediates individual differences in the vulnerability to sleep loss: Evidence from a *PER3* polymorphism

M. Maire [a], C.F. Reichert [a], V. Gabel [a], A.U. Viola [a], W. Strobel [b], J. Krebs [a], H.P. Landolt [c,d], V. Bachmann [c], C. Cajochen [a,*,1] and C. Schmidt [a,1]

[a] Centre for Chronobiology, Psychiatric University Hospital of the University of Basel, Basel, Switzerland
[b] Respiratory Medicine, Department of Internal Medicine, University Hospital Basel, Basel, Switzerland
[c] Institute of Pharmacology and Toxicology, University of Zürich, Zürich, Switzerland
[d] Clinical Research Priority Program "Sleep & Health", University of Zürich, Zürich, Switzerland

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ABSTRACT

Sleep deprivation is highly prevalent in our 24/7 society with harmful consequences on daytime functioning on the individual level. Genetically determined, trait-like vulnerability contributes to prominent inter-individual variability in the behavioral responses to sleep loss and adverse circadian phase. We aimed at investigating the effects of differential sleep pressure levels (high *vs* low) on the circadian modulation of neurobehavioral performance, sleepiness correlates, and nap sleep in individuals genotyped for a polymorphism in the clock gene *PERIOD3*.

Fourteen homozygous long ($PER3^{5/5}$) and 15 homozygous short ($PER3^{4/4}$) allele carriers underwent both a 40-h sleep deprivation and multiple nap protocol under controlled laboratory conditions. We compared genotypes regarding subjective and ocular correlates of sleepiness, unintentional sleep episodes as well as psychomotor vigilance during both protocols. Nap sleep was monitored by polysomnography and visually scored according to standard criteria.

The detrimental effects of high sleep pressure on sleepiness correlates and psychomotor vigilance were more pronounced in $PER3^{5/5}$ than $PER3^{4/4}$ carriers. Under low sleep pressure, both groups showed similar circadian time courses. Concomitantly, nap sleep efficiency and subjective sleep quality across all naps tended to be higher in the more vulnerable $PER3^{5/5}$ carriers. In addition, *PER3*-dependent sleep-loss-related attentional lapses were mediated by sleep efficiency across the circadian cycle.

Our data corroborate a greater detrimental impact of sleep deprivation in $PER3^{5/5}$ compared to $PER3^{4/4}$ carriers. They further suggest that the group with greater attentional performance impairment due to sleep deprivation ($PER3^{5/5}$ carriers) is superior at initiating sleep over the 24-h cycle. This higher sleep ability may mirror a faster sleep pressure build-

Abbreviations: SD, sleep deprivation; SE, sleep efficiency; NP, nap protocol; SL1, sleep latency to stage 1; SL2, sleep latency to stage 2; SLR, sleep latency to REM sleep; TRT, total scheduled rest time; TST, total sleep time; SEM, slow eye movement; USE, unintentional sleep episode; WMZ, wake maintenance zone; SMZ, sleep maintenance zone.

* *Corresponding author*. Centre for Chronobiology, Psychiatric University Hospital, Wilhelm Klein-Strasse 27, 4012 Basel, Switzerland.
E-mail address: christian.cajochen@upkbs.ch (C. Cajochen).

[1] These authors contributed equally to the work.

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up between the multiple sleep opportunities and thus a greater flexibility in sleep initiation. Finally, our data show that this higher nap sleep efficiency is positively related to attentional failures under sleep loss conditions and might thus be used as a marker for inter-individual vulnerability to elevated sleep pressure.

1. Introduction

Due to professional and social demands, our sleep is often curtailed or non-optimally scheduled throughout the 24h-light–dark cycle with major repercussions on cognitive processes, in particular attentional failures (reviewed in Chee & Chuah, 2008; Killgore, 2010). It is the interaction between both a sleep-wake homeostatic process and the circadian timing system which regulates cognitive performance levels across the 24-h day, as outlined in the framework of the two process model (Borbely, 1982) via an opponent interaction of these processes (for a review see Blatter & Cajochen, 2007; Schmidt, Collette, Cajochen, & Peigneux, 2007). More precisely, sleep homeostasis represents an hourglass process, with a steady build-up of sleep propensity or sleep pressure with increasing time awake, and an exponential decline during sleep. The circadian rhythmicity, exhibiting a pace of 24 h regarding the propensity of sleep and wakefulness, is set by our master clock located in the suprachiasmatic nuclei of the anterior hypothalamus (Edgar, Dement, & Fuller, 1993; Mistlberger, 2005). Some individuals are more capable to cope with the effects of sleep loss and/or non-optimally timed sleep opportunities than others – as indexed on subjective, behavioral and physiological levels (for a review see Maire, Reichert, & Schmidt, 2013; Van Dongen, Vitellaro, & Dinges, 2005). This sleep-loss related vulnerability may reflect stable traits (Frey, Badia, & Wright, 2004; Leproult et al., 2003; Van Dongen, Baynard, Maislin, & Dinges, 2004; Van Dongen, et al., 2005) depending on particular genetic variants (Dijk & Archer, 2010; Landolt, 2008). One of the most comprehensively studied of these variants is a primate-specific (Jenkins, Archer, & von Schantz, 2005) variable number tandem repeat (VNTR) polymorphism in the clock gene *PERIOD3* (*PER3*). This gene contains a 54-nucleotides unit, which in humans is repeated four (*PER3*^{4} allele) or five (*PER3*^{5} allele) times (RS57875989) (Archer et al., 2003; Ebisawa et al., 2001). Sleep deprivation (SD) and sleep restriction protocols yielded evidence for a faster sleep pressure build-up in homozygous carriers of the longer allele (*PER3*$^{5/5}$) compared to carriers of the shorter allele (*PER3*$^{4/4}$), mainly expressed by more deep sleep and slow wave activity during sleep (Archer, Viola, Kyriakopoulou, von Schantz, & Dijk, 2008; Dijk & Archer, 2009, 2010; Goel, Banks, Mignot, & Dinges, 2009; Lo et al., 2012; Viola et al., 2007). Importantly, this genetic trait of higher vulnerability to total SD is reflected in cognitive performance impairments, such that *PER3*$^{5/5}$ carriers show a greater deterioration, particularly in working memory performance (Groeger et al., 2008; Lo, et al., 2012; Viola, et al., 2007). Interestingly, differences between genotypes could also be mirrored in differential task-related activation patterns at the cerebral level during a working memory task, where *PER3*$^{5/5}$ carriers had widespread reduced cortical activations after sleep deprivation (SD) and were not able to recruit supplemental cortical regions as *PER3*$^{4/4}$ carriers were (Vandewalle et al., 2009).

So far, homozygous *PER3* VNTR allele carriers have been challenged by prolonged wakefulness (i.e., 40 h) in order to test their susceptibility to sleep loss. Thus, it is not yet known whether under conditions of low sleep pressure – achieved by multiple sleep opportunities – differences between the longer and shorter allele carriers in cognitive performance disappear, or whether such divergence still exists, particularly at times when the circadian drive for sleep is high (i.e., early morning hours). In light of the impact of the interaction of sleep homeostasis and circadian rhythmicity on cognitive performance, this is of major importance. Moreover, sleep ability throughout the entire circadian cycle has not yet been investigated with respect to inter-individual vulnerability to sleep loss, although this information would significantly contribute to the understanding of the mechanisms underlying this vulnerability.

In order to achieve differential sleep pressure conditions, we combined a 40-h SD with a 40-h short sleep–wake cycle protocol to investigate the interaction of circadian and homeostatic processes with respect to the *PER3* VNTR polymorphism. Indeed, the latter represents an intriguing tool to explore circadian and sleep homeostatic influences and their interaction on human behavior. Furthermore, the combined application of an SD and a short sleep–wake cycle protocol enables a distinct and bidirectional manipulation of the sleep homeostat, either by an increase (high sleep pressure; SD) or a decrease (low sleep pressure, naps) of sleep pressure levels, while assessing circadian sleep–wake propensity over the entire 24 h cycle. We were thus able to compare different states (sleep pressure level and time into the 24 h cycle) in the same group of individuals, presenting heterogeneous traits in response to sleep loss (*PER3*$^{5/5}$ *vs* *PER3*$^{4/4}$). We formulated the following hypotheses: *PER3*$^{5/5}$ carriers will show higher susceptibility to high sleep pressure conditions (SD protocol) than *PER3*$^{4/4}$ carriers, as indexed by higher subjective and physiological sleepiness and more attentional failures. Under consideration that naps scheduled over the 40-h protocol attenuate the sleep homeostatic drive (Cajochen, Knoblauch, Krauchi, Renz, & Wirz-Justice, 2001), and based on the observed faster build-up of homeostatic sleep pressure in *PER3*$^{5/5}$ carriers, we predict nap sleep scheduled throughout the 24 h-cycle to be more efficient in *PER3*$^{5/5}$ compared to *PER3*$^{4/4}$ carriers. This higher sleep efficiency (SE) will contribute to comparably low sleep pressure levels for both genotypes, which in turn will lead to a mitigation of the differences in subjective sleepiness and attentional failures under low sleep pressure conditions (nap protocol, NP), resulting in similar time courses for both groups. Finally, if sleep-loss related vulnerability is mediated by differences in the regulation of the homeostatic process, we assume that the

ability to initiate and maintain sleep during the NP is positively associated with performance decrements during SD, as both can be pinned down to these differences in homeostatic sleep pressure build-up.

2. Methods

2.1. Participants

Out of a large pool of approximately 650 participants, thereof 562 successfully genotyped for the *PER3* VNTR polymorphism, we selected 29 healthy volunteers between 20 and 35 years (mean age ± SD: 25.38 ± 3.3 years) for study participation based on their genotype, inclusion criteria listed hereafter, and ability to devote time for study weekends. Fifteen participants (eight males, seven females) were homozygous carriers of the short repeat allele ($PER3^{4/4}$) and 14 participants (five males, nine females) were homozygous carriers of the long repeat allele ($PER3^{5/5}$). In total, 16% of all genotyped participants were $PER3^{5/5}$ carriers, 40% $PER3^{4/4}$ carriers, and 44% were heterozygous carriers ($PER3^{4/5}$). This distribution is similar to previous studies for the European population (Lazar et al., 2012; Viola et al., 2007; Viola et al., 2012). Based on findings of previous reports, we did not include heterozygous carriers (Viola et al., 2007) to enhance the variance in vulnerability. Table 1 details the demographic data. The sex ratio between the two groups did not differ (X^2 (1) = .909, $N = 29$, $p = .34$). All participants completed a general medical questionnaire, the Morningness-Eveningness-Questionnaire (MEQ, Horne & Östberg, 1976), the Munich Chronotype Questionnaire (MCTQ; Roenneberg, Wirz-Justice, & Merrow, 2003), the Pittsburgh Sleep Quality Index (PSQI; Buysse, Reynolds, Monk, Berman, & Kupfer, 1989), the Beck Depression Inventory-II (BDI-II; Beck, Steer, & Brown, 1996) and the Epworth Sleepiness Scale (ESS; Johns, 1991). Participants did not suffer from any general medical, psychiatric and sleep disorders, and habitually slept between 7 and 9 h per night. PSQI values were requested to lie below 5, BDI-II values below 12. Furthermore, all were non-smokers, did not take any medication (except for hormonal contraceptives in female participants) or drugs.

Table 1 – Demographic data and questionnaire scores; means (SD) and *p*-values.

	$PER3^{4/4}$	$PER3^{5/5}$	*p*
N (m/f)	15 (8/7)	14 (5/9)	.34
Age (years)	24 (3.1)	25.6 (3.6)	.22
BMI (kg/m^2)	21.6 (2.3)	22.7 (2.8)	.23
Wake time (hh:min)	07:06 (61)	07:10 (43)	.79
Sleep time (hh:min)	23:06 (61)	23:10 (43)	.79
PSQI	3.2 (1.1)	3 (1.3)	.66
ESS	3.9 (2.1)	4.3 (2.7)	.67
MEQ	58 (9.2)	53.5 (10.2)	.22
MCTQ sleep duration (h)	7.8 (.7)	7.9 (1.0)	.78
MCTQ MSF sc	4.3 (.9)	4.4 (1.3)	.77
MCTQ MSF sac	7.5 (2.6)	7.2 (2.5)	.73

Note. MSF sc = Midsleep free days sleep corrected, MSF sac = Midsleep free days sleep and age corrected.
p-values were derived from X^2 (gender ratio) and *t*-tests (all other variables).

Moderate alcohol and caffeine consumption was not an exclusion criterion. Mean body mass index (BMI) was 22.13 ± 2.56 kg/m^2 (mean ± SD). To control for circadian phase misalignment, we excluded shift workers, and did not permit trans-meridian flights during three months before study participation. Before inclusion to the study, a medical examination by the physician in charge as well as a polysomnographic screening night was carried out. The latter served to rule out potential sleep disorders and to habituate participants to the new sleep environment in the laboratory setting. Women without hormonal contraceptive use (two women out of 16) were tested during the luteal phase of their menstrual cycle. The groups did not differ in terms of age, BMI, self-selected habitual bed times, ESS-, PSQI- and chronotype scores (see Table 1). The study was approved by the local ethics committee (Ethikkommission beider Basel, EKBB, Switzerland), and all procedures conformed to the standards of the declaration of Helsinki. All participants provided their written informed consent to the participation of the study.

2.2. Genotyping

DNA was extracted from saliva samples collected with the Oragene™ DNA Collection Kit using the standard procedures (DNA Genotek Inc., Ontario, Canada; http://www.dnagenotek.com/ROW/support/protocols.html). All genotypes were determined with an allele-specific PCR with 50 cycles at 60 °C. Forward primer: 5′-TTA CAG GCA ACA ATG GCA GT-3′, reverse primer: 5′-CCA CTA CCT GAT GCT GCT GA-3′. Agarose gel (2%) electrophoresis was used to identify the genotype of the individuals.

2.3. Protocol and procedure

A schematic illustration of the study design is shown in Fig. 1. Each volunteer completed two study blocks. Both comprised an ambulatory part of one week, followed by a 56-h stay in the chronobiology laboratory. During the ambulatory part of both blocks, participants were asked to maintain a regular sleep-wake cycle (8 h ± 30 min time in bed) according to their individually determined sleep–wake timing. Compliance was assessed with wrist actimetry (Actiwatch®, Cambridge Neurotechnology Ltd., UK) and sleep logs. Further, participants were requested to abstain from caffeine, alcohol, medication (except contraceptive pill), and daytime napping during this time. After each ambulatory week, volunteers entered the laboratory for the SD or the multiple NP in a randomized balanced crossover order (see e.g., Blatter et al., 2006; Cajochen, et al., 2001; Krauchi, Knoblauch, Wirz-Justice, & Cajochen, 2006; for studies applying similar protocols). Both protocols started with a baseline night (8 h time in bed). In the SD protocol, participants were scheduled to stay awake for 40 h starting after habitual wake up in order to challenge sleep pressure beyond the level of a usual 16-h waking day. In contrast, in the NP protocol, sleep pressure was kept minimal by scheduling the participants to 10 alternating cycles of 160 min wakefulness and 80 min nap sleep, starting 120 min after habitual wake up. Both blocks ended with a recovery night (minimum 8 h time in bed). In both protocols, 24-h time courses of sleep and several sleepiness, vigilance

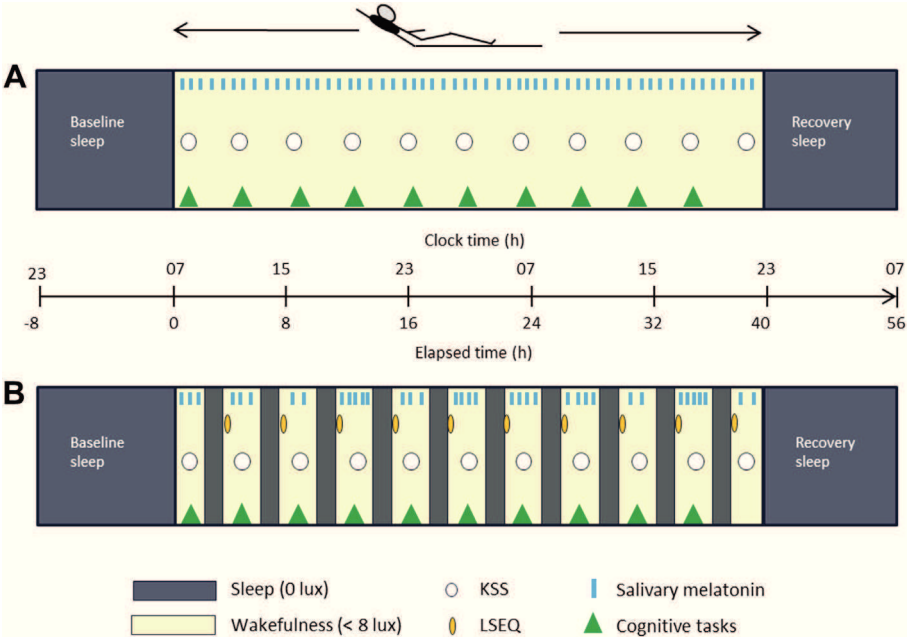

Fig. 1 – Schematic overview of the laboratory part. After baseline night (8 h), both a 40-h SD (A) and a 40-h NP paradigm (B, ten 80/160-min sleep/wake cycles) under controlled posture conditions in a within-subject design was carried out, followed by a recovery night (8 h). Gray bars in B indicate scheduled sleep episodes. Clock time-indication is relative to a 7 a.m. wake up time.

(Psychomotor Vigilance Task, PVT), and other cognitive measures (decision making and working memory tasks) were quantified under differential sleep pressure (SD *vs* NP) conditions. Here, we report psychophysiological sleepiness and vigilance measures as well as visual scorings of nap sleep; all other variables will be published elsewhere. To minimize the impact of potential masking effects on circadian and sleep–wake variables, participants stayed under highly controlled conditions; that is, semi-recumbent posture position in bed during wakefulness, regularly scheduled food intake, dim light (<8 lux) during scheduled wakefulness and 0 lux during scheduled sleep episodes, and no time-of-day information. Social interaction for participants was restricted to the contact with examiners and study helpers. Participants were allowed to get up in order to use the bathroom at scheduled times. During scheduled wakefulness, participants were allowed to read, watch pre-selected quiet movies on a laptop (screen brightness at eye level was strictly kept below 8 lux), and play card or dice games, or interact with the study helpers to prevent them from falling asleep. Participants were continuously monitored by polysomnography, in particular electroencephalography (EEG) and electrooculogram (EOG) in order to ensure wakefulness during scheduled wake episodes.

2.4. *Subjective sleepiness and subjective sleep quality (SQ)*

Participants rated their current sleepiness level on the Karolinska Sleepiness Scale (KSS; Akerstedt & Gillberg, 1990), from 1 (*extremely alert*) to 9 (*extremely sleepy, fighting sleep*). The ratings were carried out in regular intervals (56 times during SD, 37 times during NP). In order to achieve an equal number of sampling points, only corresponding samplings during NP and SD were included in the analysis (mean sampling interval length; 51 ± 33 min, mean ± SD). The KSS ratings were collapsed into 11 time bins for both the SD and NP conditions (Fig. 2A). After each nap sleep episode, subjective SQ was assessed by a modified version of the Leeds Sleep Evaluation Questionnaire (LSEQ; Parrott & Hindmarch, 1978), where we additionally asked for subjective sleep latency (SL) and number of awakenings during naps. Here we report only the items showing genotype-dependent effects, covering subjective SL, quality of sleep, and number of awakenings during the naps.

2.5. *Sleep and slow eye movement (SEM) analysis*

Nap sleep was recorded on digital V-amp EEG amplifiers (Brain Products, Gilching, Germany) using sintered Ag/AgCl ring electrodes with a 15 kOhm resistor (EasyCap GmbH in Germany), a sampling frequency of 500 Hz, and an online 50 Hz notch filter. For visual scoring, frequencies below .1 Hz (high pass) and above 20 Hz (low pass) were filtered out. Electrodes were placed according to the 10–20-system, at 10 locations (F3, F4, Fz, C3, C4, Cz, Pz, O1, O2, Oz) and referenced against averaged mastoids. Eye movements and a submental electromyogram were recorded. Polysomnographic data were scored visually on a 20-sec epoch basis according to standard criteria (Rechtschaffen & Kales, 1968). Sleep stages were expressed as percentage of total sleep time (TST), while SE, the epochs of wakefulness, and arousal were expressed as percentage of total scheduled rest time (TRT). Arousal was defined as a composite of wakefulness, epochs containing more than 50% movement and stage 1 sleep. Sleep latencies to

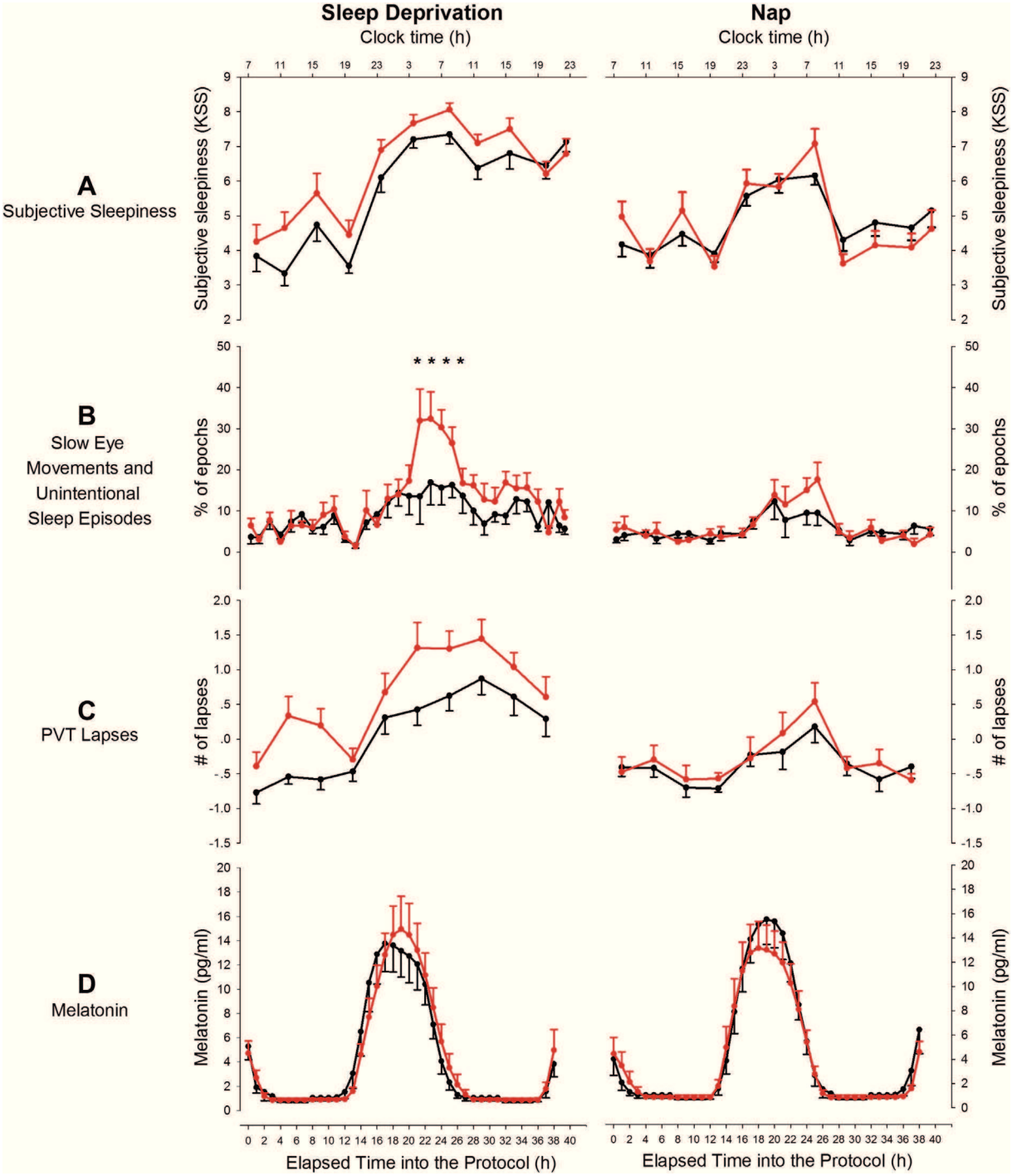

Fig. 2 – Time courses during SD (left panels) and NP (right panels) by genotype; *PER3*^{55}: red lines, *PER3*^{44}: black lines. (A) Subjective sleepiness mean values (B) SEMs and USEs % of epochs per bin (C) PVT lapses, transformed (D) Salivary melatonin. Clock time indications refer to 7 a.m. wake up time. Asterisks represent *p* values below .05 for *post hoc* comparison of values derived from the separate analysis computed for the SD protocol in (B).

stage 1 (SL1), stage 2 (SL2) or REM sleep (SLR) were defined as the first occurrence of the corresponding stage after lights off.

SEMs, a reliable physiological marker for sleepiness (Cajochen, Khalsa, Wyatt, Czeisler, & Dijk, 1999; Santamaria & Chiappa, 1987), were visually scored in 20-sec epochs according to the criteria reported by Cajochen et al. (1999) throughout both entire 40-h protocols, scheduled naps excluded. Each 20-sec epoch was scored as to whether or not at least one SEM occurred. Accordingly, unintentional sleep episodes (USE), that is, 20-sec epochs fulfilling the Rechtschaffen and Kales criteria (Rechtschaffen & Kales, 1968) for any sleep stage, were scored and pooled with the SEM data. SEMs or USEs were averaged across 80-min time-bins (except for the first and last bin with 40 min duration due to nap

scheduling within the protocol), resulting in a total of 21 bins for NP and 31 bins for the SD protocol (Fig. 2C). To investigate whether different homeostatic sleep pressure states and the *PER3* polymorphism also affect electrophysiological markers of sleepiness, we compared the time course of the composite of SEMs and USEs with respect to sleep pressure condition and genotype. In order to have an equal number of bins in both conditions for direct comparison (i.e., 21 bins; see Fig. 2C), we excluded the bins at the respective times where naps were scheduled. Other electrophysiological data collected during wakefulness (i.e., waking EEG) will be reported elsewhere.

2.6. PVT

Sustained attention was assessed by a modified version of the PVT (Dinges and Powell, 1985) in 4-h intervals at ten time points during the protocol, starting 1 h after wake up time (for average times referring to 7:00 h wake up time, see Fig. 2C). To avoid sleep inertia effects on task performance, the scheduled distance from nap to testing time was set to 115 min (Jewett et al., 1999). In this task, a white fixation cross was presented on a black screen. At random intervals (1–9 sec), a msec counter started, and participants were instructed to press a button to stop the counter as fast as possible. Feedback of their reaction time (RT) performance was displayed for 1 sec after their response. Duration of the task was set to 10 min. Here we report lapses (RTs $>$ 500 msec) and median RTs, averaged across participants per genotype for each of the ten sessions. PVT lapses were transformed (transformation by $\sqrt{x}+\sqrt{x+1}$; for details, see Graw et al., 2001), and subsequently z-transformed due to different testing environment; as every second test during both protocols took place in a functional magnetic resonance imaging scanner with a different response key pad. The median RTs were equally z-transformed.

2.7. Melatonin

The circadian secretion pattern of pineal melatonin is known to be a highly reliable marker of internal time under dim light conditions, and is closely associated with sleep propensity (Cajochen, Krauchi, & Wirz-Justice, 2003). The plasma melatonin profile provides a good evaluation of the melatonin secretion in the pineal gland (for a review, see Claustrat, Brun, & Chazot, 2005), and salivary melatonin levels correlate significantly with plasma levels (Voultsios, Kennaway, & Dawson, 1997). Saliva samples were collected at regular intervals during wakefulness (mean sampling interval: 45 ± 27 min, mean ± SD) to measure melatonin levels. Interval length was dependent on time of day, that is, sampling frequency was decreased during the biological day when melatonin secretion is low, and increased during the biological evening, night and early morning hours (Brzezinski, 1997). A direct double-antibody radioimmuno-assay was used for melatonin analysis (validated by gas chromatography–mass spectroscopy with analytical least detectable dose of .65 pm/ml; Bühlmann Laboratory, Schönenbuch, Switzerland). For amplitude estimation, first a bimodal skewed baseline cosine function (Van Someren & Nagtegaal, 2007) was fitted to raw values as described in Kolodyazhniy et al., (2012). In a next step, the peak level, which is the maximum difference of the fitted waveform to its baseline, was defined as the amplitude (see Kolodyazhniy, et al., 2012, p. 1094). The dim light melatonin onset (DLMO) and offset (DLMOff) as markers for circadian phase were determined at the 50% level of the maximal melatonin secretion for each study participant (Benloucif et al., 2008).

2.8. Relation of neurobehavioral performance during SD with nap sleep

To investigate whether the ability to initiate and maintain sleep during the multiple nap opportunities distributed over the 24 h cycle can predict performance decrement under sleep loss conditions, we considered the overall occurrence of attentional lapses in PVT performance under high sleep pressure as a marker of attentional susceptibility to sleep loss. Further, the ability to sleep over all naps (i.e., the SE) was assumed to reflect to what extent the homeostatic sleep pressure has built up during the 160 min scheduled episodes of wakefulness. Thus, we correlated *SE* during the NP protocol with *lapses* during the SD protocol, and also tested for trait-like covariance (analysis of covariance – ANCOVA) by adding the factor *genotype*.

2.9. Statistical analysis

Group analyses were performed with the statistical package SAS (SAS Institute Inc., Cary, NC; version 9.3). All variables were analyzed with mixed-model repeated measures analysis of variance (ANOVA) (ProcMixed) and *p* values were based on Kenward-Roger's corrected degrees of freedom (Kenward & Roger, 1997). Contrasts were assessed with the LSMEANS statement. If not stated otherwise, factors *genotype* ($PER3^{5/5}$ *vs* $PER3^{4/4}$), *condition* (NP *vs* SD) and *time* (Ten to 31 time points, depending on variable) were used. *Time* represents time elapsed into the protocol starting at habitual wake time. The average habitual wake time was 07:06 h ± 61 (mean clock time ± SD in min) in $PER3^{4/4}$ and 07:10 h ± 43 (mean clock time ± SD in min) in the $PER3^{5/5}$ carriers ($p > .05$). For graphs, 07:00 h was used as the average reference wake up time. We report effect sizes where trends in significance ($.05 < p > .1$) are stated. Effect sizes were indicated with Cohen's *d* for post hoc comparisons, and Cohen's f^2 for mixed-model ANOVA main effects or interactions (Cohen, 1988; Lo et al., 2012; Van Dongen, Maislin, & Kerkhof, 2001). Correlations and ANCOVAs were calculated with Statistica 9 (StatSoft Software).

3. Results

3.1. Subjective sleepiness

The time course of subjective sleepiness is illustrated for each genotype and condition separately in Fig. 2A. As expected, we observed a significant main effect of *condition* (Table 2) with significantly higher values under high (SD, 0.29 ± 0.05, mean KSS values ± SE) compared to low (NP, −0.28 ± 0.05, mean KSS values ± SE) sleep pressure conditions. Furthermore, a main effect of *time* indicated higher subjective sleepiness levels during the biological night, independent of the sleep pressure

Table 2 – Statistical results of ProcMixed ANOVA: F-values, degrees of freedom, and *p*-values.

	Condition	Time	Genotype	Genotype × condition	Genotype × time	Time × condition	Genotype × time × condition
KSS	F (1, 565) = 153.83	F (10, 565) = 46.44	F (1, 27) = .79	F (1, 565) = 10.22	F (10, 565) = 1.9	F (10, 565) = 11.49	F (10, 565) = 1.11
	$p < .0001$	**$p < .0001$**	$p = .38$	**$p = .0015$**	**$p = .0425$**	**$p < .0001$**	$p = .35$
SEM/USE	F (1, 1061) = 68.78	F (20, 1061) = 14.57	F (1, 27.1) = 2.06	F (1, 1061) = 7.49	F (20, 1061) = 2.7	F (20, 1061) = 2.98	F (20, 1061) = .82
	$p < .0001$	**$p < .0001$**	$p = .16$	**$p = .0063$**	**$p < .0001$**	**$p < .0001$**	$p = .70$
SEM/USE SD	n.a.	F (30, 774) = 8.94	F (1, 27) = 2.79	n.a.	F (30,774) = 1.85	n.a.	n.a.
		$p < .0001$	$p = .11$		**$p = .0039$**		
PVT Lapses	F (1, 512) = 163.02	F (9, 513) = 23.85	F (1, 27) = 2.72	F (1, 513) = 18.45	F (9, 513) = 1.18	F (9, 513) = 8.99	F (9, 513) = .45
	$p \leq .0001$	**$p \leq .0001$**	$p = .11$	**$p < .0001$**	$p = .30$	**$p \leq .0001$**	$p = .91$
PVT RT	F (1, 512) = 119.5	F (9, 512) = 31.33	F (1, 27) = 1.83	F (1, 512) = 6.77	F (9, 512) = .79	F (9, 512) = 5.03	F (9, 512) = .51
	$p \leq .0001$	**$p \leq .0001$**	$p = .19$	**$p = .0095$**	$p = .62$	**$p < .0001$**	$p = .87$
DLMO	F (1, 27) = 6.57	n.a.	F (1, 27) = .00	F (1, 27) = 1.14	n.a.	n.a.	n.a.
	$p = .0162$		$p = .99$	$p = .30$			
DLMOff	F (1, 27) = .01	n.a.	F (1, 27) = .51	F (1, 27) = .06	n.a.	n.a.	n.a.
	$p = .92$		$p = .48$	$p = .81$			

Note. RT represents median reaction time. Significant *p*-values are printed in bold; n.a.: not applicable.

level. The interaction between *condition* and *time* (Table 2) revealed that after 16.5 h of elapsed time (time of day 23:30 h), participants felt consistently sleepier under the SD compared to the NP condition ($p_{all} < .05$). Both interactions of *genotype* × *time* and *genotype* × *condition* were significant (Table 2). For *post hoc* results of these interactions, see Supplemental online material (SOM).

3.2. *SEMs and USEs*

Similar to subjective sleepiness levels, participants had more SEMs/USEs under SD (10.1% ± .5%, mean ± SE), compared to the NP condition (5.9% ± .3, mean ± SE; see *condition* effect Table 2). More SEMs/USEs were detected in the biological night independent of the sleep pressure levels, and a significant interaction *condition* × *time* (Table 2) indicated higher scores under SD compared to NP from 17 to 36 h into the protocol (from 24:00 h on the first day to 19:00 h on the second day), with the exception of the time window from 18 to 21 h elapsed (01:00 h to 04:00 h). A significant *condition* × *genotype* (Table 2) interaction indicated that the $PER3^{5/5}$ carriers produced significantly more SEMs/USEs under SD conditions than the $PER3^{4/4}$ carriers ($p = .029$), while the groups did not differ under NP conditions ($p = .68$). Finally, a *genotype* × *time* (Table 2) interaction indicated that, independent of the sleep pressure condition, $PER3^{5/5}$ individuals produced more SEMs/USEs during the biological night and in the beginning of the second biological day (from ca. 21 h–25 h awake; i.e., 04:00 h–08:00 h). At 37 h awake (20:00 h) the pattern reversed; $PER3^{4/4}$ carriers showed more SEMs/USEs until 39 h awake (22:00 h), where the difference disappeared again.

In order to have a closer look into the time course during SD, we additionally computed a separate analysis for the SD condition. Here, we included all available data, that is, also the bins at times where naps were scheduled during NP, which we excluded for global condition comparison. In this analysis, we observed – besides a significant effect of *time* – a significant interaction *genotype* × *time* (see Table 2), indicating higher scores for $PER3^{5/5}$ individuals than $PER3^{4/4}$ carriers at the end of the biological night and in the beginning of the second biological day. For time points revealing a trend, please see SOM.

3.3. *Sustained attention performance*

The time course of PVT lapses during the SD and NP protocol is illustrated in Fig. 2C for the $PER3^{5/5}$ and $PER3^{4/4}$ carriers. Analysis of the lapses revealed a significant main effect of *time* (more lapses occurring during the biological night) and *condition* (Table 2): During SD, more lapses occurred (SD: .33 ± .06; NP: −.33 ± .04, mean no. of lapses ± SE). The main effect *genotype* was not significant, neither was the interaction for *time* × *genotype* (Table 2). Similar to what was observed for subjective sleepiness, a *condition* × *time* interaction (Table 2) revealed that participants produced significantly more lapses under the SD compared to the NP condition from 9 h elapsed time onwards (time of day: 16:00 h), except for the test at 13 h into the protocol (time of day: 19:00 h). The significant interaction *condition* × *genotype* (Table 2) was driven by $PER3^{5/5}$ carriers who produced significantly more lapses than $PER3^{4/4}$

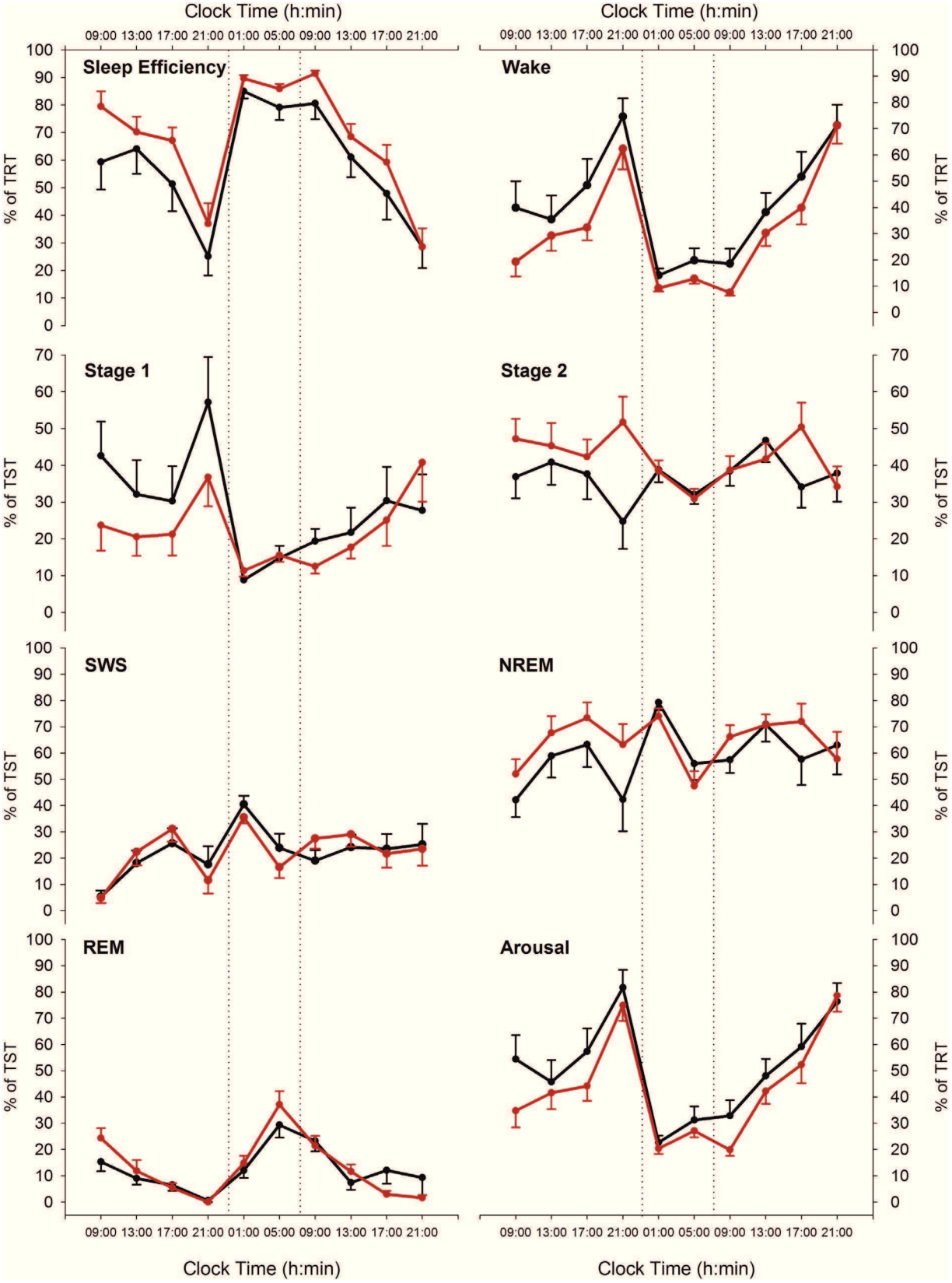

Fig. 3 – Visually scored nap sleep by genotype ($PER3^{5/5}$: red lines, $PER3^{4/4}$: black lines). SWS: Slow wave sleep. TST, SE, wake and arousal: % of TRT. Stage 1, 2, SWS, NREM and REM: % of TST. Time of day indicates start of the nap sleep episode (80 min duration) and refer to a 7 a.m. wake time. Vertical dashed lines frame the biological night.

Table 3 – Nap sleep: overall means ± SE by genotype and Statistical results of ProcMixed ANOVA: F-values, degrees of freedom, and *p*-values.

	$PER3^{4/4}$	$PER3^{5/5}$	Genotype	Nap	Genotype × nap
TRT (min)	80.05 ± .12	80.16 ± .11	$F(1, 26.5) = .36$, $p > .05$	$F(1, 238) = .46$, $p > .05$	$F(9, 238) = .81$, $p = .61$
TST (min)	46.77 ± 2.29	54.36 ± 1.84	$F(1, 27.2) = 3.13$, **$p = .088$**	$F(9, 237) = 27.08$, **$p < .001$**	$F(9, 237) = .47$, $p = .89$
SE	58.31 ± 2.85	67.85 ± 2.30	$F(1, 27.2) = 3.22$, **$p = .084$**	$F(9, 237) = 27.09$, **$p < .001$**	$F(9, 237) = .49$, $p = .88$
Wakefulness	41.05 ± 2.88	31.19 ± 2.34	$F(1, 27.2) = 3.26$, **$p = .0822$**	$F(9, 237) = 27.21$, **$p < .001$**	$F(9, 237) = .5$, $p = .88$
Stage 1	28.12 ± 2.7	21.87 ± 1.86	$F(1, 26) = 2.3$, $p = .14$	$F(9, 222) = 5.66$, **$p < .001$**	$F(9, 222) = .96$, $p = .47$
Stage 2	36.8 ± 1.75	42.05 ± 1.63	$F(1, 25.6) = 2.77$, $p = .11$	$F(9, 221) = 1.18$, $p = .30$	$F(9, 221) = 1.74$, **$p = .0819$**
Stage 3	8.20 ± .65	9.50 ± .61	$F(1, 27.3) = .72$, $p = .40$	$F(9, 223) = 6.07$, **$p < .001$**	$F(9, 223) = .37$, $p = .95$
Stage 4	13.90 ± 1.38	13.12 ± 1.24	$F(1, 27) = .2$, $p = .65$	$F(9, 223) = 5.02$, **$p < .001$**	$F(9, 223) = .75$, $p = .66$
SWS	22.09 ± 1.71	22.61 ± 1.55	$F(1, 28) = 0$, $p = .96$	$F(9, 225) = 7.16$, **$p < .001$**	$F(9, 225) = .7$, $p = .71$
NREM	58.90 ± 2.55	64.66 ± 2.00	$F(1, 26.6) = 2.11$, $p = .16$	$F(9, 223) = 3.81$, **$p = .002$**	$F(9, 223) = .95$, $p = .49$
REM	12.99 ± 1.32	13.46 ± 1.36	$F(1, 25.6) = .17$, $p = .68$	$F(9, 224) = 16.3$, **$p < .001$**	$F(9, 224) = 1.45$, $p = .17$
SL1 (min)	20.83 ± 1.89	15.58 ± 1.91	$F(1, 27.1) = 1.94$, $p = .18$	$F(9, 237) = 30.52$, **$p < .001$**	$F(9, 237) = .34$, $p = .96$
SL2 (min)	33.50 ± 2.28	27.4 ± 1.91	$F(1, 27.2) = 1.26$, $p = .27$	$F(9, 238) = 24.46$, **$p < .001$**	$F(9, 237) = .36$, $p = .95$
SLR (min)	61.13 ± 2.00	60.30 ± 2.05	$F(1, 27.3) = .01$, $p = .92$	$F(9, 238) = 20.78$, **$p < .001$**	$F(9, 238) = .7$, $p = .71$
Arousal	50.81 ± 2.66	43.39 ± 2.29	$F(1, 27.1) = 2.17$, $p = .15$	$F(9, 237) = 24.98$, **$p < .001$**	$F(9, 237) = .61$, $p = .78$
LSEQ SQ	36.32 ± 1.7	42.72 ± 1.75	$F(1, 27.7) = 4.04$, **$p = .0542$**	$F(9, 231) = 11.57$, **$p < .0001$**	$F(9, 231) = 1.04$, $p = .41$
LESQ SL	33.92 ± 2.10	28.09 ± 2.04	$F(1, 27) = 2.18$, $p = .15$	$F(9, 244) = 30.05$, **$p < .0001$**	$F(9, 244) = 1.87$, **$p = .0568$**
LESQ #w	4.26 ± .73	1.7 ± .34	$F(1, 27) = 3.48$, **$p = .0731$**	$F(9, 244) = .0114$, **$p = .0114$**	$F(9, 244) = .61$, $p = .79$

Note. #w: Number of awakenings. TST, SE, wakefulness, and arousal are expressed in percentage of TRT; sleep stages are expressed in percentage of TST. *p*-values <.1 are printed in bold.

participants during SD ($PER3^{4/4}$: .08 ± .08, $PER3^{5/5}$: .62 ± .10, mean no. of lapses ± SE), but not during NP (*Condition* × *genotype*, see Table 2).

Median RT analysis (data not shown) revealed a similar pattern as observed for the lapses. A significant main effect of *time* and *condition* was found; with slower median RT during SD (SD: .28 ± .05, NP: −.28 ± .06, mean median RT ± SE) and during the biological night. The main effect *genotype* did not reach significance, neither did the interaction for *time* × *genotype*. *Condition* × *time* was significant, with all time points beginning at 9 h elapsed time (time of day 16:00 h) being different ($p_{all} < .05$). Comparable to the lapses, *condition* × *genotype* was significant (Table 2, for post hoc results see SOM).

3.4. *Melatonin*

Fig. 2D shows the time course of melatonin secretion across each protocol in the $PER3^{5/5}$ and $PER3^{4/4}$ carriers. The overall profile of melatonin was not significantly modulated by the main factor *genotype*, nor its interaction with either *condition* (SD *vs* NP), or *time*, or both factors (Table 2). The only significant difference for the DLMO yielded the factor *condition* (Table 2) with an earlier onset (22 min; NP 22:06 ± 00:11 *vs* 22:28 ± 00:12; mean ± SE) in the NP compared to the SD protocol independent of genotype. No significant differences were found for the DLMOff (Table 2).

3.5. *Nap sleep*

Nap sleep is plotted in Fig. 3, and Table 3 details the results and means by sleep stages and genotype over all naps. Table S1 details the complete record of means by stage, genotype and nap. TRT was equal for all naps and did not differ between genotypes. As expected, the visually scored sleep stages varied over the 40-h protocol. Main effects of *nap* were disclosed for TST, SE, SL1, SL2, SLR, wakefulness, stage 1, stage 3, stage 4, REM, SWS, Non-REM sleep (NREM), and arousals (Table 3). Generally, SE was higher during the biological night and lowest in the early evening hours. The only variable showing no main effect of *nap* was stage 2 sleep. Regarding the impact of *genotype* on these variables, several trends were disclosed, as described in the SOM.

Similarly to visually scored sleep stages, subjectively estimated SQ within the naps assessed by the LSEQ revealed a significant main effect for *nap* in all investigated variables with higher SQ, lower sleep latencies, and fewer awakenings occurring during the biological night ($p_{all} < .05$, data not shown). Here, several trends were revealed for the main effect genotype (see SOM).

3.6. *Relation of neurobehavioral performance during SD with nap sleep*

In a final step, we aimed at exploring the interrelation between sleep ability during the naps and the vigilance levels during SD. Correlation analysis for overall *SE* during the nap opportunities and the occurrence of *lapses* during the PVT administered in the SD protocol revealed a significant positive correlation ($R = .4292$, $p = .02$). As illustrated in Fig. 4, an analysis of covariance indicated that this relationship was modulated by the genotype of the participant (*genotype* × *SE*, $F = 4.8$, $p = .037$). The relationship was specific for *SE* and *lapses*; as no correlation with KSS or SEMs/USEs values with *SE* was found ($p_{all} > .05$).

4. Discussion

With the *PER3* VNTR polymorphism as a tool, we prospectively created inter-individual variance by grouping participants

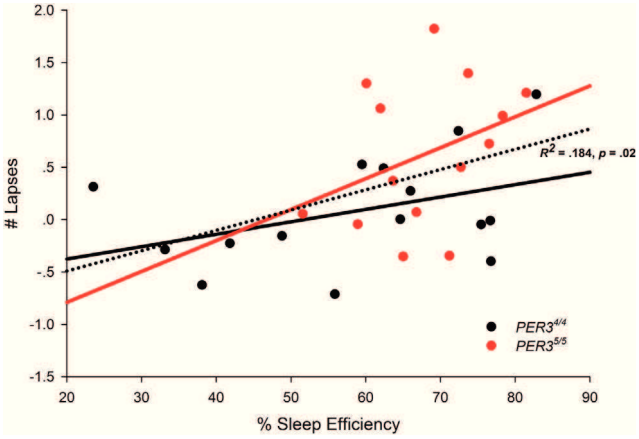

Fig. 4 – Mean SE during naps in relation to overall mean number of PVT lapses during SD. Individual values and regression lines; black line and dots represent $PER3^{4/4}$ carriers, red line and dots represent $PER3^{5/5}$ carriers. Dashed line represents regression for the whole group. R^2 and *p* values refer to the overall regression.

based on their expected vulnerability to sleep loss. Importantly, it was suggested that the source of variability is mediated by differences in the regulation of homeostatic sleep pressure (Van Dongen, Bender, & Dinges, 2012), one of the main processes underlying human sleep-wake regulation. By a direct manipulation of this process (low *vs* high) and by tracking its impact over nearly two circadian cycles, our protocol might be suitable to imply a more direct link between differences in sleep homeostatic mechanisms and neurobehavioral susceptibility to sleep loss. By applying this approach, our data confirm inter-individual variability in the modulation of sleep and neurobehavioral performance over the 24 h cycle linked to the *PER3* polymorphism. Remarkably, a congruent pattern is detected for subjective and physiological markers of sleepiness, as well as modulation in attentional failure. Finally, our data disclose an intriguing link between the ability to sleep over the 24 h cycle and sleep-loss-related vulnerability in attentional performance.

4.1. *The impact of SD on sustained attention is greater in $PER3^{5/5}$ carriers*

With the sleep homeostatic state manipulation applied in our study (low *vs* high), we were able to detect a clear genotype-dependent modulation of sustained attention performance. $PER3^{5/5}$ carriers not only had significantly more lapses across all sessions under high sleep pressure, they also tended to have higher median RTs during SD (see SOM, effect size is medium to large for this trend). This pattern was not observed when sleep pressure was kept low, nor did we observe a genotype-dependent change in the circadian pattern, suggesting a rather homeostatic genotype-dependent modulation of this neurobehavioral variable. This result goes in line with the assumption of Dijk and Archer (2010) that $PER3^{5/5}$ carriers have a faster homeostatic build-up of sleep pressure. It is worth noting that at present, four studies comparing genetic variants of *PER3* in terms of attentional PVT performance have been published (Goel, et al., 2009; Kuna et al., 2012; Lo, et al., 2012; Rupp, Wesensten, Newman, & Balkin, 2012), but only three of these included homozygous long allele carriers, applying different study designs. These three studies investigated total SD (Kuna, et al., 2012), partial sleep restriction effects (Goel, et al., 2009), and partial sleep restriction with subsequent total SD (Lo, et al., 2012), respectively. None of these studies found any genotype-specific modulation of sustained attention. However, compared to these protocols, our approach might be more appropriate for the detection of purely homeostatic state effects, since it allows a comparison of rising (SD) versus low (NP) homeostatic levels by controlling for circadian phase. Overall, this finding adds evidence to differential neurobehavioral sensitivity to total SD relative to the *PER3* polymorphism.

4.2. *Physiological markers of sleepiness are more pronounced in $PER3^{5/5}$ carriers*

Greater amounts of SEMs and USEs were particularly detected in the early morning hours in $PER3^{5/5}$ compared to $PER3^{4/4}$ carriers irrespective of protocol, but also specifically during SD. From a circadian perspective, this time window is commonly labeled the sleep maintenance zone (SMZ), because of maximal circadian-based sleep promotion or minimal circadian arousal promotion (Dijk & Czeisler, 1994, 1995). Thus, this polymorphism may also affect physiological sleepiness in a time-of-day dependent manner, irrespective of homeostatic state. Alternatively, this finding may point towards a genotype-dependent modulation of the interaction between sleep homeostasis and the circadian process. As suggested in a model by Dijk and Archer (Dijk & Archer, 2010), *PER3* impinges on the circadian output modulated by the sleep homeostat. The model postulates that the two genotypes differ in their time constants of the build-up and the dissipation of sleep pressure, which in turn affects the interaction with the circadian process, which *per se* appears to be alike in $PER3^{5/5}$ and $PER3^{4/4}$ allele carriers (Dijk & Archer, 2010). Hence, the homozygous long allele carriers experience a higher sleep promotion, especially during the biological night; this genotype feels sleepier and exhibits stronger physiological signs for sleepiness as shown in our data.

Further, on the second day of the SD protocol, $PER3^{5/5}$ carriers also tended to show more SEMs and USEs (results reported in SOM) at a time corresponding to the "post-lunch dip" (Monk, 2005; Monk, Buysse, Reynolds, & Kupfer, 1996; Strogatz, Kronauer, & Czeisler, 1987), equally pointing to a greater sleep tendency or a weaker circadian wake promotion (Strogatz, et al., 1987). Interestingly, it was shown that morning types are more likely to suffer from the post-lunch dip than evening types (Horne, Brass, & Pettitt, 1980). Studies investigating the impact of chronotype on the homeostatic build-up revealed that morning types show a pattern of a faster homeostatic build-up (Kerkhof, 1991; Mongrain, Carrier, & Dumont, 2006; Schmidt et al., 2009; Taillard, Philip, Coste, Sagaspe, & Bioulac, 2003), which is also the case for $PER3^{5/5}$ carriers (Viola, et al., 2007). Additionally, morningness has been associated with the long repeat allele in *PER3* (Archer, et al., 2003).

4.3. *Nap sleep ability and performance lapses during SD are linked and depend on the PER3 polymorphism*

Correlational and covariance analyses revealed that the higher the ability of a participant to initiate and maintain sleep throughout the naps over the 40 h is, the more his/her attentional performance will be affected by sleep loss. In other words, this link indicates that the deterioration in behavioral variables due to high sleep pressure levels is significantly related to the ability to sleep during naps scheduled over the 24-h cycle. Importantly, the strength of this effect depends on the *PER3* polymorphism. This result supports a relation between the sleep homeostatic build-up between nap opportunities and neurobehavioral vulnerability to sleep loss, which is trait-like (i.e., influenced by the *PER3* VNTR polymorphism).

In this line, it is worth noting that in accordance with our neurobehavioral performance and electrophysiological sleepiness data, nap sleep analysis revealed roughly 10% higher TST and SE over all naps in $PER3^{5/5}$ than $PER3^{4/4}$ carriers (Table 3). This effect yielded almost significance at trend level (see SOM), yet the effect size was medium. Therefore, the observed higher diurnal sleep propensity in $PER3^{5/5}$ than $PER3^{4/4}$ carriers is relevant to consider. Thus, the genotype more vulnerable to the effects of sleep loss tended to show a greater ability to sleep independent of circadian phase, along with a better subjective SQ. This finding can be interpreted within the context of a steeper build-up of sleep pressure during the scheduled 160-min wake episodes between the naps in $PER3^{5/5}$ carriers, which was proposed by the model of Dijk and Archer (Dijk & Archer, 2009, 2010). Interestingly, we also observed a greater amount of stage 2 sleep in $PER3^{5/5}$ carriers during two specific naps, one scheduled in the early evening hours of the first day (21:00 h) and one scheduled in the mid afternoon of the second day (15:00 h, see SOM). Thus again, to a certain extent, the polymorphism seems to affect sleep ability in a time-of-day-dependent manner. Interestingly, the early evening nap (21:00 h), where more stage 2 sleep was discovered in $PER3^{5/5}$ carriers, surrounds the so-called wake maintenance zone (WMZ), or "forbidden zone for sleep" (Lavie, 1986; Strogatz, et al., 1987), occurring approximately two to 3 h before habitual bedtime. There, the circadian drive for wakefulness is greatest, strongly opposes the homeostatic sleep load under entrained conditions and makes it extremely difficult to fall asleep (Strogatz, et al., 1987). In our data, the WMZ is mirrored by the lowest SE during the nap scheduled within this time zone (average SE of approximately 30%, see Table S1). Importantly, our results cannot be explained by genotype-dependent differences in circadian phase position, as the two groups did not differ in their DLMO or DLMOff, indicating that the assessments took place at equal internal times for both groups.

A limitation of our study is the relatively small group size. However, by selecting healthy, young participants without sleep complaints and controlling for gender ratio, chronotype, habitual sleep duration, and sleep timing across groups, we chose a homogenous phenotype in order to maximize potential contributions of the *PER3* polymorphism to vulnerability. In addition, due to our highly controlled laboratory conditions, we are able to control for potential masking factors such as light influence, body posture, or social and nutritional timing cues.

5. Conclusion

Our data confirm that a manipulation of the sleep homeostatic state affects sustained attention and sleepiness differentially based on *PER3*-dependent vulnerability. Even though the exact mechanism this polymorphism exerts at the molecular level leading to the observed phenotypic differences remains to be determined, this genetic variant might represent a helpful tool for the investigation of the impact and importance of inter-individual variation in physiological and behavioral responses to sleep loss. For the first time, we showed how this polymorphism modulates sleep over the whole circadian cycle and how this relates to sleep-loss induced performance decrements. We suggest that sleep ability across the circadian cycle mediates attentional differences in reaction to sleep loss, thus adding a further essential piece of evidence in the search for the mechanisms underlying trait-like inter-individual differences in sleep-wake regulation.

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Medical Screening: Thomas Götz

Supplementary data

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Supplemental Online Material for

Sleep ability mediates individual differences in the vulnerability to sleep loss: evidence from a PER3 polymorphism

Maire M[1], Reichert CF[1], Gabel V[1], Viola AU[1], Strobel W[2], Krebs J[1], Landolt HP[3,4], Bachmann V[3], Cajochen C*[1], Schmidt C*[1]

[1] Centre for Chronobiology, Psychiatric University Hospital of the University of Basel, CH-4012 Basel, Switzerland

[2] Respiratory Medicine, Department of Internal Medicine, University Hospital Basel, CH-4031 Basel, Switzerland.

[3] Institute of Pharmacology and Toxicology, University of Zürich, CH-8057 Zürich, Switzerland

[4] Clinical Research Priority Program "Sleep & Health", University of Zürich, CH-8091 Zürich, Switzerland.

* These authors contributed equally to the work.

Corresponding author:
Prof. Dr. Christian Cajochen
Centre for Chronobiology
Psychiatric University Hospital
Wilhelm Klein-Strasse 27
4012 Basel
Switzerland
0041 61 325 54 74
christian.cajochen@upkbs.ch

This file includes supplementary results.

Supplementary results

1. Subjective sleepiness

The significant *genotype x time* interaction (Table 2) revealed that, independent of the sleep pressure condition, $PER3^{5/5}$ carriers tended to be sleepier than $PER3^{4/4}$ individuals at 8.5 h (p = .07, d = 0.4) and 25 h (p= .06, d = 0.4) of elapsed time (time of day 15:30 h, resp. 08:00 h).

Furthermore, the significant *genotype x condition* interaction (Table 2) indicated that the $PER3^{5/5}$ carriers felt sleepier than $PER3^{4/4}$ individuals during the sleep deprivation (SD) condition by trend (p = .078, d = 0.6), but not during the nap protocol (NP, p =.90).

2. Slow eye movements and unintentional sleep episodes

The separate analysis of the composite of slow eye movements and unintentional sleep episodes (SEMs/USEs) during the SD condition showed a significant interaction of *genotype x time* (Table 2, Figure 2B). Besides significant higher scores for $PER3^{5/5}$ individuals during the night and the beginning of the second day, post hoc tests revealed a trend for more SEMs/USEs in $PER3^{5/5}$ carriers in the afternoon (15:00 h; p = 0.07, d = -0.19) of the second day, whereas in the evening (20:00 h; p = 0.08, d = -0.17), the pattern reversed by trend until 22:00 h.

3. Sustained attention performance

For the significant interaction *condition x genotype* (Table 2) in the median RT analysis, we discovered post hoc a trend (p = .09; d = 0.7) for higher median RT in SD for $PER3^{5/5}$ (see S1) ($PER3^{4/4}$: .002 ± .08, $PER3^{5/5}$: 0.48 ± .09, mean median RT ± SE). In NP, genotypes performed equally (p=.35).

4. Nap sleep

Total sleep time tended to be higher in $PER3^{5/5}$ than in $PER3^{4/4}$ carriers (Table 3; f^2 = 0.12). Likewise, sleep efficiency (SE) showed a trend towards higher values in $PER3^{5/5}$ carriers (Table 3; f^2 = 0.12), and opposite to SE, wakefulness tended to be lower in $PER3^{5/5}$ carriers (Table 3; f^2 = 0.12). For stage 2, the interaction *nap x genotype* showed a trend (Table 3; f^2 = 0.07), with the nap scheduled in the early evening hours (21:00 h) of the first day ($PER3^{4/4}$ 24.73 ± 7.5 % vs. $PER3^{5/5}$ 51.69 ± 6.94%, mean ± SE; p = .0008), and the nap scheduled in the afternoon (15:00 h) of the second day ($PER3^{4/4}$ 34.08 ± 5.6% vs. $PER3^{5/5}$ 50.37 ± 6.6%, mean ± SE, p = .0335) showing significantly higher values for $PER3^{5/5}$ carriers.

Moreover, in the Leeds Sleep Evaluation Questionnaire, $PER3^{5/5}$ carriers reported better sleep quality (Table 3; f^2 = 0.15) and fewer awakenings than $PER3^{4/4}$ carriers over all naps (Table 3; f^2 = 0.13)

by trend. Subjective sleep latency appeared to be modulated by *genotype* and *time* (Table 3; f^2 = 0.07), with lower latencies in $PER3^{5/5}$ carriers for the naps scheduled at 15:00 h on the first day and at 9:00 h on the second day of the protocol (Nap 3: $PER3^{4/4}$ 42.67 ± 7.2 min vs. $PER3^{5/5}$ 22.14 ± 4.6 min, p = 0.0232; Nap 7: $PER3^{4/4}$ 18.33 ± 4.8 min vs. $PER3^{5/5}$ 9.28 ± 2.3 min, p = 0.0044; mean ± SE).

Table S1: *Mean nap sleep ± SE by genotype and nap.*

	PER3$^{4/4}$					*PER* $^{5/5}$				
NAP	1	2	3	4	5	1	2	3	4	5
TRT (min)	80.07 ± .08	80.27 ± .4	80.12 ± .2	79.69 ± .7	80.23 ± .2	79.6 ± 0.9	80.03 ± .1	80.05 ± .1	80.19 ± .2	80.01 ± 0.2
TST (min)	47.56 ± 8.0	51.53 ± 7.3	41.19 ± 7.9	20.16 ± 5.6	68.16 ± 2.1	63.38 ± 4.5	56.15 ± 4.5	53.71 ± 3.9	29.6 ± 6.0	71.71 ± 1.1
SE	59.32 ± 10	64.05 ± 9.1	51.28 ± 9.9	25.08 ± 6.9	84.96 ± 2.6	79.48 ± 5.5	70.19 ± 5.6	67.07 ± 4.8	36.92 ± 7.5	89.61 ± 1.2
SL1 (min)	12.11 ± 3.2	12.51 ± 2.6	27.21 ± 7.2	42.91 ± 6.3	8.91 ± 1.8	6.27 ± 1.4	9.8 ± 2.1	12.76 ± 2.5	37.88 ± 7.3	5.08 ± 1.0
SL2 (min)	28.74 ± 7.4	29.80 ± 7.4	41.35 ± 8.0	63.58 ± 5.8	12.69 ± 2.1	20.11 ± 5.7	20.95 ± 3.8	23.84 ± 3.3	56.53 ± 5.3	10.06 ± 1.4
SLR (min)	61.51 ± 4.7	64.47 ± 5.0	67.52 ± 5.3	78.78 ± 1.2	62.02 ± 5.5	46.6 ± 6.6	64.33 ± 4.5	67.5 ± 4.7	80 ± 0	62.46 ± 3.4
Stage 1	42.58 ± 9.3	32.11 ± 9.3	30.27 ± 9.5	57.12 ±12.3	8.8 ± 0.9	23.67 ± 6.9	20.52 ± 5.1	21.22 ± 5.7	36.75 ± 7.9	11.31 ± 1.5
Stage 2	36.86 ± 5.8	40.81 ± 6.1	37.62 ± 6.8	24.73 ± 7.5	38.76 ± 3.3	47.18 ± 5.5	45.29 ± 6.2	42.3 ± 4.7	51.69 ± 7.0	38.42 ± 3.0
Stage 3	2.88 ± 1.2	7.17 ± 1.8	8.83 ± 2.0	5.95 ± 2.9	12.89 ± 2.1	3.73 ± 1.2	8.78 ± 1.5	9.45 ± 1.4	6.55 ± 2.2	15.24 ± 1.6
Stage 4	2.38± 1.9	10.94 ± 4.1	16.81 ± 4.5	11.63 ± 5.3	27.56 ± 3.7	1.1 ± 1.1	13.58 ± 4.0	21.62 ± 4.8	5.01 ± 3.3	20.3 ± 2.5
REM	15.3± 3.6	8.98 ± 2.3	6.48 ± 2.1	0.57 ± 0.6	11.98 ± 2.8	24.32 ± 3.8	11.83 ± 4.1	5.41 ± 1.8	0 ± 0	14.73 ± 2.9
SWS	5.26± 2.4	18.11 ± 4.8	25.63 ± 5.8	17.58 ± 6.9	40.45 ± 3.3	4.83 ± 2.0	22.36 ± 5.1	31.07 ± 4.8	11.56 ± 5.0	35.54 ± 2.2
NREM	42.12± 6.5	58.91 ± 8.3	63.25 ± 8.6	42.31 ± 12.1	79.21 ± 2.9	52.01 ± 5.6	67.65 ± 6.4	73.38 ± 5.9	63.25 ± 7.9	73.97 ± 3.0
Arousal	54.39 ± 9.2	45.75 ± 8.3	57.29 ± 8.9	81.61 ± 6.8	22.64 ± 2.6	34.74 ± 6.4	41.57 ± 6.3	44.12 ± 5.5	74.88 ± 5.9	20.38 ± 2.1
Wake	39.87 ± 10.1	35.4 ± 9.2	48.43 ± 10.0	74.58 ± 7.0	14.09 ± 2.7	19.32 ± 5.6	29.2 ± 5.7	32.27 ± 4.8	62.31 ± 7.8	9.19 ± 1.2

	PER3$^{4/4}$					*PER* $^{5/5}$				
NAP	6	7	8	9	10	6	7	8	9	10
TRT (min)	80.18 ± .6	80.23 ± .15	80.17 ± .15	80.05 ± .1	79.51 ± .5	80.04 ± .2	80.04 ± .2	80.45 ± .2	80.77 ± .5	80.38 ± .3
TST (min)	63.84 ± 3.7	64.69 ± 4.6	48.95 ± 5.8	38.29 ± 7.6	22.56 ± 6.0	68.74 ± 1.4	73.14 ± 0.8	55.02 ± 3.7	47.76 ± 5.1	22.92 ± 5.4
SE	79.46 ± 4.4	80.56 ± 5.6	61.04 ± 7.2	47.88 ± 9.5	28.5 ± 7.7	85.87 ± 1.8	91.38 ± 1.1	68.43 ± 4.7	59.21 ± 6.3	28.57 ± 6.7
SL1 (min)	9.65 ± 1.5	5.34 ± 1.0	22.29 ± 5.8	19.19 ± 5.4	48.56 ± 7.9	6.79 ± 1.2	3.83 ± 1.0	16.31 ± 3.1	19.94 ± 5.2	37.68 ± 8.3
SL2 (min)	19.62 ± 4.3	16.45 ± 3.6	30.41 ± 5.9	32.17 ± 7.4	60.38 ± 5.8	16.69 ± 3.9	11.56 ± 3.6	24.07 ± 3.0	29.68 ± 4.8	61.68 ± 5.3
SLR (min)	40.62 ± 6.1	31.4 ± 7.6	67.12 ± 5.2	62.19 ± 7.0	76.64 ± 2.3	34.81 ± 5.1	30.37 ± 7.1	64.19 ± 5.3	73.95 ± 5.2	79.5 ± 0.4
Stage 1	14.77 ± 3.3	19.36 ± 3.4	21.71 ± 6.8	30.36 ± 9.2	27.7 ± 9.8	15.52 ± 1.8	12.47 ± 1.9	17.68 ± 3.0	25.04 ± 7.0	40.74 ± 10.6
Stage 2	32.07 ± 2.6	38.42 ± 4.0	46.73 ± 5.9	34.08 ± 5.6	37.84 ± 7.7	30.92 ± 2.7	38.75 ± 3.8	41.7 ± 4.3	50.38 ± 6.6	34.16 ± 5.6
Stage 3	7.98 ± 1.9	6.88 ± 1.3	9.24 ± 1.4	10.12 ± 2.2	11.63 ± 3.3	7.08 ± 1.5	10.41 ± 1.2	11.67 ± 1.87	9.1 ± 1.8	12.8 ± 3.5
Stage 4	15.87 ± 4.8	12.12 ± 3.3	14.93 ± 4.0	13.4 ± 4.4	13.54 ± 6.3	9.43 ± 3.0	17.02 ± 3.7	17.33 ± 3.85	12.54 ± 4.3	10.72 ± 4.3
REM	29.31 ± 4.8	23.22 ± 4.0	7.4 ± 2.7	12.05 ± 5.1	9.27 ± 6.6	37.05 ± 5.9	21.36 ± 3.8	11.63 ± 2.64	2.93 ± 1.4	1.58 ± 1.2
SWS	23.85 ± 5.5	19.00 ± 4.0	24.16 ± 4.7	23.52 ± 5.7	25.18 ± 7.9	16.51 ± 4.1	27.42 ± 3.8	28.99 ± 4.6	21.64 ± 5.3	23.52 ± 6.4
NREM	55.91 ± 6.1	57.42 ± 5.0	70.89 ± 6.6	57.59 ± 9.8	63.02 ± 11.2	47.43 ± 5.7	66.17 ± 4.4	70.7 ± 4.0	72.02 ± 6.7	57.68 ± 10.4
Arousal	30.91 ± 5.1	32.88 ± 5.8	48.01 ± 6.5	59.12 ± 8.8	76.33 ± 7.1	27.08 ± 2.0	19.87 ± 2.3	42.21 ± 4.8	52.26 ± 7.0	78.61 ± 6.2
Wake	19.48 ± 4.5	18.54 ± 5.7	38.18 ± 7.3	51.68 ± 9.6	71.24 ± 7.8	12.83 ± 1.8	7.49 ± 1.1	30.21 ± 4.9	39.86 ± 6.4	71.12 ± 6.8

Note. TRT: Total rest time, TST: Total sleep time, SE: sleep efficiency, NREM: Non-REM sleep, SL1: Sleep latency to stage 1, SL2: Sleep latency to stage 2;
Total sleep time, sleep efficiency, wakefulness, and arousal are expressed in percentage of TRT; sleep stages are expressed in percentage of TST.

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Time-on-task decrement in vigilance is modulated by inter-individual vulnerability to homeostatic sleep pressure manipulation

Micheline Maire[1], Carolin F. Reichert[1], Virginie Gabel[1], Antoine U. Viola[1], Julia Krebs[1], Werner Strobel[2], Hans-Peter Landolt[3,4], Valérie Bachmann[3], Christian Cajochen[1]*† and Christina Schmidt[1†]

[1] *Centre for Chronobiology, Psychiatric University Hospital of the University of Basel, Basel, Switzerland*
[2] *Respiratory Medicine, Department of Internal Medicine, University Hospital Basel, Basel, Switzerland*
[3] *Chronobiology and Sleep Research, Institute of Pharmacology and Toxicology, University of Zürich, Zürich, Switzerland*
[4] *Clinical Research Priority Program "Sleep & Health", University of Zürich, Zürich, Switzerland*

Edited by:
Shigeyoshi Itohara, RIKEN Brain Science Institute, Japan

Reviewed by:
Philippe Peigneux, Université Libre de Bruxelles, Belgium
Kazuo Mishima, National Institute of Mental Health, Japan

****Correspondence:***
Christian Cajochen, Centre for Chronobiology, Psychiatric, University Hospital, Wilhelm Klein-Strasse 27, 4012 Basel, Switzerland
e-mail: christian.cajochen@upkbs.ch
† These authors have contributed equally to this work.

Under sleep loss, vigilance is reduced and attentional failures emerge progressively. It becomes difficult to maintain stable performance over time, leading to growing performance variability (i.e., state instability) in an individual and among subjects. Task duration plays a major role in the maintenance of stable vigilance levels, such that the longer the task, the more likely state instability will be observed. Vulnerability to sleep-loss-dependent performance decrements is highly individual and is also modulated by a polymorphism in the human clock gene *PERIOD3 (PER3)*. By combining two different protocols, we manipulated sleep-wake history by once extending wakefulness for 40 h (high sleep pressure condition) and once by imposing a short sleep-wake cycle by alternating 160 min of wakefulness and 80 min naps (low sleep pressure condition) in a within-subject design. We observed that homozygous carriers of the long repeat allele of *PER3 (PER3*$^{5/5}$*)* experienced a greater time-on-task dependent performance decrement (i.e., a steeper increase in the number of lapses) in the Psychomotor Vigilance Task compared to the carriers of the short repeat allele *(PER3*$^{4/4}$*)*. These genotype-dependent effects disappeared under low sleep pressure conditions, and neither motivation, nor perceived effort accounted for these differences. Our data thus suggest that greater sleep-loss related attentional vulnerability based on the *PER3* polymorphism is mirrored by a greater state instability under extended wakefulness in the short compared to the long allele carriers. Our results undermine the importance of time-on-task related aspects when investigating inter-individual differences in sleep loss-induced behavioral vulnerability.

Keywords: time-on-task, *PER3* polymorphism, sleep deprivation, inter-individual variability, psychomotor vigilance, behavioral vulnerability, sleep loss

INTRODUCTION

In modern 24/7 society, sleep loss is part of our daily lives, and many professions come along with night or shift work nowadays. The detrimental effects of too little sleep on various domains of cognitive performance have long been known (for a review, see Killgore, 2010). Nevertheless, people are often still able to successfully accomplish complex tasks under such circumstances. Indeed, rather than to lead to a complete loss in the ability to perform, sleep loss induces increasingly greater performance variability (Doran et al., 2001; Durmer and Dinges, 2005; Van Dongen and Dinges, 2005). In other words, optimal performance is still possible even after many hours of sleep deprivation, but at the cost of increasing intermittence of performance lapses e.g., leading to greater standard deviations in reaction times (RT; Doran et al., 2001).

To understand how cognitive performance variation emerges, the two main oscillators involved in the regulation of sleep and wakefulness need to be considered (Borbely, 1982; Daan et al., 1984). On one side, an hourglass-like sleep homeostatic process tracks our sleep-wake history and leads to a rise in sleep propensity or sleep pressure with increasing time awake. On the other side, a circadian process represents a nearly 24-h oscillation, promoting wakefulness and sleep at specific times of the day. The interplay of both processes leads to consolidated states of sleep and wakefulness and contributes to the modulation of cognitive performance over the 24 h light-dark cycle (Cajochen et al., 2004; Dijk and Von Schantz, 2005; Cajochen et al., 2010). Throughout a regular 16-h waking day, cognitive performance remains relatively stable, followed by a steep decrease once wakefulness is extended into the biological night. Most detrimental effects emerge in the

early morning hours, when the circadian pacemaker promotes maximal sleep drive and the homeostatic sleep pressure is rather high (after ca. 21–24 h of prior wakefulness) (Wright et al., 2012). With increasing sleep propensity, a certain "wake state instability" (Durmer and Dinges, 2005) is observed, that is, sleep initiating mechanisms tend to progressively interfere with wakefulness. This leads to an increasing performance variability including task disengagement, and a dependency on compensatory mechanisms (Doran et al., 2001; Rogers et al., 2003; Dorrian et al., 2005). To specifically observe this increasing attentional failure, task duration plays a key role—the longer the task, the more likely the growing variability will be detected. This is based on the fact that potential compensatory mechanisms are more likely to fail after a certain time (Doran et al., 2001). Hence, performance variability such as momentary task disengagement does not only depend on prior wakefulness, but also on the duration of the task (Doran et al., 2001).

Vulnerability to performance decrements caused by sleep deprivation and/or adverse circadian phase has been reported to be trait-like (Leproult et al., 2003; Van Dongen et al., 2004, 2005) and to some extent, genetically determined (Landolt, 2008). An increasing body of evidence points toward a variable number tandem repeat (VNTR) polymorphism in the human clock gene *PERIOD3* (*PER3*) to be involved in the modulation of this vulnerability, indicated by a faster build-up and subsequent dissipation of homeostatic sleep pressure in homozygous carriers of the long repeat allele (*PER3*$^{5/5}$carriers) (Viola et al., 2007, 2012; Dijk and Archer, 2009, 2010). In our study, we aimed at investigating the effect of the *PER3* VNTR polymorphism on state instability in vigilance; more precisely, whether the wake-dependent homeostatic increase in the number of performance lapses throughout a 10-min psychomotor vigilance task (PVT) is different in homozygous *PER3* short vs. long allele carriers. The PVT (Dinges and Powell, 1985) has been shown to be sensitive to both sleep deprivation and adverse circadian phase (Wyatt et al., 1999; Graw et al., 2004). With a duration of 10 min, it provides an optimal tool to investigate the time course of vigilance (Doran et al., 2001). We experimentally varied sleep pressure by extending wakefulness to 40 h in one branch of the study (sleep deprivation protocol, SD) and by imposing a short sleep wake-cycle (10 cycles of 160 min of wakefulness and 80 min nap, NP) in the other branch of a balanced cross-over design. We were thus able to investigate momentary attentional failures under systematic homeostatic sleep pressure manipulation over the entire circadian cycle, all in relation to the *PER3* polymorphism. By applying this approach, we previously observed a global increase in the number of lapses during SD compared to NP, and moreover detected a greater number of lapses for *PER3*$^{5/5}$carriers than *PER3*$^{4/4}$carriers during SD. These results confirmed the adequacy of our protocol to study the trait- and state-like modulation of sleep homeostasis (Maire et al., 2013). However, even though time-on-task decrement has been described to be highly dependent on sleep homeostatic processes and has a significant impact on daily life, the effect of sleep-loss-related trait-like vulnerability has never been reported under this angle. Here, we assumed a generally greater time-on-task effect during SD compared to NP. Further, when compared to the more resilient genotype (*PER3*$^{4/4}$), we expect the more vulnerable genotype (*PER3*$^{5/5}$) to present higher susceptibility to the time-on-task effect when sleep pressure is at high levels, but not when sleep pressure is kept at low levels.

MATERIALS AND METHODS

PARTICIPANTS

Twenty-nine healthy volunteers (mean age ± *SD*: 25.38 ± 3.3 years) participated in the study. **Table 1** details the demographic data. Fifteen (eight males, seven females) were homozygous carriers of the short repeat allele (*PER3*$^{4/4}$), and 14 (five males, nine females) were homozygous carriers of the long repeat allele (*PER3*$^{5/5}$). The selection of this group was based on the individual's genotype and ability to devote time to participation; the applied exclusion criteria are listed below. All participants completed questionnaires regarding their general and mental health, sleep habits and quality, and chronotype. We excluded participants with general medical, current or past psychiatric and sleep disorders, and usual sleep duration of less than 7 or more than 9 h. Further exclusion criteria encompassed smoking, medication (except oral contraceptives), or drug consumption. To control for circadian phase misalignment, we excluded shift workers, and study applicants who had trans-meridian flights during three months before study participation. A physical examination by the physician in charge as well as a screening night was carried out to exclude sleep disorders, and to habituate the participants to sleep in laboratory conditions with electrodes before study participation. Women who did not use contraceptives (2 out of 16) were tested during the luteal phase of their menstrual cycle. The groups did not significantly differ in terms of sex ratio, age, BMI, bed times preceding study weekends, and questionnaire scores (**Table 1**). The local ethics committee (Ethikkomission beider Basel, EKBB, Switzerland) approved the study, and all procedures conformed to the standards of the declaration of Helsinki. All participants provided their written informed consent to the participation.

Table 1 | Demographic data, questionnaire scores (*M* ± *SD*) and *p*-values derived from X^2- (gender) and *t*-tests (other variables).

	PER3^{44}	*PER3*^{55}	*p*
N (m, f)	15 (8, 7)	14 (5, 9)	0.34
Age (y)	24.76 (3.38)	25.99 (3.30)	0.22
BMI (kg/m^2)	21.22 (2.23)	22.62 (2.09)	0.23
Wake time (clock time)	06:49 (56 min)	07:03 (41 min)	0.79
Sleep time (clock time)	22:49 (56 min)	23:03 (41 min)	0.79
PSQI	3.11 (0.99)	2.82 (1.34)	0.66
ESS	3.83 (1.72)	4.09 (1.94)	0.67
MEQ	57.78 (6.94)	55.34 (10.09)	0.22
MCTQ sleep duration (h)	7.93 (0.77)	7.70 (0.60)	0.78
MCTQ MSFsc	4.33 (0.89)	4.02 (1.14)	0.77
MCTQ MSFsac	6.77 (2.90)	6.39 (1.96)	0.73

PSQI, Pittsburgh Sleep Quality Index (Buysse et al., 1989); ESS, Epworth Sleepiness Scale (Johns, 1991); MEQ, Morningness-Eveningness Questionnaire (Horne and Östberg, 1976); MCTQ, Munich Chronotype Questionnaire (Roenneberg et al., 2003); MSFsc, Mid sleep free days sleep corrected; MSFsac, Mid sleep free days sleep and age corrected.

GENOTYPING

As reported in Maire et al. (2013), DNA was extracted from saliva samples collected with the Oragene DNA sample collection kit using standard procedures (DNA Genotek Inc., Ontario, Canada). All genotypes were determined with an allele-specific PCR with 50 cycles at 60°C. Forward primer: 5′-TTA CAG GCA ACA ATG GCA GT-3′, reverse primer: 5′-CCA CTA CCT GAT GCT GCT GA-3′. Agarose gel (2%) electrophoresis was used to identify the genotype of the individuals.

PROTOCOL AND PROCEDURE

Figure 1 illustrates the study design. Each volunteer completed two study blocks; both comprising an ambulatory part of one week, followed by a 56-h stay in the chronobiology laboratory. During both ambulatory weeks, participants were asked to maintain a regular sleep-wake cycle (8 h ± 30 min time in bed) according to their self-selected sleep-wake timing. Sleep logs and wrist actimetry (Actiwatch®, Cambridge Neurotechnology Ltd., UK) served to control for compliance to the regimen. Participants were requested to abstain from caffeine, alcohol, medication intake (except contraceptive pill), and daytime napping. After each ambulatory part, volunteers reported to the laboratory and underwent the SD and the NP protocol according to a randomized and balanced crossover design. Both protocols started with a baseline night (8 h time in bed at usual bedtimes). After a baseline night, participants stayed awake for 40 h after habitual wake time in the SD; in the NP they underwent 10 alternating cycles of 160 min of scheduled wakefulness (except for the first [120 min] and last wake period [40 min]) and 80 min of scheduled sleep (i.e., naps). Both blocks ended with a recovery night (minimum 8 h time in bed at usual bedtimes) and implied stringently controlled conditions, that are, semi-recumbent posture position in bed during wakefulness, regularly scheduled food intake, dim light (<8 lux) during scheduled wakefulness and zero lux during scheduled sleep episodes (i.e., naps), and no time-of-day indication. Participants' social interaction was restricted to the experimental staff. Getting up was allowed at scheduled times to use the bathroom. During scheduled wakefulness, participants were allowed to read, play card or dice games, and watch selected films. Participants were continuously monitored by electroencephalography (EEG). Data on melatonin, subjective and physiological sleepiness parameters, and polysomnographic nap sleep obtained in this study have been published in Maire et al. (2013).

PSYCHOMOTOR VIGILANCE TASK

Vigilance was assessed by a modified version of the PVT (Dinges and Powell, 1985) at ten time points within a test session of approximately 30 min duration, also encompassing an unrelated working memory test. The first session started after 1h awake and testing was subsequently repeated every 4 h until the end of the protocol (clock times see **Figure 1**). The PVT was the second test in each session and started at about 20 min into the test bout, after the working memory task. Every second cognitive test session took place in a functional magnetic resonance imaging (fMRI) scanner. In the PVT, a fixation cross was presented on a black screen. At random intervals (2–10 sec), a millisecond counter started, and participants were instructed to press a button to stop the counter as fast as possible (clock event). Modification of the original task consisted in the inclusion of null events, where the clock event was replaced by the fixation cross (25% of the trials at random) due to fMRI experimental design compatibility. Feedback of RT performance was displayed for one sec after the participants' response. Altogether, the task duration was 10 min. Here we report lapses (RT > 500 ms), optimal performance (the fastest 10% of the RTs between 150 and 500 ms, to exclude anticipatory responses and lapses, respectively), and standard deviations of the RTs. According to Basner and Dinges (2011), lapses represent the most sensitive measure to investigate the effects of acute total sleep deprivation, whereas the fastest RTs often remain unaffected by SD (Graw et al., 2004). Standard deviations of RTs were analyzed as a marker of performance variability within subjects (Doran et al., 2001).

EFFORT SCALES

After every test session, visual analog scales (VAS) ranging from 0–100 were used to assess subjectively perceived effort during the task, ranging from "*little*" to "*much*." Participants had to indicate experienced *strain*, *concentration*, *fatigue*, and *motivation* during the test.

STATISTICAL ANALYSIS

All analyses were performed using the statistical package SAS (SAS Institute Inc., Cary, NC; version 9.3). Variables were analyzed with mixed-model repeated measures ANOVAs (PROC MIXED) and *p*-values were based on Kenward-Roger's corrected degrees of freedom (Kenward and Roger, 1997). Alpha was set at 0.05. Contrasts were assessed using the LSMEANS statement. For *post-hoc* analysis, the Tukey-Kramer test was applied for alpha-adjustments of multiple comparisons, and corrected *p*-values are reported. For global PVT analysis (lapses and 10% fastest RTs), the factors *genotype* ($PER3^{5/5}$ vs. $PER3^{4/4}$), *condition* (NP vs. SD), and *time* (10 sessions) were used. *Time* represents time elapsed into the protocol starting at habitual wake time (see **Table 1** for average wake times per genotype). For the time-on-task analysis, we included the factors *genotype, time*, and *time-on-task* (first three minutes vs. last three minutes), and analyzed each condition separately for lapses, fastest RTs, and standard deviations. For graphs, 7 a.m. was used as the average reference wake up time. The lapses were transformed (transformation by $\sqrt{x} + \sqrt{x+1}$; for details, see Graw et al. (2001), and subsequently *z*-transformed due to different testing environment (every second session took place in the fMRI scanner with a different response keypad). Fastest RTs and standard deviations were equally *z*-transformed to account for the reason stated above. The first two trials of each test were excluded from analysis to eliminate effects of orienting to the task.

RESULTS

GLOBAL PVT PERFORMANCE

Lapses

$PER3^{5/5}$ carriers produced significantly more lapses than $PER3^{4/4}$ carriers in the SD (interaction: *condition x genotype* [$F_{(1,\ 513)} = 18.17$, $p < 0.0001$]; see **Figure 2A** ($PER3^{5/5}$; 0.62 ± 0.10, vs. $PER3^{4/4}$; 0.08 ± 0.08; mean ± SE; $p = 0.0323$), while during the NP protocol, no significant difference between the two genotypes was observed (see also Maire et al., 2013).

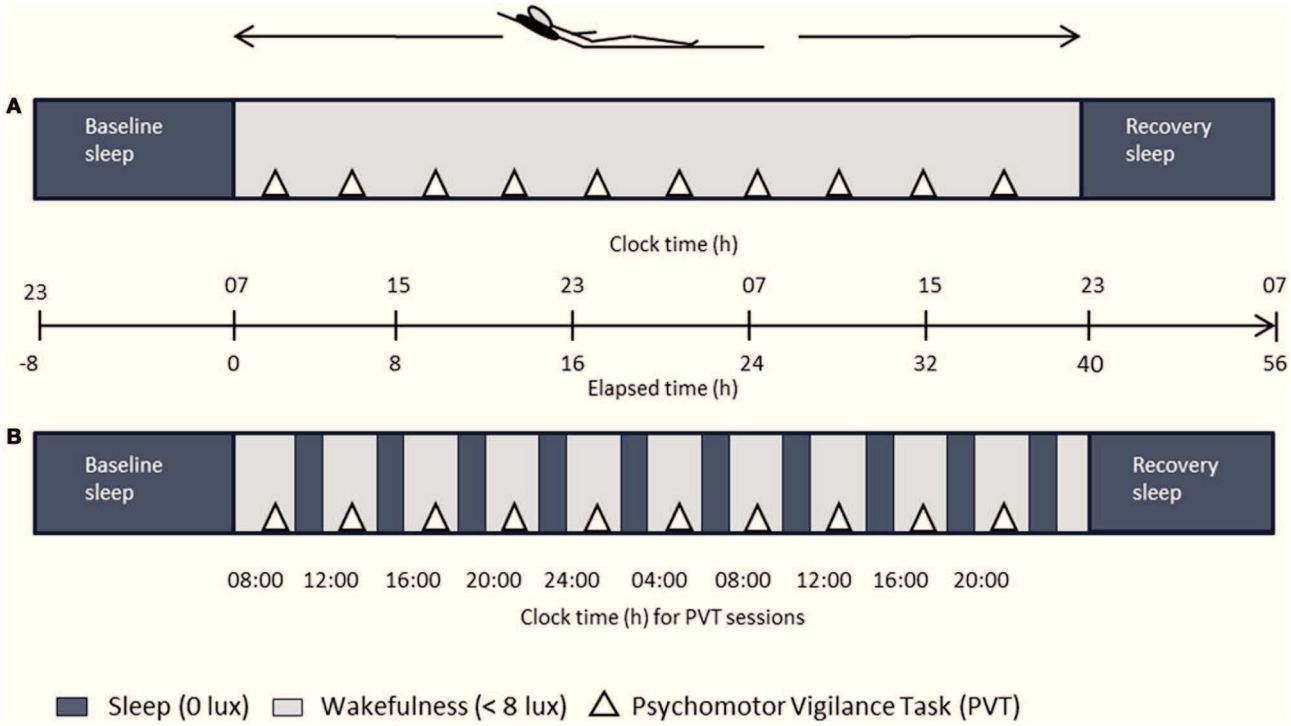

FIGURE 1 | Overview of the laboratory part. After baseline sleep (8 h), either a 40-h sleep deprivation **(A)** or a 40-h multiple nap paradigm (**B**, ten 80/160-min sleep/wake cycles) under controlled posture conditions was carried out in a within-subject design, followed by recovery sleep (8 h). Dark gray bars in **(B)** indicate scheduled sleep episodes. Elapsed time indication is relative to 7 a.m. wake up time.

Optimal Performance

Although lapses in performance increase under SD, normal RTs are still possible (Doran et al., 2001). Therefore, we were interested in the 10% fastest RTs representing the optimal performance levels in the respective task session. Analyses (**Figure 2A**) revealed main effects of *condition* [$F_{(1,\ 512)} = 23.27$, $p \leq 0.0001$] and *time* [$F_{(1,\ 512)} = 9.94$, $p \leq 0.0001$], with faster optimal RTs during NP (-0.14 ± 0.05, mean $\pm$ SE) than SD (0.14 ± 0.06, mean $\pm$ SE), and during the biological day compared to night time. The interaction *time* $\times$ *condition* was significant [$F_{(1,\ 512)} = 2.42$, $p = 0.011$], indicating that during the last session of the SD protocol (8 p.m., 37 h awake), participants had significantly higher (slower) optimal RTs ($p = 0.0031$) than during NP. There was no main effect of *genotype* or significant interactions were revealed regarding this factor ($p_{all} > 0.05$).

TIME-ON-TASK EFFECTS

Lapses

The time course of the lapses during SD over the 10-min task duration and for all sessions is shown in **Figure 3**. (**A**) depicts the whole group; (**B**) shows each genotype separately and (**C**) illustrates the difference between genotypes. The analysis yielded a significant main effect of *genotype* with $PER3^{5/5}$ carriers showing overall more lapses during SD (**Table 2**; $PER3^{5/5}$: 0.35 ± 0.05 vs. $PER3^{4/4}$: 0.03 ± 0.03; mean $\pm$ SE), confirming the global PVT performance results. Both factors, *time* and *time-on-task*, were significant, showing that lapses varied with test timing and were more numerous during the last portion of the 10-min PVT task (**Table 2**; first section: -0.009 ± 0.03; last section: 0.37 ± 0.05; mean $\pm$ SE). Also, the interaction *time* $\times$ *time-on-task* was significant (**Table 2**), such that during the night session (clock time: 4 a.m.) as well as during two sessions at noon and in the afternoon of the second day during the SD (clock times: 12 p.m. and 4 p.m.), the lapses during the last test part were more numerous ($p_{all} < 0.05$). Interestingly, the effect of *time* was modulated by *genotype* (**Table 2**), indicating that $PER3^{5/5}$genotypes produced significantly more lapses in the session during the night compared to $PER3^{4/4}$ carriers (21 h awake, clock time 4 a.m., $p < 0.05$; $PER3^{5/5}$; 0.36 ± 0.07, vs. $PER3^{4/4}$; 0.13 ± 0.05; mean $\pm$ SE). Likewise, a significant interaction *time-on-task* $\times$ *genotype* (**Table 2**) revealed that while both groups showed a time-on-task-dependent increase in lapses, $PER3^{5/5}$carriers had significantly more lapses during the last test section when compared to $PER3^{4/4}$carriers (**Figure 2B;** $p < 0.01$; $PER3^{5/5}$; 0.59 ± 0.08, vs. $PER3^{4/4}$; 0.17 ± 0.06; mean $\pm$ SE), whereas both groups did not differ in the first test section ($p > 0.1$).

Under low sleep pressure (NP), significant *time* and *time-on-task* effects revealed a time-of-day-dependent pattern and an increase in lapses over the course of the task (**Table 2**; first section: -0.18 ± 0.02; last section: -0.09 ± 0.03; mean $\pm$ SE, **Figure 2B**). The interaction *time x time-on-task* (**Table 2**) showed that especially in session 7 (8 a.m. on the second day of the

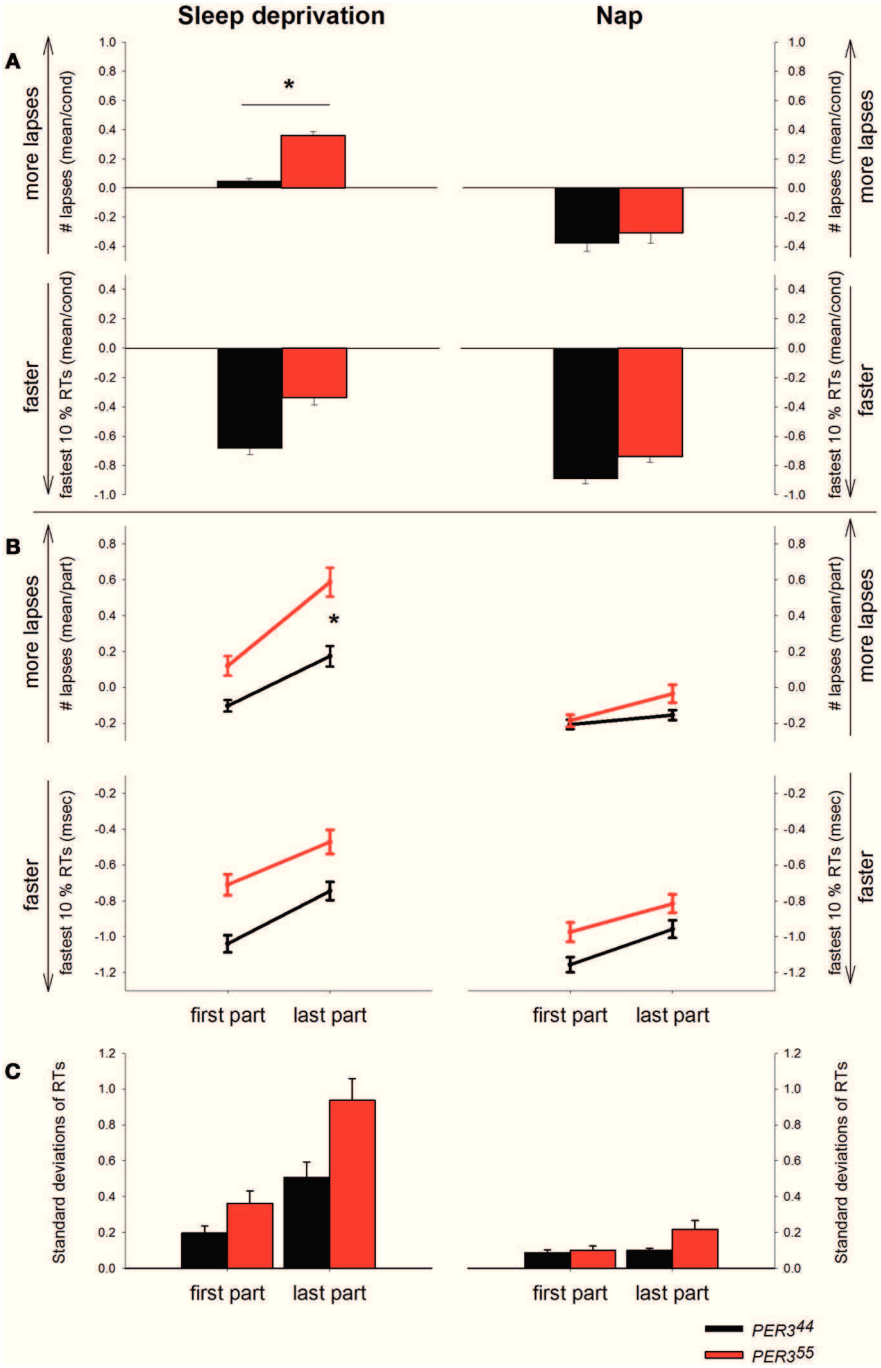

FIGURE 2 | Psychomotor Vigilance Task (PVT) performance displayed by genotype and condition. *RT* = Reaction time. **(A)** Mean number of lapses (transformed) and mean of the 10% fastest *RT*s (*z*-scores) during sleep deprivation and during the nap protocol by genotype ($PER3^{5/5}$: red bars, $PER3^{4/4}$: black bars). Asterisk represents *p*-value < 0.05. **(B)** PVT lapses and fastest RTs displayed by genotype and condition over the first and the last part of the task over all sessions. $PER3^{5/5}$: red lines, $PER3^{4/4}$: black lines. **(C)** Standard deviations of RTs plotted by genotype and condition for the first and the last test part. $PER3^{5/5}$: red bars, $PER3^{4/4}$: black bars.

protocol), lapses increased from the first to the last section ($p = 0.0006$). However, opposed to what was seen during SD, we observed no significant main effect of *genotype* or *genotype* × *time-on-task* (**Table 2**).

Optimal Performance

The analysis of the 10% fastest RTs (**Figure 2B**) during SD revealed a significant effect of *time* and *time-on-task* (**Table 2**), indicating, as expected, that RTs were lower during the biological

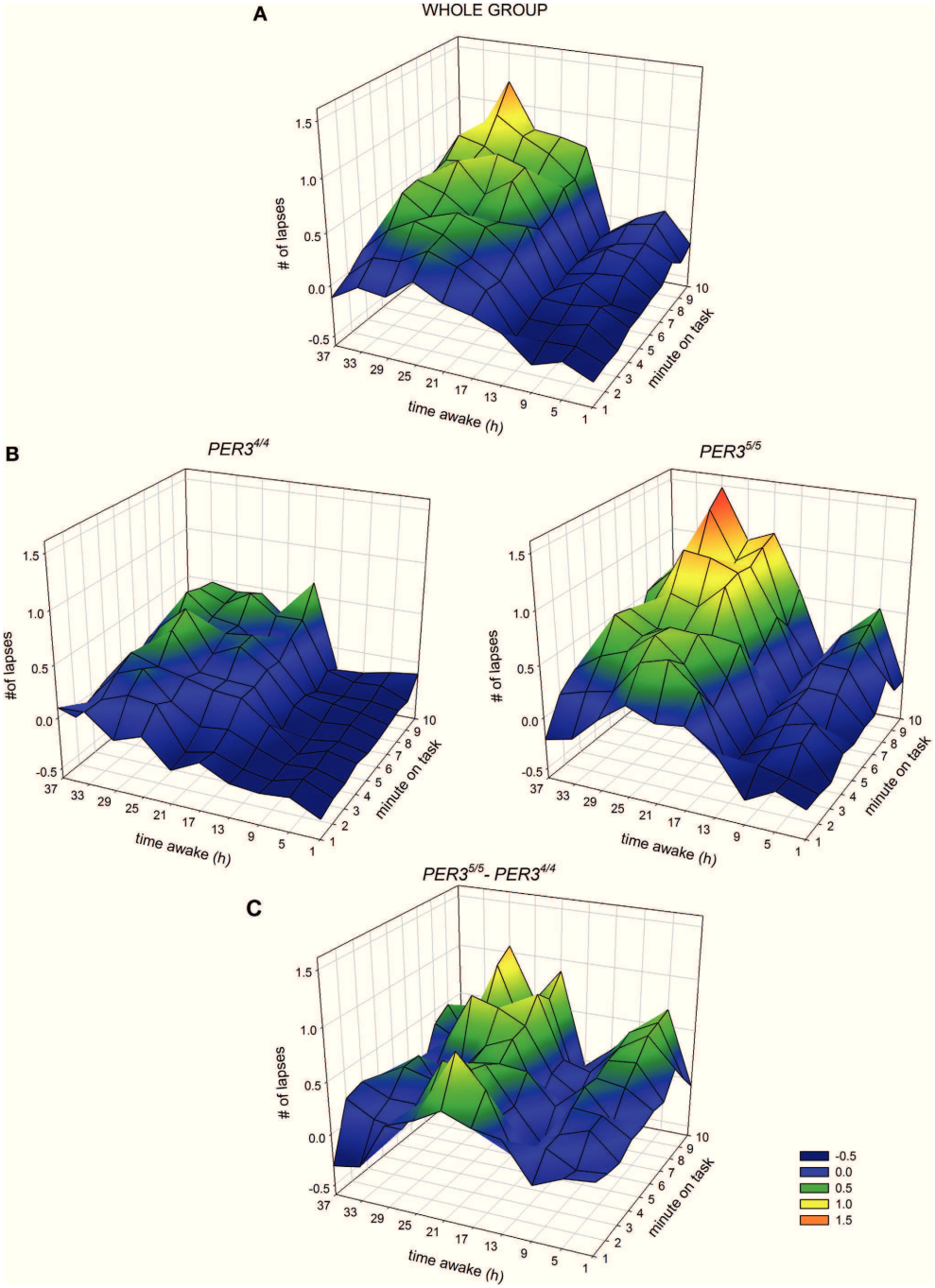

FIGURE 3 | Interaction between hours of scheduled wakefulness (time awake) **during sleep deprivation** (y-axis of each panel) **and time-on-task** (minutes on task, *x*-axis of each panel) **in the modulation of the number of lapses on the Psychomotor Vigilance Task** (PVT, *z*-axis of each panel) **(A)** for the whole group, **(B)** for $PER3^{4/4}$ carriers (left) and $PER3^{5/5}$ carriers (right) and **(C)** the difference between the two genotypes ($PER3^{5/5}$–$PER3^{4/4}$). Higher values on the *z*-axis indicate higher levels of impairment.

day and within the first part of the test (First part: -0.88 ± 0.03 vs. last part: -0.61 ± 0.04, mean $\pm$ SE). No significant interaction was found for *time* × *time-on-task* (**Table 2**). Although there was a trend for a main effect of *genotype*, no significant interactions were revealed regarding this factor (**Table 2**, **Figure 2B**).

During NP, we observed a significant effect of *time* and *time-on-task*, equally showing faster RTs during the biological day and the first test part (**Table 2**). Here, the interaction *time* × *time-on-task* was significant, indicating that during the tests at 8 a.m. on both days, RTs were significantly lower in the first test part

Table 2 | Results of mixed model ANOVA for time-on-task effects; *F*-values (df), and *p*-values.

		PER3	T	ToT	T × ToT	*PER3* × T	ToT × *PER3*	T × ToT × *PER3*
SD	Lapses	$F_{(1,\ 27)} = 5.59$ **$p = 0.0255$**	$F_{(9,\ 511)} = 20.97$ **$p < 0.0001$**	$F_{(1,\ 511)} = 80.99$ **$p < 0.0001$**	$F_{(9,\ 511)} = 2.14$ **$p = 0.0248$**	$F_{(9,\ 511)} = 2.95$ **$p = 0.002$**	$F_{(1,\ 511)} = 5.6$ **$p = 0.0184$**	$F_{(9,\ 511)} = 1.47$ $p = 0.16$
	Fast RT	$F_{(1,\ 27)} = 3.36$ $p = 0.07$	$F_{(9,\ 507)} = 16.31$ **$p < 0.0001$**	$F_{(1,\ 507)} = 44.55$ **$p < 0.0001$**	$F_{(9,\ 507)} = 0.21$ $p = 0.99$	$F_{(9,\ 507)} = 0.54$ $p = 0.85$	$F_{(1,\ 507)} = 0.53$ $p = 0.47$	$F_{(1,\ 507)} = 1.12$ $p = 0.35$
NP	Lapses	$F_{(1,\ 27)} = 0.78$ $p = 0.3842$	$F_{(9,\ 513)} = 12.86$ **$p < 0.0001$**	$F_{(1,\ 513)} = 10.66$ **$p = 0.0012$**	$F_{(9,\ 513)} = 2.54$ **$p = 0.0096$**	$F_{(9,\ 513)} = 2.13$ **$p = 0.0259$**	$F_{(1,\ 513)} = 3.01$ $p = 0.08$	$F_{(9,\ 513)} = 0.32$ $p = 0.97$
	Fast RT	$F_{(1,\ 27)} = 1.04$ $p = 0.31$	$F_{(9,\ 512)} = 16.32$ **$p < 0.0001$**	$F_{(1,\ 512)} = 35.08$ **$p < 0.0001$**	$F_{(9,\ 512)} = 1.91$ **$p = 0.048$**	$F_{(9,\ 512)} = 1.76$ $p = 0.07$	$F_{(1,\ 512)} = 0.22$ $p = 0.64$	$F_{(9,\ 512)} = 0.65$ $p = 0.75$

Significant results ($p < 0.05$) are printed in bold for factors genotype (PER3), time, T; time on task, ToT; and interactions.

(**Table 2**). *Genotype* and the interactions with this factor were not significant (**Table 2**, **Figure 2B**).

STANDARD DEVIATIONS OF REACTION TIMES

The analysis of the standard deviations of RTs (**Figure 2C**) throughout the task during SD revealed significant main effects of *time* [$F_{(9,\ 511)} = 14.54$, $p \leq 0.0001$] and *time-on-task* [$F_{(1,\ 511)} = 41.21$, $p \leq 0.0001$], as well as the interaction of these two factors [$F_{(9,\ 511)} = 3.39$, $p = 0.0005$]. In other words, the standard deviations were increasing with time awake, to reach a maximum at noon on the second sleep deprived day (12 p.m.), and decreased again toward the biological evening. This pattern was more pronounced in the last part of the test. Although showing a trend, the main effect of *genotype* was not significant [$F_{(1,\ 27)} = 3.28$, $p = 0.0812$]. The interaction of *time* × *genotype* was significant [$F_{(9,\ 511)} = 2.9$, $p = 0.0024$], as well as the interaction of *time-on-task* × *genotype* [$F_{(9,\ 511)} = 3.89$, $p = 0.0491$]. Post hoc tests revealed that the standard deviations of the RTs differed between the genotypes mainly in the noon-session of the second day (12 p.m., $p < 0.0001$). Moreover, the genotypes did not differ did in terms of their variability of RTs in first test part ($p = 0.36$), but showed a trend for a difference in standard deviations during the last test part ($p = 0.0734$). The three-way interaction between all factors was not significant ($p > 0.1$).

In the NP, the main effects of *time* [$F_{(9,\ 513)} = 8.25$, $p < 0.0001$] and *time-on-task* [$F_{(1,\ 513)} = 6.09$ $p = 0.0139$] were significant, as was the interaction of these two factors [$F_{(9,\ 513)} = 2.12$, $p = 0.0263$], showing an increase of the standard deviations toward the biological morning, which was more pronounced in the last test minutes. The effect of the factor *genotype* was not significant [$F_{(1,\ 27)} = 1.68$, $p = 0.2059$]. However, the interaction of *genotype* with *time* [$F_{(9,\ 513)} = 3.97$, $p < 0.0001$] was significant, showing greater standard deviations in $PER3^{5/5}$carriers during the session at 8 a.m. on the second day compared to the short allele carriers ($p < 0.0001$). Although a significant interaction of *genotype* × *time-on-task* [$F_{(1,\ 513)} = 4.1$, $p = 0.0435$] was revealed, none of the post hoc comparisons showed significant differences between genotypes.

EFFORT SCALES

None of the items on the VAS questionnaire regarding perceived strain, extent of concentration, fatigue, or motivation of participants during task performance differed significantly between genotypes ($p_{all} > 0.05$; data not shown). Neither did the genotypes differ significantly in terms of these indicators across time (*genotype* × *time*, $p > 0.05$). However, we observed significant main effects of *time* ($p_{all} < 0.05$) for all four variables, indicating a time-of-day-dependent variation for the whole group. Significant main effects of *condition* for strain, concentration, and fatigue ($p_{all} < 0.0001$) revealed higher values during SD, whereas motivation for the task was comparable during both conditions ($p > 0.05$).

DISCUSSION

As hypothesized, $PER3^{5/5}$carriers had significantly more difficulties to maintain stable attentional performance over a period of 10 min than $PER3^{4/4}$carriers, particularly under conditions of high sleep pressure and at times when the circadian pacemaker promotes sleep. When sleep pressure was kept at low levels by multiple naps, the groups performed equally and no genotype-modulated pattern of a time-on-task decrement was observed. Momentary task disengagement seems to be more pronounced in $PER3^{5/5}$than in $PER3^{4/4}$ carriers under SD—thus, they suffered more from elevated sleep pressure conditions. Importantly, no genotype-related difference in subjectively perceived strain, effort or motivation was found in either of the protocols. By analysing the 10% fastest RTs (i.e., optimal performance), we showed that the time course of optimal performance levels did not differ in function of genotype, indicating that a temporary mobilization of effort is still possible for both vulnerable and more resilient participants. The differential extent of the resulting variability in RTs is mirrored in the standard deviations being greater for $PER3^{5/5}$carriers. A faster homeostatic build-up of sleep pressure in $PER3^{5/5}$carriers than in $PER3^{4/4}$carriers has been reported (Viola et al., 2007, 2012; Goel et al., 2009), as indexed by more slow wave sleep and more EEG slow-wave activity in $PER3^{5/5}$carriers. Moreover, the deterioration in cognitive performance, operationalized as a composite of several cognitive tasks (Viola et al., 2007) as well as working memory (Groeger et al., 2008), was shown to be greater in $PER3^{5/5}$compared to $PER3^{4/4}$carriers under SD, which was paralleled by an increase in physiological correlates of sleepiness, such as EEG theta activity and the incidence of slow eye movements (SEM) (Viola et al., 2007; Groeger et al., 2008). Likewise, we have previously reported that our $PER3^{5/5}$sample produced a greater number of PVT lapses, and higher values on subjective and physiological

indicators of sleepiness under high sleep pressure conditions (Maire et al., 2013). However, other authors could not find differences in PVT performance between the genotypes (Goel et al., 2009; Kuna et al., 2012 [lapses]; Lo et al., 2012 [lapses and inverse of the 10% slowest RT]). This discrepancy could be related to the fact that in contrast to others, we strictly controlled for the amount of prior wakefulness and circadian phase by systematically manipulating these two processes in a SD and a nap protocol which allowed for an accurate titration and quantification of the circadian and homeostatic influence on attentional failures over a rather long time span (40 h).

Task duration is an important feature of the demand level a cognitive task exerts. The interplay between sleep deprivation, state instability and task duration has been described before (for an overview, see Doran et al., 2001). However, this phenomenon is rarely reported when studying the impact of sleep loss on cognition, and has not yet been investigated with respect to inter-individual differences in the behavioral vulnerability to sleep loss. Early theories associated vigilance decrement over a certain time span on the task with the monotonous and repetitious nature of vigilance tasks (for a review, see Warm et al., 2008). More recent studies show that the maintenance of stable vigilance levels also depends on task type and its workload, and that the temporal irregularity of the stimuli contributes majorly to the level of demands such a task has (Warm et al., 2008). Zhou et al. (2011) recently showed that performance variability is greater the longer one has been awake prior to performance and the closer to the circadian nadir (i.e., early morning hours). Although the variability detected in their study was not related to the duration of the task *per se*, the authors suggest that state instability acts as an explanation for the responsiveness of neurobehavioral performance to increasing sleep drive already during a habitual wake period.

Importantly, Doran et al. (2001) state that lapses will progress into uncontrolled sleep attacks due to increasing homeostatic sleep pressure. In line with this, we have recently shown that *PER3*$^{5/5}$carriers indeed have more incidental SEM as well as unintentional sleep attacks during SD, particularly during the biological night (Maire et al., 2013). Thus, with our findings of *PER3*$^{5/5}$ carriers showing a greater increase in attentional lapses, we confirm that the responsiveness to SD is greater in this group and that their stronger sleep homeostatic process might be mirrored in the time course of performance. Interestingly, genotypes did not differ in terms of their optimal RTs, although we observed a general time-on-task effect for this measure, too. Indeed, optimal RTs in the PVT seem to be only marginally affected by elevated sleep pressure during a 40-h SD protocol (Graw et al., 2004).

Several studies (Drummond et al., 2005; Weissman et al., 2006; Chee et al., 2008) have linked lapses in performance to a lower deactivation of the so-called brain default mode network initially presented in Raichle et al. (2001). Furthermore, a recent study by Asplund and Chee (2013) showed that both sleep deprivation and time-on-task lead to reduced activation in overlapping brain areas, suggesting that these effects have shared neural and psychological causes. An fMRI study by Vandewalle et al. (2009) showed differences in activations for *PER3*$^{5/5}$carriers compared to *PER3*$^{4/4}$ carriers after 25 h of SD during a working memory task. Specifically, *PER3*$^{4/4}$ carriers showed no reductions in activations, but were able to recruit supplemental brain areas, while *PER3*$^{5/5}$carriers showed widespread reductions in brain activations after SD. The recruitment of supplemental brain areas might mirror compensatory effects (Drummond et al., 2000) that are necessary to prevail against task disengagement. It remains to be determined how the greater vulnerability of *PER3*$^{5/5}$carriers to time-on-task-dependent attentional failures is mirrored at the cerebral level, and whether brain activation differs where optimal performance can be sustained under sleep loss.

Motivation plays a major role in successfully performing a task, and might even mask the more serious effects of sleep deprivation through compensatory effort (Doran et al., 2001). Indeed, the mobilization of effort to keep attentional performance stable despite challenging sleep loss conditions seems to depend largely on motivation (see Sarter et al., 2006 for a review). According to our data, subjectively perceived motivation was comparable between genotypes, also indicated by the fact that "normal" RTs still occurred (Doran et al., 2001). Thus, we conclude that the difference we observed results mainly from divergent sleep homeostatic forces acting on wake state instability, as it is obviously not obscured by discrepancies in motivation. Besides the sleep homeostatic forces only, the interplay between homeostatic and circadian sleep promotion in the early morning could also be altered in the more vulnerable genotype (i.e., *PER3*$^{5/5}$), since most of the differences in attentional failures between the two groups occurred after 21–25 h of extended wakefulness, which corresponded to the circadian sleep maintenance zone between 4 and 8 a.m. in our subject sample. Presumably, these differences in sleep homeostatic and/or circadian drives might allow or hinder the activation of attentional top-down mechanisms at the cerebral level. A possible explanation could be increased prefrontal cortex (PFC) cholinergic activity that might activate the anterior attention system, favoring the top-down optimization of input processing in sensory regions (Sarter et al., 2006). Hence, cholinergic PFC control may optimize goal-directed behavior and cognitive processes, despite performance challenges, such as time-on-task, circadian phase shifts, and sleep loss (Sarter et al., 2006).

Taken together, we show that attentional performance lapses in the PVT reflect the failure to stay focused on the task—which was significantly more difficult for *PER3*$^{5/5}$than *PER3*$^{4/4}$carriers. However, optimal performance and thus temporary mobilization of effort throughout the task did not depend on genotype. A probable limitation of our study is the rather small sample size. However, by carefully selecting young, healthy participants without sleep complaints and controlling for gender ratio, chronotype, sleep duration, and timing across groups, we chose a rather homogenous phenotype to maximize potential contribution of the *PER3* polymorphism to vulnerability in combination with highly controlled laboratory conditions that restrict potential masking factors such as light influence, body posture, or social and nutritional timing cues.

This is the first study to report time-on-task effects modulated by the *PER3* polymorphism by combining two protocols with low and high sleep pressure levels. Our results provide further evidence that the *PER3* polymorphism is implicated in

inter-individual differences in the susceptibility to sleep loss. As momentary lapses in attention can have severe consequences in professional and daily live, our results undermine the importance of considering the time course of performance in further investigations of the nature of sleep loss-related inter-individual differences in cognitive performance.

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How the brain copes to sustain attention at night-time: Impact of sleep pressure and genetic vulnerability to sleep loss.

Maire M[1], Reichert CF[1], Gabel V[1], Viola AU[1], Phillips C[4], Krebs J[1], Scheffler K[5,6], Klarhöfer M[2], Strobel W[3], Cajochen C[1], Schmidt C[1]

1 Centre for Chronobiology, Psychiatric Hospital of the University of Basel, Basel, Switzerland
2 Department of Medical Radiology, MR-Physics, University of Basel, Basel, Switzerland
3 Respiratory Medicine, Department of Internal Medicine, University Hospital Basel, Basel, Switzerland
4 Cyclotron Research Centre, Department of Electrical Engineering and Computer Science, University of Liège, Liège, Belgium
5 MRC-Department, MPI for Biological Cybernetics, Tübingen, Germany
6 Department of Biomedical Magnetic Resonance, University of Tübingen, Tübingen, Germany

Abstract

Even though wakefulness at night leads to prominent performance deterioration and is regularly experienced by shift workers, the cerebral correlates underlying attentional decrement at night remain virtually unexplored. We assessed brain activity during a sustained attention task under high and low sleep pressure during night-time, coinciding with strongest circadian sleep promotion. We examined differential sleep-loss-related vulnerability by considering a PERIOD3 polymorphism impinging on sleep homeostasis. Our results link higher vulnerability to cortical and subcortical deactivation patterns. Thalamic regions were progressively less recruited with time-on-task and functionally less connected to task-related and arousal-promoting brain regions in those volunteers showing higher attentional instability in their behavior. The data finally suggest that the latter is linked to shifts into a task-inactive default-mode network in between task-relevant stimulus occurrence. We provide a multifaceted view on cerebral correlates of sleep loss at night and suggest that genetic predisposition leads to differential cerebral coping mechanisms.

Sleep loss affects cognitive performance most strongly during the biological night[1]. At the same time, night work is particularly common in professions where the lives of human beings are at stake. Even though adequate decisions can still be taken in most situations encountering night work, serious consequences are regularly reported, such as traffic accidents being more likely fatal[2] and medical errors to occur more frequently[3]. Performance decrement during the biological night originate from a misalignment of sleep-wake regulatory mechanisms: During night time, the circadian timing system, pacing a near-24h-rhythm to endogenously regulate sleep-wake propensity, no longer encourages wakefulness as it does during the biological day, but promotes sleep reaching maximal sleep propensity levels towards the end of the night[4]. This system interacts with a sleep homeostatic process tracking previous sleep-wake history, which is reflected by increasing sleep propensity or „pressure" levels the longer one is awake[5], and dissipates solely when sleep is allowed. Thus, during the night, the achievement of consolidated wakefulness and associated adequate performance levels is particularly challenging, as both the circadian and homeostatic processes promote sleep[4]. This is strongest during a time window at the end of the biological night, hereafter labeled the "sleep maintenance zone" (SMZ). Behaviorally, adequate night work might be compromised by attentional lapses, a slowing of response times, and stronger state instability reflected by increased response variance[6]. It appears that the ability to phasic responses to behaviorally relevant stimuli is preserved to a certain degree under sleep loss conditions[7], while decrement is detected most strongly in maintaining appropriate attentional levels continuously over time[6]. The cerebral basis of different attentional processes under sleep deprivation (SD) has been investigated before (for a review, see[8]), but critically, never during night time.

A second question that needs clarification addresses how differential vulnerability to sleep loss, that is, how well someone copes with the detrimental effects on cognition, becomes evident at the cerebral level. Although reports differentiating participants more vulnerable and more resilient to SD at the cerebral level do exist[9], it is unclear how this manifests during this very critical time point. A primate-specific[10] variable number tandem repeat (VNTR) polymorphism in the human clock gene *PERIOD3* (*PER3*) has been associated with susceptibility to SD in neurobehavioral performance[11-15] and electro-physiology[13, 15], most likely through influence on the sleep homeostatic process[16]. In congruence with previous reports, $PER3^{5/5}$ carriers seemed more vulnerable to sleep loss than $PER3^{4/4}$ carriers on the physiological and behavioral level. One fMRI study investigating working memory performance after normal night sleep compared to the morning after 24h of SD[22] showed that differences between the *PER3* genotypes also become evident at the cerebral level. However, likewise to other SD studies[8], data were collected during the biological day. The impact of sleep loss and associated vulnerability on cerebral correlates underlying cognitive performance during nighttime remains thus virtually unexplored. Furthermore, although sleep loss-related attentional drifts might be most likely detected during monotonous tasks[17] at adverse circadian phase, this issue remains without consideration up to date, even though such circumstances might be particularly prone to drifting into task-inactive networks and its associated behavioral consequences (e.g., failures to react to relevant stimuli)[18].

Within this context, our aim was to investigate the neural bases of sleep-loss related attentional decrements during night time in two groups (14 $PER3^{5/5}$ versus 14 $PER3^{4/4}$ carriers) previously reported to differ in their behavioral and electrophysiological response to sleep loss[12, 13]. We combined a 40h SD and a multiple nap protocol (NP; see e.g.[19] for a study applying a similar approach) allowing to compare brain activity at the same critical circadian time point once under

sleep loss, and once when homeostatic sleep pressure is kept low by multiple naps. We thus approach towards a disentanglement of sleep homeostatic and circadian influences at the cerebral level. Body posture, light influence and meal intake were kept constant in both protocols to control for masking effects on the circadian system. As the *PER3* polymorphism has been associated with vulnerability to sleep loss through its action on the sleep homeostatic process[14], we expected group differences to be mainly present under high sleep pressure conditions. The fMRI sessions were individually scheduled to occur 21 hours after habitual wake time in the biological night. We used the Psychomotor Vigilance Task[20] (PVT), a sustained attention task highly sensitive to SD and circadian variation[7]. During this relatively monotonous task, participants have to continuously sustain attention in order to react as fast as possible with a simple button press to stimuli which occur at random intervals. As sleep loss has been associated with a slowing down in reaction time (RT), we categorized stimuli into fast (RTs < 25th percentile), intermediate (percentile 75 > RTs < percentile 25), slow (RTs > 75th percentile) and lapse (>500ms) events. We hypothesized that the *PER3*-related vulnerability to sleep loss will mainly lead to a selective change in brain responses associated with the slow RT domain, as behavioral[7] and cerebral[21] changes in this RT range are considerably more prominent after sleep loss than for optimal performance (fast RT domain). Furthermore, we assumed that sleep loss will additionally affect the ability to sustain attention in between the phasic occurrence of stimuli. Particularly during the periods preceding slow events, a sleep-loss related drift into task-inactive networks[22] was assumed in those participants behaviorally more vulnerable to sleep loss (i.e., *PER3*$^{5/5}$ carriers). Our findings confirmed our hypotheses and provide a multi-faceted view on the neural bases of attention-related slowing down induced by night-time sleep loss and also shed light on potential cerebral mechanisms of behavioural vulnerability, assessed under stringently controlled conditions in the laboratory.

Results

Time course of vigilance, and subjective and physiological markers of sleepiness over 40 hours

Participants' characteristics are listed in **Table 1**. The typically observed[19] circadian- and sleep-pressure-level-dependent time course for subjective and physiological sleepiness was detected throughout the 40 h SD and multiple nap protocol. As depicted in Figure 1, slow rolling eye movements and unintentional sleep episodes as a physiological correlate for sleep drive, significantly increased once passing into the biological night. Maximal levels were reached towards the end of the night to the beginning of the biological day (i.e., during the SMZ), stabilizing thereafter on the second day under SD and decreasing again under NP. A similar pattern was observed for attentional lapses (>500 ms)[23] during PVT performance. Importantly, as reported previously, these measures were affected by genotype, such that *PER3*$^{5/5}$ carriers participants felt significantly sleepier, produced more slow eye movements and unintentional sleep episodes as well as attentional lapses under SD compared to NP[13].

Subjective Sleepiness and Vigilance during night-time

Participants were significantly sleepier (assessed with the Karolinska Sleepiness Scale, KSS; t_{27} = -1.74; $p_{\text{one-sided}}$ = 0.04) and had more lapses (t_{27} = -2.92; $p_{\text{one-sided}}$ = 0.03), and longer RTs in the slow, fast and intermediate domains during the night under SD compared to NP (condition: p_{all} < 0.05; **Supplementary Table S1**). The *PER3*$^{5/5}$ carriers tended to be sleepier than the *PER3*$^{4/4}$ carriers (t_{26} = -1.49; $p_{\text{one-sided}}$ = 0.07; mean ± SE: *PER3*$^{4/4}$ 6.6 ± 0.3 *PER3*$^{5/5}$ 7.3 ± 0.3), and produced significantly more

attentional lapses (t_{26} = -2.05; $p_{\text{one-sided}}$ = 0.03; mean ± SE: *PER3*$^{4/4}$ 4.6 ± 0.7 *PER3*$^{5/5}$ 7.2 ± 1.1) during night time under SD. However, RT did not differ in any of the domains (fast, intermediate, slow) according to genotype, nor was the interaction with condition (i.e. SD and NP) significant (**Supplementary Table S1**).

fMRI data:

Brain responses were modeled for each subject at each voxel using a general linear model (GLM). Our model included four regressors for each condition (fast, slow, intermediate, lapses) convolved with the canonical hemodynamic response function (HRF; see online Methods). We processed the data in a two-step analysis to take into account the intra- and inter-individual variance, respectively. In this study, we will focus on the slow RT domain because this RT range has been shown to be most sensitive to homeostatic sleep pressure[7, 21].

General brain responses to the PVT in the slowest reaction time range (Supplementary Results)
Brain areas generally associated with the slowest RTs during the night independent of sleep pressure levels are listed in **Supplementary Table 2**. Several frontal, parietal and occipital areas, as well as the cerebellum, and a region within the pons, compatible with the reticular formation in the brainstem, were recruited independent of genotype during the slowest responses (data not shown). Generally, these results are in line with the report of Drummond et al. [21] that the PVT recruits areas which are part of the fronto-parietal attention network as well as subcortical motor systems. Deactivations during task performance were found in areas related to the default-mode network[24], such as the precuneus and the posterior cingulate cortex (**Supplementary Table 2**, data not shown).

Sleep pressure modulates night-time brain responses to slow reaction times
Under high sleep pressure, a bilateral thalamic region as well as the putamen showed higher blood-oxygen-level-dependent (BOLD) activity compared to NP (**Supplementary Table 3**). During NP, several cortical regions, that is, the left cuneus, right middle temporal gyrus, and bilateral temporal pole, as well as insular regions, were more active than during SD (**Supplementary Table 3,** data not shown).

Genetic vulnerability to sleep loss acutely affects sleep-pressure dependent BOLD activity and functional connectivity to arousal-promoting subcortical regions during night time vigilance
Similar to what was observed behaviorally (sleepiness and attentional lapses), the *PER3* polymorphism significantly modulated brain responses to the task according to sleep pressure level. Strongest effects were observed for the slow RT domain (events associated with a RT > Percentile 75), previously reported to be most sensitive to sleep loss conditions[7]. We will detail results of this RT range in the following sections.

Generally, *PER3*$^{4/4}$ individuals showed increased activation during SD compared to NP in multiple attention-related cortical and subcortical brain areas, whereas the *PER3*$^{5/5}$ carriers showed the opposite pattern (**Supplementary Table 4**; see also **Figure 2** for selected regions with corresponding parameter estimates). More precisely, higher BOLD activity under SD was observed for *PER3*$^{4/4}$ compared to *PER3*$^{5/5}$ carriers in frontal, temporal and parietal areas as well as in a brainstem region (SD 44>55; **Table 2).** No regions were activated more in *PER3*$^{5/5}$ than *PER3*$^{4/4}$ carriers during SD (SD 55>44). To add, separate analyses by genotype revealed that the *PER3*$^{4/4}$ group showed BOLD activity

increases from NP to SD in a series of cortical and thalamic structures, as well as areas of the basal ganglia and the cerebellum **(see Table 2; 44 SD>NP).** The *PER3*$^{5/5}$ group yet showed the opposite pattern: None of the areas showed higher activations during SD compared to NP (55 SD>NP), but a series of areas were more activated during NP (55 NP>SD), that is, frontal, temporal, parietal and several occipital areas, as well as a brainstem area compatible with the location of the reticular formation (midline pons**, Table 2)**. Functional connectivity assessed by a psycho-physiological interaction (PPI) analysis revealed that, under sleep loss (SD), activity in the latter region was more connected to cortical (left IFG and right inferior occipital area (BA18)), thalamic (bilateral anterior and posterior thalamus, also encompassing the pineal gland) and a more superior located brainstem region (**Supplementary Table 5**) in *PER3*$^{4/4}$ compared to the *PER3*$^{5/5}$ carriers.

Our data indicate that while the more resistant individuals (*PER3*$^{4/4}$) react to sleep loss by increasing task-related cortical BOLD activity and by additionally recruiting thalamic resources, the more vulnerable genotype (*PER3*$^{5/5}$) mainly reacts by activity decreases and reduced connectivity between brainstem, thalamic and task-related cortical regions.

Brain responses underlying time-on-task modulation: Greater state instability by decreased thalamic recruitment

According to the state-instability hypothesis[6], SD strongly affects the ability to maintain appropriate attentional levels continuously over time. In a previous report[12], we reported that *PER3*$^{5/5}$ carriers presented higher response variability and stronger time-on-task decrements in vigilant attention under sleep loss. To investigate this at the cerebral level, we added a time modulation regressor (first order polynomial) to account for time-on-task effects for each trial type. We detected that BOLD activity throughout the 10-min PVT differed between genotypes in the anterior and posterior thalamus as well as the anterior cingulate cortex (**Supplementary Table 6, Figure 3A**) under SD for the slow RT range. *PER3*$^{5/5}$ carriers showed activity decreases in these brain areas over the course of the task (p<0.05), whereas activation in *PER3*$^{4/4}$ carriers remained stable (p>0.05; **Figure 3B** depicts the course of thalamic brain activity for two representative subjects of each genotype). In fact, the group presenting higher state instability at the behavioral level was hallmarked at the cerebral level by reduced task-related cingulate and thalamic recruitment with increasing time-on-task.

Subjective sleepiness modulates brain responses to slow events in the left inferior frontal gyrus under night-time SD depending on genetic vulnerability to sleep loss.

To link night time BOLD activity modulation by sleep pressure to the observed differences in subjective sleepiness, KSS values assessed before the test session were added at the random-effect level as a covariate of interest. Depending on genotype when sleep deprived, BOLD activity associated to slow events were significantly related to subjective sleepiness in the left inferior frontal gyrus (IFG; peak coordinates: -46, 38, 12; Z = 3.8, p_{svc} < 0.001): *PER3*$^{5/5}$ carriers showed a negative relation (the more they felt tired, the less they recruited this region), whereas in *PER3*$^{4/4}$ carriers, the relation was positive (the more they felt tired, the more they activated this region; **Figure 2G**).

Key structures of the default mode network are more activated prior to the slow events under SD in the vulnerable genotype.

Successful PVT performance requires the participants to sustain attention between stimulus appearance (here, as long as the cross is presented). Critically, as it has been shown previously that brain activity before stimulus appearance is predictive for subsequent lapses in attention[22], we were

interested in brain activity immediately prior to stimulus appearance. We added finite-impulse-response regressors modeling BOLD activity immediately preceding (one TR; i.e., 2.2 s) the occurrence of the relevant stimulus. Results showed that for slow RTs and compared to *PER3*$^{4/4}$, the *PER3*$^{5/5}$ carriers had higher activation in regions commonly assigned to the default mode network (DMN) [25-27] (**Table 3 and Figure 4**; e.g., middle frontal gyrus, dorsal anterior cingulate cortex (ACC), posterior cingulate (PCC), precuneus, gyrus angularis). No areas were more active in the *PER3*$^{4/4}$ compared to the *PER3*$^{5/5}$ (**Table 3**). Importantly, prior to the fastest RTs, we did not observe any genotype related-differences (SD 55 > 44 n.s. at p = 0.001 uncorrected level).

Discussion

This is the first study to investigate the cerebral correlates underlying performance during night-time, when the cognitive consequences of acute sleep loss are strongest. A major strength of our study is the combination of the SD with the multiple nap protocol, allowing for the investigation of critical circadian time windows under different sleep pressure conditions with the aim to level out their respective influences. Behavioral decrement under SD is most consistently observed in monotonous tasks of long duration and relatively unpredictable stimulus appearance[17]. By applying a simple paradigm (i.e., PVT) fulfilling these criteria, we detected a consistent pattern of deactivated cortical brain areas under high sleep pressure in the more vulnerable group (*PER3*$^{5/5}$*)* indicative for a relative loss of top-down control[28] during sustained attention performance. Contrariwise, the resilient group (*PER3*$^{4/4}$) showed increased activity, suggesting a higher ability to initiate "compensatory" recruitment of attention-related brain areas in response to SD. In the same perspective, the IFG, thought to participate in stimulus-driven reorienting of attention[29] increased BOLD activity with increasing sleepiness levels in the resilient *PER3*$^{4/4}$ group, while activity in the same region decreased with increasing sleepiness in the vulnerable group. We further observed that arousal-promoting areas were progressively less recruited with time-on-task in vulnerable subjects under SD, as well as less functionally connected to other arousal- and attention-related brain structures, potentially responsible for their greater state instability at the behavioral level. Finally, we showed that vulnerable subjects are more likely to "drift away", that is; activate areas of the default-mode networks before a slow response is given.

Independent of genotype, our data are compatible with previous reports collected under SD during daytime. Portas et al.[30] showed thalamic activity increases in a selective attention task after SD. Another study by Tomasi et al.[31] reported compensatory thalamic activity after SD during a visual tracking task. Accordingly, we observed that high sleep pressure levels during night-time are associated with increased activation in subcortical structures, whereas under low sleep pressure, several cortical regions were more active. However, many studies observed higher activations in fronto-parietal areas after SD when performance was preserved, and a decrease in activation when performance declines (reviewed in[8]). Although we observe significantly lower slowest RTs in SD and thus a performance decline, we did not observe such deactivations in fronto-parietal regions under high sleep pressure compared to low sleep pressure when pooling both genotype groups. This could be due to the fact that compared to other reports; our data was collected during night time, whereas previous studies reported data collected during the biological day. Alternatively; including two groups of subjects showing opponent patterns (as discussed below) might have evened out such an effect.

Taking into account the differential vulnerability based on the *PER3* genotype, we consistently detected deactivation in brain areas under high sleep pressure in the more vulnerable group (*PER3*$^{5/5}$), whereas the resilient group (*PER3*$^{4/4}$) showed increased activity in several brain regions. These findings stay in congruence with data from Vandewalle et al.[32], who investigated the impact of the *PER3* genotype during daytime after SD in a working memory task. Drummond et al.[21] , who investigated the neural basis of the PVT, interpreted brain activity increases associated with slow RTs after SD as a possible correlate of attentional recovery. This assumption is based on the finding that compensatory behavior (e.g., anticipatory responses or button presses without stimulus appearance) often follows slow RTs or lapses[6]. The *PER3*$^{4/4}$ thus might be more able to show supplemental recruitment of attention-related brain areas. Similarly, Chee and Tan[33] showed that vulnerable subjects (i.e., those performing less accurate based on a median split) are unable to increase fronto-parietal activation during a visual selective attention task when sleep deprived, whereas resilient subjects were able to do so. In another study, Chee et al. [28] differentiated brain activity during lapses (conceptually corresponding to the slow RT domain reported here) after SD with those under well-rested conditions. They showed that the ability to raise activation in response to lapses was lower under SD, and during lapses, visual sensory cortex activation as well as thalamic activation was reduced. Remarkably, what we observe in the *PER3*$^{5/5}$ carriers resembles this pattern, whereas *PER3*$^{4/4}$ carriers' activity corresponds more to what was observed under well-rested conditions. These authors interpret deactivations as a sign for loss of top-down attentional control[28]. We were able to relate this genotype-specific pattern to subjective sleepiness: the more resilient participants showed an increase in their activation in the left IFG, whereas the more vulnerable displayed decreased activity in this region with increasing sleepiness. Interestingly, the IFG has been associated with the alerting component [34] and stimulus-driven reorienting of attention[29].

Behaviorally, RTs did not differ between genotypes in the slowest, fastest and intermediate range. However, *PER3*$^{5/5}$ carriers did not only show more lapses (RTs > 500ms) in average, but also differ from *PER3*$^{4/4}$ with increasing time-on-task[12, 13]. Intriguingly, on the cerebral level, the genotypes also differ in their activity time course of slow events over the task. More precisely, *PER3*$^{5/5}$ carriers show a decrease in thalamic and ACC activity, supposedly mirroring a greater time-on-task-related vigilance decrement, whereas the *PER3*$^{4/4}$ carriers show relatively stable levels of activation within these regions. In the same line, Chee and Tan[33] showed that vulnerable subjects suppress thalamic activity during lapses (defined as long RTs) after SD, whereas the resistant ones show a trend towards elevation. Thus, the *PER3*$^{5/5}$ genotype might be less able to sustain attention by greater "mental effort" as mirrored on the cerebral level[30]. Notably, the thalamus has numerous projections to different cortical areas, but also receives input from ascending arousal systems, thereby mediating bottom up arousal[35]. We observed increased connectivity between a part of the reticular formation (paramedian part of the midbrain) and a more superior brainstem area, the thalamus, the left IFG and an occipital area in the resilient genotype. Importantly, activity in a brainstem region close to this seed region has previously been shown to negatively correlate with slow wave sleep[36], further indicating that this area is potentially implicated in sleep-wake-related arousal promotion. This strengthens the plausibility that the differences between vulnerable and resilient genotypes might be the consequence of a stronger ascending arousal promotion in *PER3*$^{4/4}$.

Considering the greater number of lapses during SD and the higher amount of slow eye movements and unintentional sleep episodes in the more vulnerable *PER3*$^{5/5}$ genotype, it is tempting to interpret the failure to recruit "compensatory" brain areas as a greater tendency to drift into an "off-mode" or

default mode during the absence of a stimulus. A compatible observation was made by Weissman et al. [22] who found areas of the DMN to be activated more prior to attentional lapses. Our data are in line with these findings: immediately before to the appearance of a target stimulus areas commonly assigned to the DMN (e.g., dorsal ACC, PCC, precuneus, medial frontal areas)[26, 27, 37] were more activated in the vulnerable *PER3*$^{5/5}$ carriers than in the resilient group under high sleep pressure. Interestingly, if taking into account the specific pattern during the slowest RT for each group separately, only *PER3*$^{5/5}$ carriers exclusively show deactivations under SD, all in areas compatible with the DMN[26, 27, 37]. In a broad view, this suggests that these participants are less able to sustain an active attentional network while they are waiting for stimulus appearance, and might switch into a more passive default mode[18], which in turn has to be more actively suppressed as soon as the stimulus appears. Such a mechanism might reflect a coping strategy of the more vulnerable participants and leads to similar RTs for both genotypes on the behavioral level. With the *PER3*$^{5/5}$ carriers having more RTs categorized as lapses, this coping process seems hence less successful or frailer to behavioral instability. This observation was specific to the slow RT range; which was also the domain that differed most between genotypes at the cerebral level. Hence, these assumptions account for non-optimal performance only. In every-day conditions, this can be particularly harmful, since response slowing down is typically observed under SD and can be fatal, especially when appearing as an insidious process, eventually resulting in total lapses.

Finally, under low sleep pressure, the comparison of the two genotypes did not reveal any difference in brain activity. This corresponds to what we found in our behavioral and electrophysiological data[12, 13], that is, genotypes do not differ substantially to adverse circadian phase in the absence of high homeostatic sleep pressure levels. In this line, it is important to notice that the genotypes have a roughly 10 % higher nap sleep efficiency over the entire circadian cycle[13], suggesting that between the naps, the homeostatic build-up is faster for the *PER3*$^{5/5}$ carriers, and that higher sleep efficiency might be necessary to reach equally low homeostatic levels than more resilient *PER3*$^{4/4}$. Homeostatic sleep pressure, operationalized here by a manipulation of the state (SD vs. NP) and presumably also by trait (*PER3*$^{4/4}$ vs. *PER3*$^{5/5}$)[16] seems thus to potentiate the harmful effects of adverse circadian phase at the cerebral level. Whether similar potentiating effects during maximal circadian wake promotion at the end of the biological day[38] can also be detected at the cerebral level remains to be addressed by future analyses.

The relatively small sample size in our study might present a limitation. Nevertheless, by selecting healthy participants without sleep complaints and controlling for several confounding variables (gender ratio, chronotype, habitual sleep duration, and sleep timing across groups), we chose a homogenous phenotype across both genotypes. By implementing stringently controlled conditions (e.g., control of light influence, body posture, social and nutritional timing cues), we were able to minimize potential masking factors.

To conclude, for the first time, we present data on the cerebral basis of vigilance during the SMZ, adding further to the literature. By comparing two groups that were extensively characterized with regard to their vulnerability with a multi-method approach (i.e., electrophysiology, subjective sleepiness, nap sleep efficiency, vigilance[12, 13]), we are able to infer patterns of resilience and vulnerability at the cerebral level. Our study further reveals that the good sensitivity to sleep loss observed for relatively monotonous tasks with low stimulus predictability might be due to a shift into the task-inactive DMN in between the phasic appearance of relevant stimuli. Given that some people

seem to have a greater predisposition to drift into these networks competing with goal-directed attention under SD, our findings might have implications to optimally adapt environment or instructions for night workers.

Online Methods

Thirty-three healthy volunteers participated in the study. One subject was excluded due to fMRI data loss, two subjects dropped out of the study, and two subjects were not included in this sample because of matching criteria (final N = 28, mean age ± SD: 24.9 ± 3.3 years). **Table 1** in the main text details the demographic data. Fourteen (seven males, seven females) were homozygous carriers of the short repeat allele (*PER3*$^{4/4}$), and 14 (five males, nine females) homozygous carriers of the long repeat allele (*PER3*$^{5/5}$). Besides the applied exclusion criteria listed below, the selection of this group was based on genotype and the ability to devote time to participation. Every volunteer completed questionnaires regarding his/her general and mental health, sleep habits and quality, and chronotype. General medical, current or past psychiatric and sleep disorders, and habitual sleep duration of less than seven or more than nine hours were all exclusion criteria. Further exclusion criteria encompassed smoking, medication (except oral contraceptives), or drug consumption, and pregnancy. We excluded shift workers and study applicants who had trans-meridian flights in the last three months before study participation to control for circadian phase misalignment. All participants underwent a medical interview and examination by a qualified physician to ensure aptitude to study participation. A screening night served to exclude sleep disorders and to habituate the participants to sleep in laboratory conditions with electrodes before study participation. Women without contraceptives (two out of 16) were scheduled during the luteal phase of their menstrual cycle for study participation. The groups did not significantly differ in terms of gender ratio, age, BMI, bed times during study weekends, and questionnaire scores (**Table 1**). The local ethics committee (Ethikkomission beider Basel, EKBB, Switzerland) approved the study, and all procedures conformed to the standards of the declaration of Helsinki. All participants provided their written informed consent to the participation.

Genotyping

DNA was extracted from saliva samples collected with the Oragene DNA sample collection kit using standard procedures (DNA Genotek Inc., Ontario, Canada). An allele-specific PCR was applied with 50 cycles at 60°C. Forward primer: 5'-TTA CAG GCA ACA ATG GCA GT-3', reverse primer: 5'-CCA CTA CCT GAT GCT GCT GA-3'. Agarose gel (2%) electrophoresis was used to identify the genotype of the individuals.

Procedure

The study procedure is illustrated in **Figure 1.** Behavioral and electrophysiological data of this design characterizing our sample according to genotype have been published elsewhere (see also below) [1, 2].

Each volunteer completed two study blocks (56h duration each) in the laboratory. Both blocks were preceded by a seven-days ambulatory part during which participants were asked to maintain an individually fixed regular sleep-wake cycle (8 h ± 30 min time in bed at self-selected times). Compliance was controlled with sleep logs and wrist actimetry (Actiwatch®, Cambridge

Neurotechnology Ltd., UK). Participants were asked to refrain from caffeine and alcohol, medication intake (except contraceptive pill), and daytime napping. Volunteers reported to the laboratory after each ambulatory part, and underwent the sleep deprivation (SD) and the nap protocol (NP) in a randomized, balanced, crossover order. We implemented stringently controlled conditions, that are, semi-recumbent posture position in bed during wakefulness, regularly scheduled light meals, dim light (< 8 lux) during scheduled wakefulness and zero lux during scheduled sleep episodes (i.e., naps), and no time-of-day indication. Both protocols started with a baseline night (8 h time in bed at the respective habitual bedtimes). In the SD, participants were awake for 40 h after habitual wake up time. In the NP, they underwent 10 alternating cycles of 160 min of scheduled wakefulness and 80 min of scheduled sleep (i.e., naps). Both blocks then ended with a recovery night (minimum 8 h time in bed at usual bedtimes). The combination of the two protocols allows investigating a continuous rise in homeostatic sleep pressure on one hand, whereas on the other hand, by multiple naps, sleep pressure remains at relatively low levels (illustrated in **Figure 1B**). Social interaction during the study was restricted to the experimental staff. Participants were allowed to get up to use the bathroom at scheduled times. They were allowed to read, play card or dice games with the experimental staff, and watch selected films during the scheduled wake episodes. Participants were continuously monitored by electroencephalography (EEG). Data on melatonin, psycho-physiological sleepiness, and nap sleep collected in this study have been published in [1, 2].

Here, we focus on the functional imaging data acquired during the night, that is, 21h after wake up (approx. 4 am for a 7 am wake up time). **Figure 1** illustrates the overlap of this time point with highest levels of physiological sleepiness (slow eye movements and unintentional sleep episodes) especially under high sleep pressure (**Figure 1A**) and peak levels of melatonin secretion during our study.

Behavior

Psychomotor Vigilance Task

We assessed sustained attention at ten time points during both the NP and SD protocol with the PVT [3] within a test session of approximately 30 min duration. After an unrelated working memory task, the PVT was the second test in each session and started at about 20 min into the test bout. Duration of the task was 10 min. Five of the 10 sessions took place in a functional magnetic resonance imaging (fMRI) scanner; here we report data collected during the session during the biological night (see Figure 1). The original design of the PVT [3] was modified to suit fMRI design and task admission within the MRI scanner. A fixation cross was presented on a black screen, at random intervals (2-10 sec), a millisecond counter started. Participants were instructed to press a button to stop the counter as fast as possible (clock event). Null events, where the clock event is replaced by the fixation cross, were included in the task (25 % of the trials) to add further jittering. Feedback of reaction time (RT) performance was displayed for one sec after the participants' response.

Subjective sleepiness

Participants indicated their subjective sleepiness levels on the Karolinska Sleepiness Scale (KSS[4]), from 1 (*extremely alert*) to 9 (*extremely sleepy, fighting sleep*). Ratings were given regularly (56 times during SD, 37 times during NP). We reported in detail in an earlier study[2] that genotypes differed in their KSS values during SD, but not NP. Here, we report an average value (average sampling time ± SD: 03:22 a.m. ± 10 min) of the three sampling points during SD (approximate times: 02:30; 03:30

and 04:00 am; times similar for genotypes, $p>0.05$) preceding the fMRI acquisition within the biological night. The values differed on the trend-level between the two groups ($t_{26} = -1.49$; $p_{\text{one-sided}} = 0.07$). The mean of the individually averaged values was used as a covariate to investigate the relation between BOLD activity and sleepiness during SD (see below) and for regression analysis.

Functional MRI data acquisition.

A 3 Tesla MR Scanner (MAGNETOM Verio, Siemens Healthcare) with a standard twelve-channel head coil was used to acquire functional MRI time series. Multislice T2*-weighted fMRI images were obtained with a gradient echo-planar sequence using axial slice orientation (32 slices; voxel size: 3 x 3 x 3 mm^3 with 0.75 mm interslice gap; matrix size 76 x 76 x 32; repetition time = 2200 ms; echo time = 32 ms; flip angle = 82°). Structural T1-weighted images were acquired for anatomical reference 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 = 256 x 256 x 176, voxel size = 1 x 1 x 1 mm^3). 176 contiguous axial slices covering the entire brain were assessed in sagittal direction.

Behavioral Data Analysis

Group analyses of the behavioural data (i.e., PVT performance and subjective sleepiness) were performed with the statistical package SAS (SAS Institute Inc., Cary, NC; version 9.3). We used one-sided t-tests where a priori hypotheses were stated, and mixed-model repeated measures ANOVA (PROC MIXED), and *p* values were based on Kenward-Roger's corrected degrees of freedom[5]. Contrasts were assessed with the LSMEANS statement. The factors *genotype* (*PER3*$^{5/5}$ vs. *PER3*$^{4/4}$) and *condition* (NP vs. SD) were used. Regression analyses were calculated with Statistica 9 (StatSoft Software).

FMRI Data analysis

General approach

Data from the NP and the SD condition were analyzed with SPM8 (http://www.fil.ion.ucl.ac.uk) implemented in MATLAB 12. Realignment of functional scans with iterative rigid body transformations minimized the residual sum of square between the first and subsequent images. The scans were normalized to the Montreal Neurological Institute (MNI) EPI template (third-degree spline interpolation; voxel size 2 x 2 x 2 mm^3) and spatially smoothed with a Gaussian kernel with full width at half maximum (FWHM) of 8 mm. We modeled the data in a two-step analysis to take into account the intra- and inter-individual variance, respectively. Brain responses were modeled for each subject at each voxel using a GLM. Our model included four regressors for each condition: events associated with RTs lower than the 25th percentile (fast RTs), events associated with RTs higher than the 75th percentile (slow RTs), events linked to the RT range between the 25th and 75th percentile (intermediate RT) as well as lapses (RTs >500 ms). Every event of each trial type was modeled as a function representing its onset. The ensuing vector was convolved with the canonical hemodynamic response function (HRF), and used as a regressor in the individual design matrix. For each trial type, we additionally added a time modulation regressor (first order polynomial) to account for time-on-task effects. Head motion parameters that were estimated during realignment (translations in x, y, and z directions and rotations around x, y, and z axes) and a constant vector were also included in the matrix as a variable of no interest. The main effect of task performance during the different test sessions was estimated with linear contrasts. We focused on the RT range encompassing the mean of the slowest 25 % of RTs. The slowest RT range is very sensitive to increased sleep pressure[6-8] and its

neural basis has been previously described[8]. Further, at the between-subject level, we included subjective sleepiness as a covariate to investigate the relation to cerebral activity.

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) or over small spherical volumes (radius 10 mm) around structures of interest at a priori locations derived from the literature (small volume correction, svc).

Functional connectivity analysis

To assess functional connectivity between regions of interest (ROI), we conducted a psychophysiological interaction (PPI) analysis[9, 10] implemented in SPM8. We defined a seed region-of-interest within a region in of the brainstem compatible with a part of the reticular formation (x =0, y = -30, z = -36) which we identified based on the GLM analysis described above. In this seed region, the first eigenvariate of the BOLD time-series was extracted from voxels within a 6mm radius sphere around the peak voxel derived from the contrast SD 44 > 55, slowest RT. We set the individual threshold at p = 0.9 to extract data from the same volume for each subject. One male subject (genotype: *PER3*$^{5/5}$) was excluded from this analysis because the individual activation mask did not cover this brain area (i.e., no data available). Thus, the *PER3*$^{5/5}$group had n = 13 in this analysis, N = 27.

BOLD activity before stimulus appearance

The PVT requires that participants sustain their attention in between stimulus appearance (in our case, i.e. as long as the cross is presented). We were interested in brain activity immediately before stimulus appearance. For this purpose, we further added finite-impulse-response (FIR,[11]) regressors for each RT range and its time modulation (fastest, slowest and intermediate RTs) to our initial model described above. Generally, with FIR analysis, the HRF is modeled without assumption of its shape by using successive boxcar functions (mini-boxcars or “sticks”). Here, in order to capture BOLD signal right before stimulus appearance, we added one single prestimulus-stick with a duration of 2.2 s (i.e., one TR). With this approach, it is assured that we are investigating pre-stimulus BOLD activity, as the HRF associated with the stimulus per se has a 4-6 sec lag.

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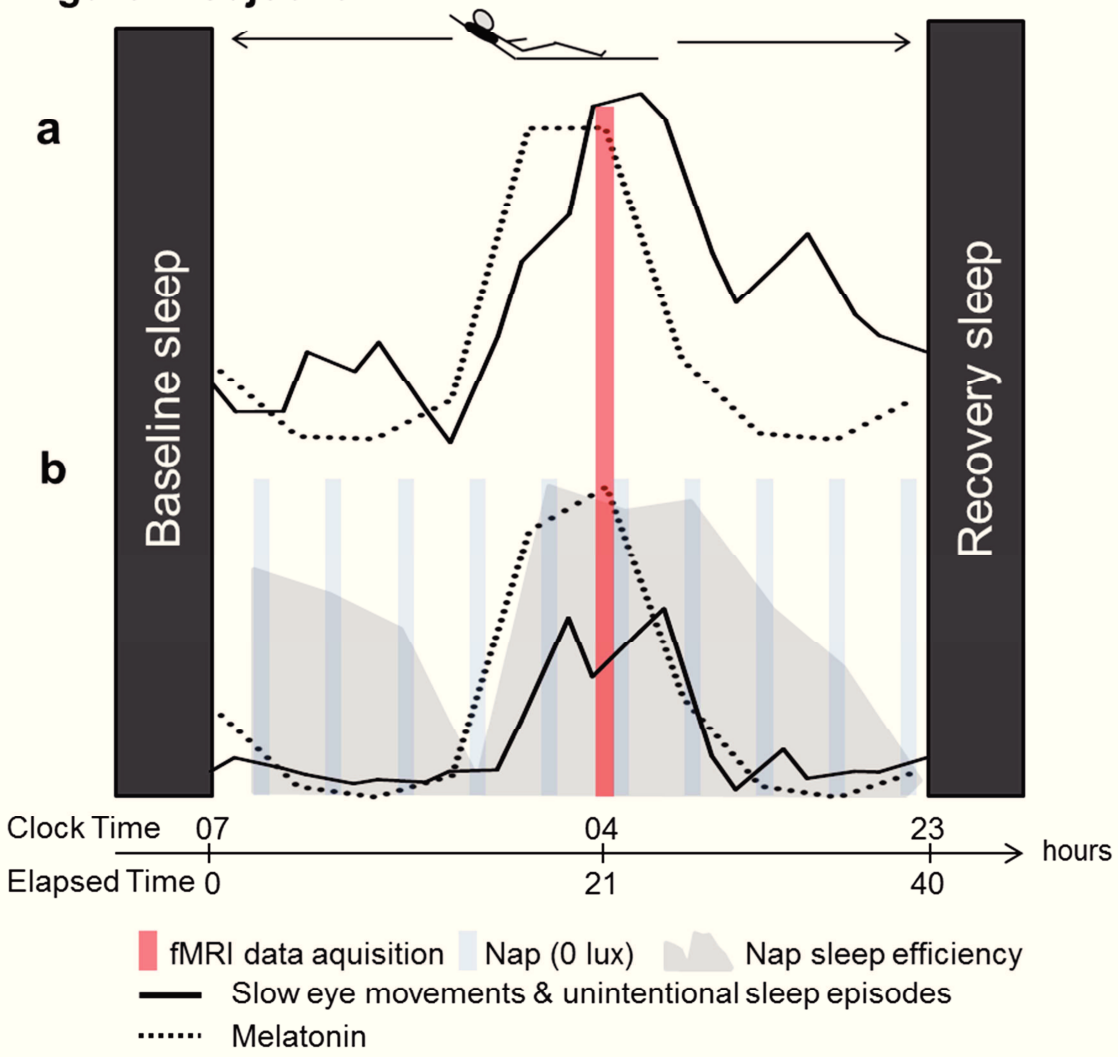
Figure 1. Schematic illustration of the laboratory part. (a) 40-h sleep deprivation. **(b)** 40-h multiple nap protocol (ten 80/160-min sleep/wake cycles). Clock time-indication is relative to a 7 a.m. wake time. In **(a)** and **(b)**, the solid line represents the time course of the averaged amount of slow eye movements and unintentional sleep episodes; the dashed line represents the time course of melatonin secretion; the filled grey area in **(b)** depicts the time course of the nap sleep efficiency (see Maire et al., 2014a for details on this data). **(c)** shows the schematic homeostatic build-up for each condition, SD = Sleep deprivation, NP = Nap protocol.

Figure 2. Brain activities SD > NP, *PER3*^{44} > *PER3*^{55} and relation to subjective sleepiness. (a-f) Left : Selected brain areas during slowest reaction times showing a significant condition x genotype interaction (see also Table 2 and S4). Depicted areas show higher activations under high sleep pressure (sleep deprivation, SD) compared to low sleep pressure (nap protocol, NP), depending on genotype. Right: Bar plots display parameter estimates for regions A-F by genotype and condition. The observed pattern in regions A-F is representative for brain areas listed in Table 2 and S4: the *PER3*^{55} group consistently shows a decrease in activation in SD vs. NP, whereas the *PER3*^{44} group shows the opposite pattern. Red bars: *PER3*^{55}, black bars: *PER3*^{44} carriers, dashed bars: NP, filled bars: SD. **(g)** Left panel: Regression analysis of the relation between estimated BOLD responses during slowest RTs in the left inferior frontal gyrus and the Karolinska Sleepiness Scale (KSS) values during the biological night preceding fMRI acquisition (R^2 = 0.56, p < 0.001, n = 28). Right panel: Display of the brain area showing a significant covariance pattern with subjective sleepiness depending on genotype: Left inferior frontal gyrus [peak voxel: -46, 38, 12]; $p_{corrected}$ < 0.000; Z = 3.8). **(a-f):** Activity overlay on population mean structural image.

Figure 3. Brain areas showing a significantly different time-on-task effect in the slowest RT domain between genotypes during sleep deprivation. (a) Overlay of statistical results on population mean structural image. **(b)** schematically displays the time course of thalamic brain activity [-8 -16 -4] over the task for a representative subject of each group.

Figure 4. Brain areas showing significantly more activation 2.2 sec (equals one TR) prior to stimulus appearance with a subsequent slow response in *PER3*^{55} than *PER3*^{44} during sleep deprivation.
Overlay of statistical results on population mean structural image. TR = Time of Repetition.

Figure-1 Cajochen



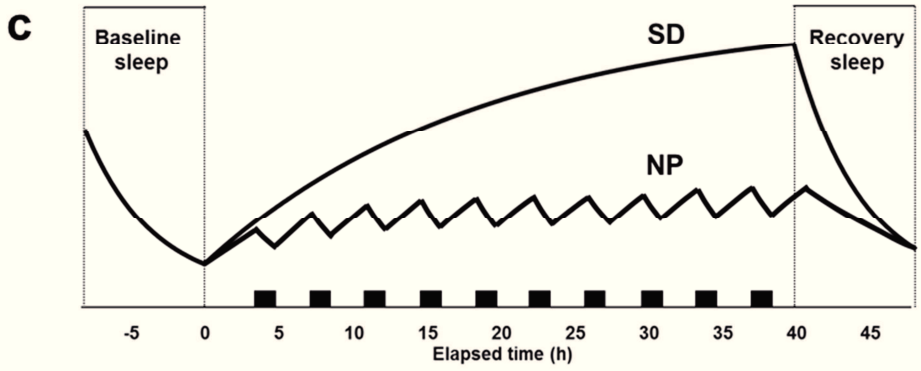
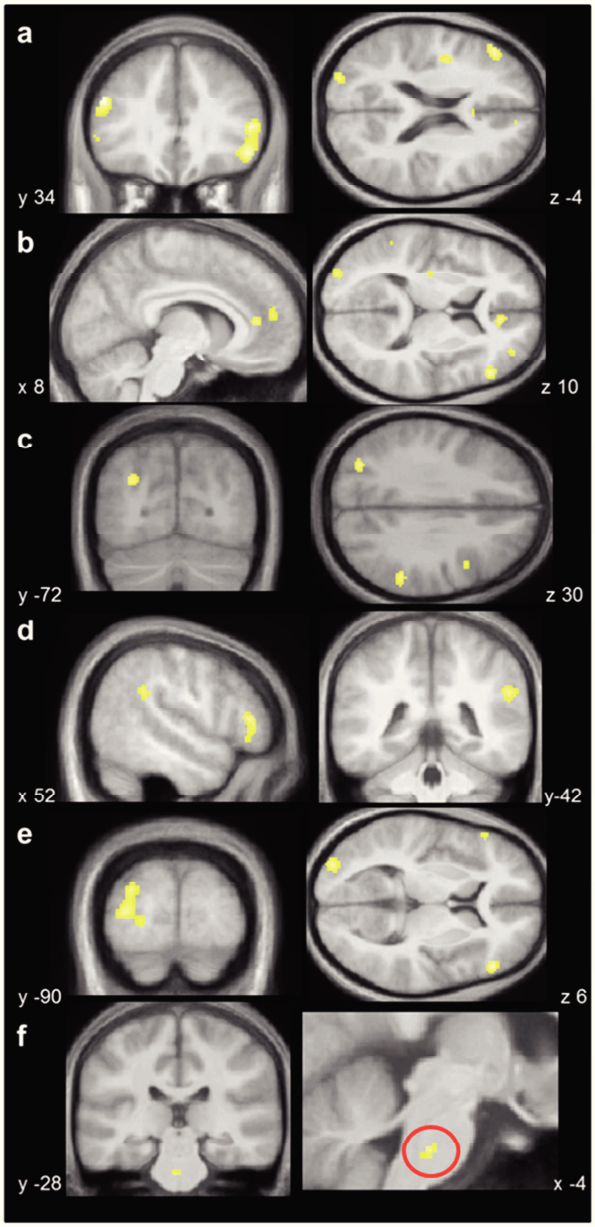
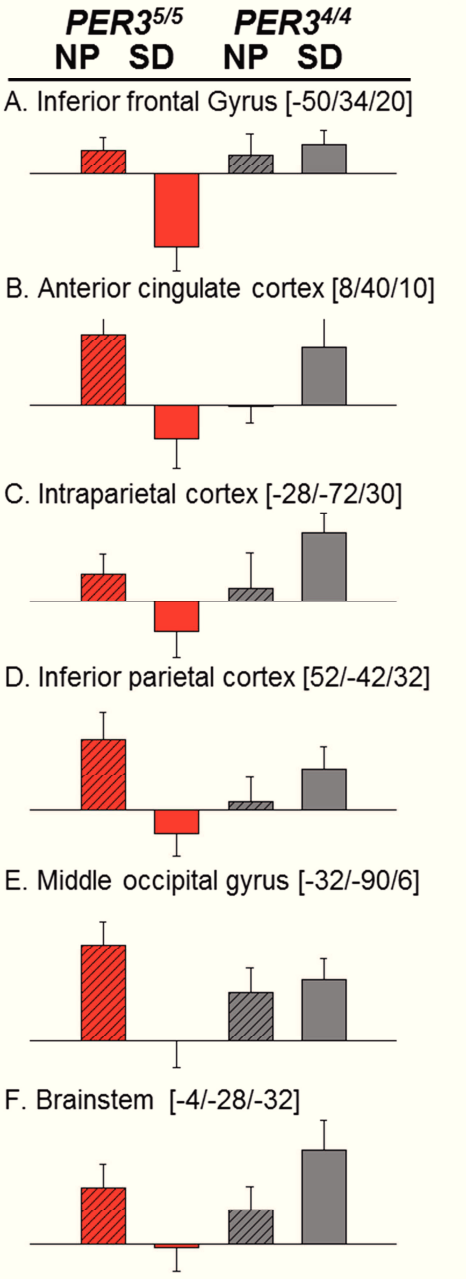
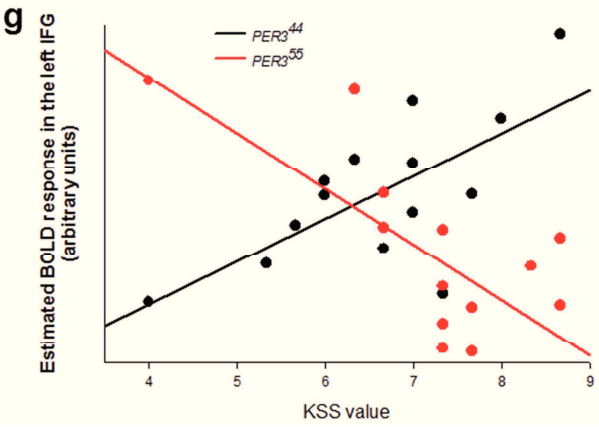


Figure-2 Cajochen







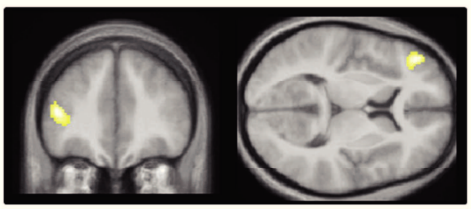


Figure-3 Cajochen

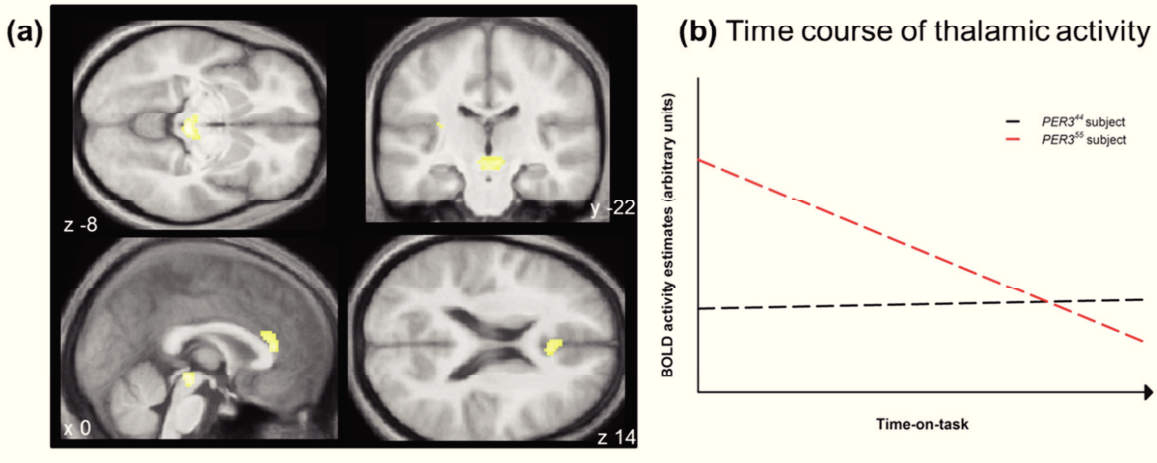


Figure-4 Cajochen

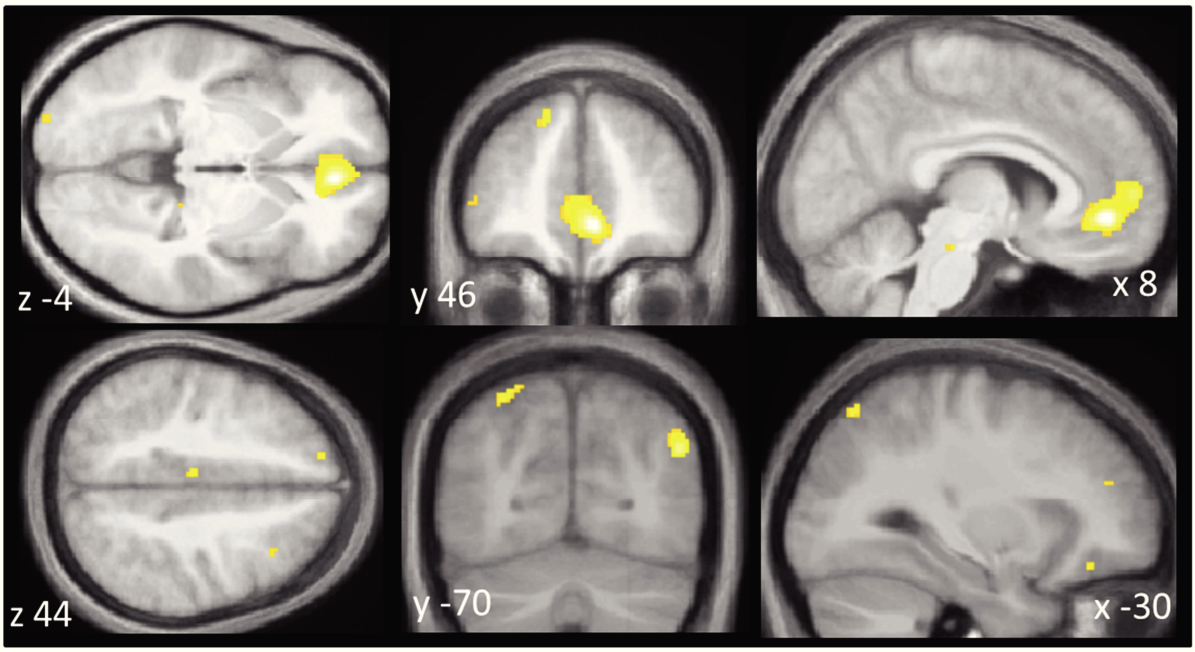


Table 1. Means (± SD) of demographic data and questionnaire scores by *PER3* genotype.

	PER3$^{4/4}$	*PER3*$^{5/5}$	*P*
N [m, f]	14 [7, 7]	14 [5, 9]	0.44
Years of age	24.3 (3.0)	25.6 (3.6)	0.31
BMI [kg/m^2]	21.9 (1.9)	22.7 (2.8)	0.38
Wake Time during study [clock time]	07:10 (61 min)	07:10 (44 min)	1.00
Sleep Time during study [clock time]	23:10 (61 min)	23:10 (44 min)	0.97
PSQI	3.3 (1.1)	3.0 (1.4)	0.54
ESS	3.6 (1.9)	4.3 (2.7)	0.47
MEQ	58.1 (9.5)	53.5 (10.2)	0.23
MCTQ Sleep duration [h]	7.8 (0.7)	7.9 (1.0)	0.69
MCTQ MSFsc	4.3 (1.0)	4.4 (1.3)	0.81
MCTQ MSFsac	7.5 (2.7)	7.2 (2.5)	0.54
BDI-II	2.6 (2.5)	1.3 (1.9)	0.13

P-values were derived from X^2- (gender) and *t*-tests (all other). PSQI = Pittsburgh Sleep Quality Index[39], ESS = Epworth Sleepiness Scale[40], MEQ = Morningness-Eveningness Questionnaire[41], MCTQ = Munich Chronotype Questionnaire[42], MSFsc= Mid sleep free days sleep corrected, MSFsac = Mid sleep free days sleep and age corrected, BDI = Becks Depression Inventory-II[43].

Supplementary Table S1. Results of mixed model ANOVA for different reaction time (RT) ranges in the Psychomotor Vigilance Task; F-values (df), and p-values. Significant results are printed in bold.

RT Range	Genotype	Condition	Condition x Genotype
Intermediate RTs	F (1,26) = 1.07, p = 0.3	F (1,26) = 12.2, p = 0.002	F (1,26) = 0.06, p = 0.8
25 % Slowest RTs	F (1,26) = 0.95, p = 0.3	F (1,26) = 4.9, p = 0.04	F (1,26) = 0.2, p = 0.7
25 % Fastest RTs	F (1,26) = 0.67, p = 0.4	F (1,26) = 11.2, p = 0.003	F (1,26) = 0.01, p = 0.9

Supplementary Table S2. General task-related activity during slowest reaction times

Brain area	Side	Z score	p_{FWE}	x	y	z
Task-related increases in activation (T +)						
frontal/cingulate/insula						
middle frontal gyrus	R	5.80	< 0.001	40	40	16
IFG (p. opercularis)	R	4.68	0.02	48	10	26
middle cingulate gyrus	R	4.55	0.04	8	28	32
Insula	R	5.22	< 0.01	30	18	-2
parietal/occipital						
angular gyrus	R	5.08	0.005	36	-58	44
supramarginal gyrus	R	4.51	0.045	52	-36	44
parahippocampal gyrus	L	4.56	0.04	-16	-30	-10
calcarine gyrus	R	6.27	< 0.0001	16	-90	0
	R	4.65	0.03	8	-72	10
	L	4.56	0.04	-10	-72	10
area striata (BA 17)	L	5.75	< 0.001	-10	-92	0
cerebellum/subcortical						
cerebellum	L	5.95	< 0.0001	-36	-70	-22
Pons/Brainstem	R	5.13	< 0.01	2	-32	-40
Task-related decreases in activation (T -)						
frontal						
superior frontal gyrus	L	4.83	0.01	-12	50	36
	L	5.60	<0.001	-16	20	60
	L	5.13	<0.01	-20	36	50
middle frontal gyrus	L	5.08	<0.01	-32	18	54
posterior cingulate/parietal						
posterior cingulate cortex	L	4.76	0.02	0	-52	22
precuneus	L	5.67	<0.001	-42	-72	34
	L	4.76	0.02	-50	-62	42

Coordinates (x, y, z) are expressed in mm in the Montreal Neurological Institute (MNI) space. P_{FWE}: p-value after family-wise-error correction over the entire volume. IFG = inferior frontal gyrus R = right, L = left, B = bilateral, BA = Brodman area.

Supplementary Table S3. Significant differences between brain activity under high vs. low sleep pressure.

Brain area	Side	Z score	p_{FWE}	x	y	z	Ref.
Brain areas showing higher activation under low sleep pressure (NP > SD)							
cuneus	L	3.49	0.0002	-6	-94	20	[31]
	L	3.32	0.0004	-8	-92	24	[31]
middle temporal gyrus	R	3.91	0.0000	64	-30	-4	[32]
temporal pole	L	3.44	0.0003	-38	6	-18	[44]
	L	3.28	0.0005	-42	20	-30	[44]
	R	3.67	0.0001	44	4	-16	[45]
insula	L	3.20	0.0007	-42	-18	8	[32]
	L	3.10	0.0010	-42	-6	4	[46]
	R	3.15	0.0008	40	-14	10	[46]
Brain areas showing higher activation under high sleep pressure (SD > NP)							
putamen	L	3.78	0.0001	-22	10	12	[46]
thalamus	B	3.55	0.0002	0	-10	6	[46]

Coordinates (x, y, z) are expressed in mm in the Montreal Neurological Institute (MNI) space. p_{svc}: p-value after correction for multiple comparisons over small volumes of interest taken from the literature. Ref. = references for coordinates, R = right, L = left, B = bilateral.

Supplementary Table S4. Significant differences between genotypes under high and low sleep pressure.

Brain area	Side	Z score	p_{FWE}	x	y	z	Ref.
Brain areas more active in *PER3*^{44} carriers during SD than NP compared to *PER3*^{55} carriers resp. more active during NP than SD in *PER3*^{55} compared to *PER3*^{44} carriers. (SD > NP 44 > 55 resp. NP > SD 55 > 44)							
frontal							

IFG	L	3.39	0.0004	-54	30	2	[47]
	L	3.53	0.0002	-50	34	20	[32]
	L	3.49	0.0002	-56	28	4	[32]
	R	3.42	0.0003	46	36	4	[32]
	R	3.24	0.0006	44	34	-4	[32]
IFG p. opercularis	R	3.31	0.0005	32	48	10	[32]
insula/IFG p. opercularis	L	3.57	0.0002	-44	-4	16	[46]
middle frontal gyrus	R	3.10	0.0010	36	24	22	[46]
	R	3.22	0.0006	44	10	30	[21]
cingulate/precentral/medial							
anterior cingulate cortex	R	3.52	0.0002	8	40	10	[32]
	R	3.19	0.0007	2	16	22	[46]
middle cingulate cortex	L/B	3.16	0.0008	-6	-18	44	[32]
precentral gyrus	L	3.15	0.0008	-34	2	48	[46]
superior medial gyrus	R	3.33	0.0004	10	52	14	[46]
	R	3.33	0.0004	10	52	14	[32]
parietal							
inferior parietal cortex/ intraparietal sulcus	L	3.13	0.0009	-28	-72	30	[20]
inferior parietal cortex	R	3.20	0.0007	52	-42	32	[21]
temporal/occipital							
superior temporal gyrus	L	3.19	0.0007	-54	-48	12	[32]
middle occipital gyrus	L	3.85	0.0001	-20	-84	-2	[32]
	L	3.53	0.0002	-32	-90	6	[32]
subcortical							
putamen	L	3.28	0.0005	-30	-16	8	[21]
brainstem/pons	L	3.12	0.0009	-4	-28	-32	[48]

Brain areas more active in *PER3*^{44} carriers during NP than SD compared to *PER3*^{55} carriers resp. more active during SD than NP in *PER3*^{55} compared to *PER3*^{44} carriers.
(SD > NP 55 > 44 resp. NP > SD 44 > 55)
n.s. at p = 0.001 uncorrected level

Table 2. Significant differences between genotypes under high and low sleep pressure.

Brain area	Side	Z score	p_{FWE}	x	y	z	Ref.
Higher brain activity in *PER3 [44]* than *PER3[55]* carriers during SD (SD 44 > 55)							
frontal/precentral							
IFG	R	3.45	0.0003	44	34	-4	[32]
	L	3.21	0.0007	-50	34	16	[32]
	L	4.07	0.0000	-48	32	26	[32]
IFG (p.opercularis)	L	3.21	0.0007	-50	28	4	[32]
	L	3.19	0.0007	-44	34	-6	[32]
	R	3.99	0.0000	40	10	28	[32]
IFG (p.triangularis)	L	3.45	0.0003	-42	18	20	[32]
middle frontal gyrus	R	3.53	0.0002	34	30	20	[21]
	R	3.09	0.0010	38	36	18	[21]
superior frontal gyrus	L	3.24	0.0006	-14	50	32	[21]
	R	3.39	0.0004	14	52	26	[32]
superior medial frontal gyrus	R/B	3.52	0.0002	2	48	28	[32]
	B	3.66	0.0001	0	48	28	[21]
dorsolateral prefrontal gyrus/precentral gyrus	L	3.80	0.0001	-52	6	32	[49]
precentral gyrus	L	3.30	0.0005	-42	-2	40	[50]
	L	3.35	0.0004	-36	-4	60	[50]
insula/temporal							
insula	L	3.30	0.0005	-34	22	-2	[51]
middle temporal gyrus	L	3.18	0.0007	-46	-60	10	[50]
	L	3.15	0.0008	-54	-22	-12	[32]
superior/middle temporal gyrus	L	3.21	0.0007	-64	-24	-4	[32]
parietal							
temporo-parietal junction	R	3.16	0.0008	52	-72	10	[50]
superior parietal/precuneus	R	3.34	0.0004	34	-70	38	[52]
subcortical							
brainstem	B	3.20	0.0007	0	-30	-36	[48]
Higher brain activity in *PER3[55]* than *PER3 [44]* carriers during SD (SD 55 > 44)							
n.s. at p = 0.001 uncorrected level							
Higher brain activity in *PER3 [44]* than *PER3[55]* carriers during NP (NP 44 > 55)							
n.s. at p = 0.001 uncorrected level							
Higher brain activity in *PER3[55]* than *PER3 [44]* carriers during NP (NP 55 > 44)							
n.s. at p = 0.001 uncorrected level							

Higher brain activity in *PER3*^{44} carriers during SD vs NP (44 SD > NP)							
frontal/cingulate							
orbital frontal gyrus	R	3.50	0.0002	32	40	-6	46
middle cingulate cortex	L	3.22	0.0007	-12	24	34	52
subcortical/cerebellum							
thalamus	L	3.27	0.0005	-4	-10	6	46
nucleus caudatus	R	3.25	0.0006	12	12	0	46
Putamen	L	3.21	0.0007	-22	8	10	32
cerebellum	L	3.13	0.0009	-26	-64	-32	52
Higher brain activity in *PER3*^{44} carriers during NP vs SD (44 NP > SD)							
n.s. at p = 0.001 uncorrected level							
Higher brain activity in *PER3*^{55} carriers during SD vs NP (55 SD > NP)							
n.s. at p = 0.001 uncorrected level							
Higher brain activity in *PER3*^{55} carriers during NP vs SD (55 NP > SD)							
frontal							
IFG	L	4.10	0.0000	-50	36	20	32
	L	3.61	0.0002	-56	28	4	32
	L	4.05	0.0000	-50	32	22	32
	L	4.04	0.0000	-50	30	26	32
	L	3.66	0.0001	-44	34	22	32
IFG (p. triangularis)	L	4.13	0.0000	-46	38	22	46
	L	4.05	0.0000	-40	40	18	46
middle cingulate cortex	R	3.40	0.0003	6	-22	36	32
precentral gyrus	R	3.29	0.0005	50	-14	48	46
insula/temporal							
insula	R	3.33	0.0004	40	0	4	46
	L	3.64	0.0001	-40	-18	10	32
	L	3.56	0.0002	-42	-2	10	32
superior temporal gyrus	L	3.14	0.0008	-50	-30	20	52
	L	3.26	0.0006	-60	-28	4	32
temporal pole	R	3.34	0.0004	48	4	-18	32
medial temporal pole	R	3.27	0.0005	54	10	-28	32
middle temporal	R	4.24	0.0000	62	-28	-6	32
parietal							

inferior parietal lobe	L	3.17	0.0008	-60	-20	44	[32]
supramarginal gyrus	R	3.90	0.0000	54	-40	26	[46]
occipital							
middle occipital/lingual gyrus	L	3.67	0.0001	-20	-82	-2	[32]
inferior occipital /lingual gyrus	L	3.52	0.0002	-20	-84	0	[46]
lingual gyrus	R	3.46	0.0003	18	-54	-4	[46]
inferior/middle occipital gyrus	L	4.30	0.0000	-32	-92	6	[46]
middle occipital gyrus	L	3.46	0.0003	-28	-88	2	[32]
middle/superior occipital gyrus	L	4.20	0.0000	-36	-92	4	[32]
early visual cortex	R	3.49	0.0002	2	-86	4	[47]
	L	3.41	0.0003	-16	-82	-10	[47]
subcortical							
brainstem	L	3.39	0.0004	-4	-32	-36	[48]

Coordinates (x, y, z) are expressed in mm in the Montreal Neurological Institute (MNI) space. p_{svc}: *p*-value after correction for multiple comparisons over small volumes of interest taken from the literature. SD = sleep deprivation, NP = nap protocol, Ref. = references for coordinates, IFG = inferior frontal gyrus, R = right, L = left, B = bilateral.

Supplementary Table S5. Significant differences in functional connectivity with a brainstem area during SD between genotypes

Brain area	Side	Z score	p_{FWE}	x	y	z	Ref.
Brain areas more connected to the seed region in *PER3*^{44} compared to *PER3*^{55} carriers (SD 44 > 55)							
IFG	L	3.12	0.0009	-44	28	-4	[32]
inferior occipital lobule (BA 18)	R	3.27	0.0005	18	-84	-16	[28]
thalamus	R	3.70	0.0001	26	-30	-4	[32]
	R	3.40	0.0003	20	-18	-10	[32]
	R	3.23	0.0006	18	-14	-10	[32]
	B	3.97	<0.0001	0	-8	2	[46]
thalamus/pineal gland	B	3.84	0.0001	-6	-32	2	[21]
	B	3.50	0.0002	2	-32	6	[21]
brainstem	L	3.28	0.0005	-10	-22	-16	[46]
Brain areas more connected to the seed region in *PER3*^{55} compared to *PER3*^{44} carriers (SD 55 > 44) n.s. at p = 0.001 uncorrected level							

Coordinates (x, y, z) are expressed in mm in the Montreal Neurological Institute (MNI) space. p_{svc}: *p*-value after correction for multiple comparisons over small volumes of interest taken from the literature. SD = sleep deprivation, Ref. = references for coordinates, IFG = inferior frontal gyrus, R = right, L = left, B = bilateral.

Supplementary Table S6. Brain activity modulated by time-on-task during sleep deprivation

Brain area	Side	Z score	p_{FWE}	x	y	z	Ref.
44 > 55							
anterior cingulate cortex	B	3.39	0.0004	0	32	14	[21]
	R	3.23	0.0006	4	26	22	[21]
thalamus	R	3.41	0.0003	8	-22	-8	[50]
	R	3.29	0.0005	10	-18	-6	[50]
	L	3.32	0.0004	-8	-16	-4	[46]
	L	3.29	0.0005	-6	-20	-6	[46]
	L	3.33	0.0004	-30	-26	14	[32]
55 > 44							
n.s. at p = 0.001 uncorrected level							

Coordinates (x, y, z) are expressed in mm in the Montreal Neurological Institute (MNI) space. p_{svc}: *p*-value after correction for multiple comparisons over small volumes of interest taken from the literature. Ref. = references for coordinates, R = right, L = left, B = bilateral.

Table 3. Differences in brain activity prior to slowest RTs during sleep deprivation according to genotype

Brain area	Side	Z score	p_{FWE}	x	y	z	Ref.
Areas with greater activity one TR (2.2s) before stimulus appearance in *PER3*^{55} than *PER3*^{44} carriers (SD 55 > 44)							
superior/middle frontal gyrus	R	3.40	0.0003	32	22	46	[22]
superior occipital gyrus	L	3.18	0.0007	-38	-78	28	[22]
medial frontal gyrus	R	4.20	0.0000	8	46	-4	[53]
	R	3.48	0.0002	2	54	10	[27]
	L	3.48	0.0002	-2	56	10	[27]
	L	3.48	0.0003	-6	58	14	[27]
	L	3.33	0.0004	-2	50	8	[27]
dorsal ACC	R	4.26	0.0000	6	40	-6	[27]
dorsal ACC	R	3.93	0.0000	2	42	-8	[27]
posterior /middle cingulate	L	3.39	0.0004	-6	-20	44	[54]
superior parietal gyrus/	R	3.93	0.0000	46	-66	32	[53]
gyrus angularis/prencuneus	L	3.56	0.0002	-28	-70	56	[32]
	L	3.29	0.0005	-24	-68	60	[32]
	L	3.25	0.0006	-20	-66	60	[32]
medial temporal gyrus	L	3.70	0.0001	-62	-14	-22	[37]
	R	3.85	0.0001	56	-6	-16	[37]
pontine reticular formation	R	3.41	0.0003	8	-24	-20	[55]
Areas with greater activity one TR (2.2s) before stimulus appearance in *PER3*^{44} than *PER3*^{55} carriers (SD 55 > 44)							
n.s. at p = 0.001 uncorrected level							

Coordinates (x, y, z) are expressed in mm in the Montreal Neurological Institute (MNI) space. p_{svc}: *p*-value after correction for multiple comparisons over small volumes of interest taken from the literature. SD = sleep deprivation, Ref. = references for coordinates, R = right, L = left, B = bilateral; ACC = Anterior cingulate cortex.

5. Discussion

With the three studies presented in this thesis, we aimed to investigate sleep-loss related decrements in sleepiness, sustained attention, and the underlying cerebral correlates by taking genetic vulnerability to sleep loss into account, which was based on a VNTR polymorphism in the clock gene *PER3*. We combined a 40h SD protocol with a multiple nap protocol using a multi-method, within-subject approach.

In the first publication, we showed that variations in the modulation of subjective and electrophysiological sleepiness, lapses in attention, and nap sleep over the 24 hour cycle are modulated by the *PER3* polymorphism mainly due to differences in the sleep homeostatic process, which stronger affects $PER3^{5/5}$ than $PER3^{4/4}$ carriers (Maire, Reichert, Gabel, Viola, Strobel, et al., 2014), corroborating previous reports (Dijk & Archer, 2010; Viola et al., 2007). Intriguingly, nap sleep efficiency (i.e., the amount of sleep per 80-min nap opportunity) throughout the 24 hour cycle was associated with attentional vulnerability to total SD in a genotype-dependent manner.

Our second publication sheds light on the influence of vulnerability to sleep loss and sleep homeostatic mechanisms on "state instability" in sustained attention. We observed more intermittent lapses and an increasing variability in performance along the task (PVT) were observed in the vulnerable $PER3^{5/5}$ carriers under sleep pressure conditions (Maire, Reichert, Gabel, Viola, Krebs, et al., 2014). Low sleep pressure mitigated the time-on-task effect in general as well as genotype-related differences. Importantly, optimal performance levels (fast RTs) were still achieved by both vulnerable and more resilient volunteers.

In our third publication, we show that during night time, when it is most difficult to maintain adequate attentional levels, vulnerability is mirrored at the cerebral level under SD (Maire et al., submitted). Focusing on non-optimal performance (slowest RT range), we observed a consistent deactivation pattern in several task-related cortical and subcortical areas in the vulnerable $PER3^{5/5}$ carriers after SD compared to NP, whereas $PER3^{4/4}$ carriers showed increases in brain activations. These patterns co-varied with subjective sleepiness in an inferior frontal region. Furthermore, arousal-promoting structures were functionally less interconnected, and also less connected to task-related inferior frontal and occipital brain areas in the vulnerable group. Additionally, a time-on-task related decrease became evident in thalamic and anterior cingulate regions for $PER3^{5/5}$ carriers, whereas BOLD activity in $PER3^{4/4}$ carriers remained stable throughout the task. Finally, we detected that prior to stimulus appearance; $PER3^{5/5}$ carriers were more likely to shift into a task-inactive default-mode network. Behaviorally, genotypes did not differ in their mean RTs per se, but showed more failures to respond in time (lapses).

Together, our results show that more vulnerable participants experience greater sleep propensity, might have a lower arousal promotion and a different, potentially less efficient cerebral coping mechanism to counteract sleep loss-related attentional decrement. Below, we will integrate these findings from different perspectives.

Differences in sleep homeostatic build-up: Physiological and subjective correlates, and relation to performance decrement

Regarding mechanisms underlying sleep-wake regulation, we reappraise previous reports proposing a higher build-up of sleep pressure in *PER3*$^{5/5}$ carriers compared to *PER3*$^{4/4}$ carriers, but similar circadian profiles in both genotypes (Dijk & Archer, 2009, 2010; Viola et al., 2007). Considering the stronger physiological sleepiness mirrored in more SEMs and unintentional sleep under SD, a greater sleep propensity and/or weaker arousal promotion is suggested, specifically during the night when circadian sleep promotion is greatest. This time-of-day dependent accentuation points towards a genotype-dependent modulation of the interaction between sleep homeostasis and the circadian process, as postulated in the conceptual model of Dijk and Archer (2010), see also Figure 7: *PER3* impinges on the circadian output modulated by the sleep homeostat. According to the model, the two genotypes differ in their time constants of the build-up and the dissipation of sleep pressure, which then affects the interaction with the circadian process, although the latter appears to be similar in *PER3*$^{5/5}$ and *PER3*$^{4/4}$ allele carriers. Findings from Hasan and colleagues (Hasan et al., 2014) corroborate this concept: Homer1a, a molecular correlate of sleep loss (Maret et al., 2007), was differentially expressed in the cortex and hypothalamus of mice carrying the human *PER3*$^{5/5}$ genotype, suggesting that the VNTR polymorphism impacts on the neurochemical cascade in response to sleep loss. Consequently, the *PER3*$^{5/5}$ genotype feels sleepier, exhibits stronger physiological signs for sleepiness, and experiences greater attentional decrement, all mirrored in our data. Interestingly, the homeostatic build-up seems to be greater already within the 160 min wakefulness between the sleep opportunities in the nap protocol, as overall nap sleep efficiency was roughly 10 % higher in the *PER3*$^{5/5}$ carriers. Although no continuous full night of sleep was provided during the 40 h of the nap protocol, naps were sufficient to attenuate vulnerability-related differences – yet with different sleep efficiencies. We also found an intriguing link between the overall higher ability to sleep throughout the naps to greater impairment of attentional performance, which in turn was dependent on the *PER3* VNTR polymorphism. This supports a link between the sleep homeostatic build-up and neurobehavioral vulnerability to sleep loss which seems to be trait-like (i.e., influenced by the *PER3* VNTR polymorphism). Notably, subjective measures of sleepiness indicated that participants were aware of their greater sleep propensity. This suggests that the subjective estimation for sleep drive in both genotypes is appropriate. As an important potential implication, this awareness might lead more vulnerable persons to take appropriate

countermeasures earlier when sleep loss is experienced in everyday life, such as taking naps or drinking coffee.

Altered arousal promotion? Cerebral correlates, wake-state instability and neurochemical processes

Besides greater homeostatic sleep drive as a unidirectional force, altered cerebral arousal promotion depending on the *PER3* genotype might also account for the observed differences, likely in interaction with the sleep homeostat. The greater impact of SD in the vulnerable group on sustained attention - which is to a large extent supported by arousal-promoting areas (Sarter et al., 2001) - speaks in favor of this assumption. Indeed, we showed a task-related decrease in thalamic and brainstem regions, both part of the ascending arousal system (Jones, 2003), in *PER3*$^{5/5}$ carriers under SD. Studies investigating SD reported that activity in the thalamus declines when performance deteriorates (e.g., Chee et al., 2008; Portas et al., 1998), or to increase when performance levels are maintained (e.g., Muto et al., 2012; Tomasi et al., 2008). Recently, a decrease in thalamic grey matter was observed consequent to SD and correlated with performance decrement (Liu, Kong, Liu, Zhou, & Wu, 2014). Thalamo-cortical circuits were likewise shown to be less functionally connected during resting state after SD (Shao et al., 2013). In line with this, we found that the brainstem and thalamus were less functionally connected to each other and to frontal cortical regions in the vulnerable genotype during task performance (Maire et al., submitted). Thalamic activity moreover decreased with time-on-task only in the *PER3*$^{5/5}$ carriers, pointing towards a weaker arousal promotion along the task (Portas et al., 1998). Favoring this assumption, at the behavioral level we observed more attentional failures mainly towards the end of the task over all 10 test sessions, along with increased performance variability (higher standard deviations of mean RTs) in this group (Maire, Reichert, Gabel, Viola, Krebs, et al., 2014). During the night session (at approx. 4 a.m.), visual inspection of the mean RTs (including RTs > 500 ms classified as lapses) per genotype clearly revealed a steeper increase for the vulnerable participants throughout the 10 minutes of the task (Figure S1). Time-on-task related decrements are thought to reflect wake-state instability, which likely results from sleep initiating mechanisms interfering with wakefulness (Doran et al., 2001), or as suggested here, from a failure of arousal-promoting systems. Together, this presents a possible mechanism causing the higher attentional vulnerability to sleep loss in *PER3*$^{5/5}$ carriers indicated by our data. Alternatively, local use-dependent sleep associated with sleep homeostasis (Huber et al., 2004; Kattler et al., 1994) could also play a role, presumably shutting down areas selectively involved in top-down or bottom-up attentional processes (Chee & Tan, 2010; Van Dongen et al., 2011). According to the synaptic-downscaling hypothesis (Tononi & Cirelli, 2003), this would raise the question if more vulnerable participants experience higher synaptic potentiation during wakefulness and also with time-on-task

(Van Dongen et al., 2011). Future studies could investigate this by simultaneously assessing fMRI and high-density EEG or using transcranial magnetic stimulation approaches.

The above mentioned finding of Hasan et al. (Hasan et al., 2014) indicate a genotype-dependent regulation of Homer1a, a gene previously related to sleep-loss (Maret et al., 2007), and its transcripts. To speculate, neurochemicals involved in arousal promotion could also be differentially regulated according to genotype. For instance, ascending excitatory cholinergic neurotransmission might play a role, contributing to less wake-promotion (Jones, 2003). The decreased activity and lower thalamo-cortical connectivity of the brainstem region encompassing a part of the reticular formation in our data might parallel altered neurotransmission in *PER3*$^{5/5}$ carriers. Considering that the brainstem reticular formation contains numerous cholinergic neurons (Jones, 2003), and cholinergic neurotransmission plays an important role in modulating sustained attention (Himmelheber, Sarter, et al., 2000; Sarter et al., 2001), this assumption seems not too far-fetched. Additionally, the interplay of cholinergic with noradrenergic projections (Sarter et al., 2001) might play a role. It was suggested that noradrenergic neurons do not directly mediate sustained attention, but are implicated in generating a certain arousal level which is a prerequisite for sustained attention (reviewed in Sarter et al., 2001), also by excitation of cholinergic cells in the forebrain (Jones, 2008), and through the thalamus (Coull, Frith, Dolan, Frackowiak, & Grasby, 1997). Alternatively, orexin/hypocretin implicated in the flip-flop mechanism of sleep-wake-regulation could be differentially regulated according to genotype. The “finger” on the switch upholding wakefulness (Saper et al., 2001) might be weaker in *PER3*$^{5/5}$ carriers, leading to easier transitions to sleep especially when sleep pressure is high. The increased sleep efficiency in the naps in fact points to a heightened “sleep ability”, even at circadian phases where circadian wake promotion is maximal (Maire, Reichert, Gabel, Viola, Strobel, et al., 2014). Using pharmacological interventions such as by administering wake-promoting drugs such as Modafinil or a cholinesterase inhibitor (such as Chuah & Chee, 2008; Chuah et al., 2009), or sleep-promoting drugs such as the new orexin antagonists (Michelson et al., 2014) in both genotypes could generate new insights regarding neurochemical processes.

Failure to sustain attention: Cerebral and behavioral aspects and the default-mode network

Lapses in attention might be a consequence of transient failures of top-down regulation and control processes, or impaired bottom-up processing (Chee & Tan, 2010; Van Dongen et al., 2011). They do also occur in well-rested states, but sleep loss accentuates these failures in function of individual vulnerability (Chee & van Dongen, 2013). Investigating the neural basis of lapses, Weissman et al. (Weissman, Roberts, Visscher, & Woldorff, 2006) found increased activation prior to lapses in a circumscribed task-inactive network, the default-mode network (DMN; Raichle et al., 2001). This is a subset of brain regions active during resting state, and deactivated during task engagement (Buckner,

Andrews-Hanna, & Schacter, 2008; Fransson & Marrelec, 2008; Guldenmund, Vanhaudenhuyse, Boly, Laureys, & Soddu, 2012; Laird et al., 2009; Raichle et al., 2001). It was associated with mind-wandering, introspection, episodic memory retrieval, and general stimulus-independent thoughts (Guldenmund et al., 2012; Gusnard, Akbudak, Shulman, & Raichle, 2001; Sonuga-Barke & Castellanos, 2007). Likewise, its deactivation during task engagement was related to task difficulty (more deactivated with increasing challenge) (Gould, Brown, Owen, Bullmore, & Howard, 2006). This is particularly interesting, as our more vulnerable participants showed a greater deactivation of DMN regions during slowest RTs, indicating that they were challenged more at this point than the resilient ones. Alternatively, the deactivation could result from the mere fact that between stimuli, when attention had to be sustained tonically, the *PER3*$^{5/5}$ carriers drifted more into task-inactive networks and subsequently had to deactivate these regions to a greater extent when reacting. In fact, we were able to confirm this assumption by investigating brain activity prior to stimulus appearance; *PER3*$^{5/5}$ carriers had greater activation in regions of the DMN than *PER3*$^{4/4}$ carriers, exclusively before slowest RTs.

In our fMRI data, we did not analyze[3] the lapses in attention (events with RTs >500ms) and thus cannot conclude on brain activity during these. Nonetheless, on the behavioral level, lapses were more frequent in vulnerable participants under SD. Focusing on the interplay between default-mode network activity and failures in sustained attention, Sonuga-Barke and Castellanos (2007) have formulated an interesting hypothesis. They suggested that spontaneous very low frequency activity (<0.1 Hz), associated with the DMN, might interfere with task-related active processing, especially under suboptimal conditions (such as increased fatigue). Together with the finding that the more vulnerable *PER3*$^{5/5}$ carriers showed higher activity in regions assigned to the DMN when no stimulus was present (i.e., in between phasic and irregular appearance of stimuli) compared to *PER3*$^{4/4}$ carriers, several speculations are possible. Keeping in mind that this latter finding was specific for slow answers, but not observed for optimal (fast) RTs, some neural processes underlying sustained attention, or its failure respectively, might emerge along a continuum, and more so in vulnerable participants. Notably, before and during optimal performance, no default-mode activity was observed for either genotype. As one possible scenario, in vulnerable participants such default mode-activity at one point prevails between the stimuli. This taxes, but does not abolish, the readiness to react timely, and is counteracted with the effortful strategy to deactivate these DMN regions, resulting in a relatively slow answer. At some point, this strategy might not suffice anymore, resulting in response failures, likely also accompanied by micro-sleep (Poudel, Innes, Bones, Watts, & Jones,

[3] We did model the lapses in our fMRI analysis, but did not analyze them statistically due to two main reasons. First, conceptualized as a discrete variable (i.e., counting the no. of RTs > 500 ms), lapses do not necessarily appear in each test session or for each participant. For analysis, a certain number of events is however necessary. Second, we do not know whether a participant is awake or fell asleep during a lapse, and thus would not be able to disentangle sleep- from of task-related activity.

2014) or local sleep (Huber et al., 2004). Presumably, the more resilient participants are generally more able to recruit supplemental forces to compensate after a slow response, preventing them better from drifting into the lapse-domain. A finding by Chee and Tan (2010) supports this, showing that resilient participants are more able to increase activation in response to a lapse after SD. Interestingly, shown in our data, subjective sleepiness seems to co-vary with these opposite patterns, as *PER3*$^{5/5}$ volunteers decreased activation in a task-related inferior frontal area, whereas *PER3*$^{4/4}$ carriers increased. Earlier, Chee et al. (2008) differentiated brain activity during lapses (slow RTs) after SD with those under well-rested conditions. They showed that the ability to raise activation in response to lapses was lower under SD, and during lapses, visual sensory cortex and thalamic activation was reduced. Together, it seems that the cerebral pattern underlying the slow responses in our resilient volunteers is similar to the pattern Chee et al. (2008) observed under well-rested conditions, and the pattern discovered in the vulnerable group resembles the one under SD. Opposed to other studies, where vulnerability was derived from the amount of performance decrement (e.g., by a median split; Chee & Tan, 2010; Chuah, Venkatraman, Dinges, & Chee, 2006; Mu et al., 2005), we did not find differences for mean RTs in the slowest range between genotypes. With our approach, confounds with performance levels per se can be ruled out (similar to Vandewalle et al., 2009). Clearly, the neural basis of sustained attention is modulated by genetic vulnerability. Metaphorically - at the cerebral level, together with the different amount of unintentional sleep, the resilient participants fight, whereas the vulnerable ones take flight.

Limitations, implications and outlook

Our approach bears several limitations. Using a candidate gene approach, we are not able to exclude the impact of other genes on the observed group differences. Especially because the phenotype of sleep-wake regulation is complex, it is certainly not determined by one single gene. However, we selected this polymorphism based on several previous reports which pointed out its implication in vulnerability to sleep loss. We were able to confirm that the two groups reacted differentially to sleep loss, consistently mirrored in subjective and objective variables, and characterized them extensively according to their sleep-wake behavior. The exact implication of the *PER3* polymorphism itself remains speculative, yet it seems legitimate to draw general conclusions about individual vulnerability patterns and their cerebral correlates. More insight about specific molecular processes is certainly warranted.

The potential lack of statistical power due to the small sample size might have obscured actually present differences or led to the accentuation of insignificant differences. However, by controlling several factors impinging on sleep-wake regulation, we tried to minimize error sources. A further limitation rendering our results less translatable to the general population is the exclusion of the heterozygous genotype resulting from the VNTR polymorphism (*PER3*$^{4/5}$), which is present in

approximately 50 % of the population (Lazar et al., 2012; Maire, Reichert, Gabel, Viola, Strobel, et al., 2014; Viola et al., 2007). To enhance generalizability, further studies investigating functional implications of the *PER3* polymorphism should include the heterozygous genotype.

The present data set also includes waking EEG patterns during both protocols, which will be examined next. Previous reports (Viola et al., 2007) and our preliminary data (see Figure S2) indicate a clear vulnerability-related modulation of several frequency bands, with a strong expression in the alpha (8-11 Hz) range. Interestingly, differences are visually detectable in both protocols, although more pronounced during SD. A comprehensive analysis of this data seems worthwhile, also taking into account sleep inertia after the naps (Ferrara & De Gennaro, 2000; Marzano, Ferrara, Moroni, & De Gennaro, 2011). In addition, the night sleep architecture during baseline and recovery nights will be investigated in detail. So far, visual scoring of the sleep stages according to standard criteria (Rechtschaffen & Kales, 1968) over the entire night did not reveal genotype-dependent differences. Nonetheless, indications for a different distribution of sleep stages over the several sleep cycles are present. In a next step, the data will be subjected to spectral analysis. Considering the different amount of unintentional sleep during SD, as well as the better nap sleep efficiency and its relation to performance decrement, the EEG spectrum during the recovery nights is of particular interest. For the present fMRI dataset, the next step consists in the analysis of the circadian time-point where wake-promotion is maximal (the "wake-maintenance-zone"; Lavie, 1986; Strogatz, Kronauer, & Czeisler, 1987). It is not yet known how differential vulnerability manifests at the cerebral level during this time of day. Moreover, we assessed fMRI data during working memory performance with the N-Back paradigm (Cohen et al., 1997). Vandewalle et al. (2009) already used this task and reported differences between *PER3* genotypes at the cerebral level after a normal waking day and in the morning after 25h of SD. In line with these authors, we did not observe any differences for accuracy measures at the behavioral level, at neither time-point. This speaks in favor of several studies showing that higher cognitive processes are often less compromised than basic vigilance (e.g. Lo et al., 2012), yet the underlying cerebral processes might still be different. Up to now, no study investigated whether and to what extent the cerebral correlates of working memory are modulated by vulnerability during the night, when circadian sleep promotion is greatest.

Our findings add substantially to the literature and have implications for future research and might eventually be translated into the field. First, as the ability to sleep during naps distributed over the circadian cycle seems to be linked to attentional decrement under SD, the former could serve as a predictor for how much someone is cognitively impaired by sleep loss. Future studies should investigate if this also relates to actual industrial accidents or medical errors during night and shift work, and if self-assessment of "sleep ability" would correspond. We likewise show that self-ratings of sleepiness are in line with physiological indications of vulnerability, suggesting that more

vulnerable participants are able to take earlier countermeasures when facing sleep loss. Second, vulnerability to sleep loss is detectable at the cerebral level, even underlying similar performance, potentially preceding changes in behavior. If vulnerable persons are more likely to drift into task-inactive cerebral networks when attending a monotonous task during the biological night, night work task characteristics or environment could be adapted to reduce error rates, because obviously optimal performance is still possible despite higher vulnerability. Finally, it would be of interest if self-rated vulnerability to sleep loss would correspond to what is observed both in the laboratory and in the field. The latter could lead to provocative consequences for occupational selection.

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7. Appendix

Supplementary Figures

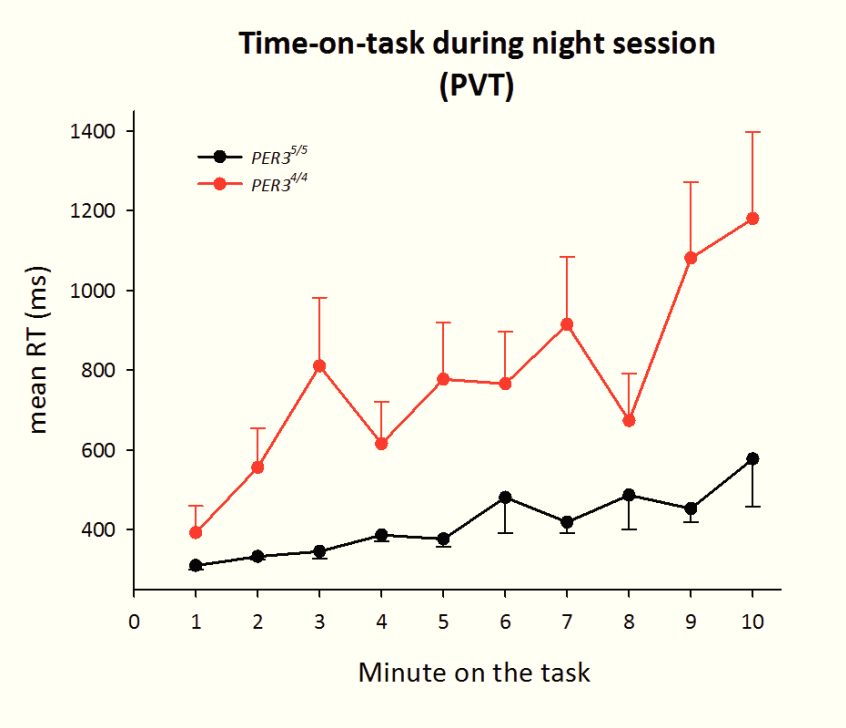

Figure S1. Time course of mean reaction times (RT) per genotype over the 10-min Psychomotor Vigilance Task (PVT) during night-time. Red line: *PER3*$^{5/5}$ carriers (n = 14), black lines: *PER3*$^{4/4}$ carriers (n = 14). All RTs > 500ms are considered lapses, and RTs can reach a maximum length of 10'000 ms when no button is pressed during stimulus presentation. The graphs show that for *PER3*$^{5/5}$ carriers, a very high group mean is reached toward the end of the task, suggesting that often these participants do not answer at all or considerably late. For the *PER3*$^{4/4}$ carriers, an increase towards the lapse-domain at the end of the task is also observed, but with less extreme values. Moreover, performance seems more stable in *PER3*$^{4/4}$ carriers, as indexed by lower standard errors of the mean and less minute-to-minute fluctuation.

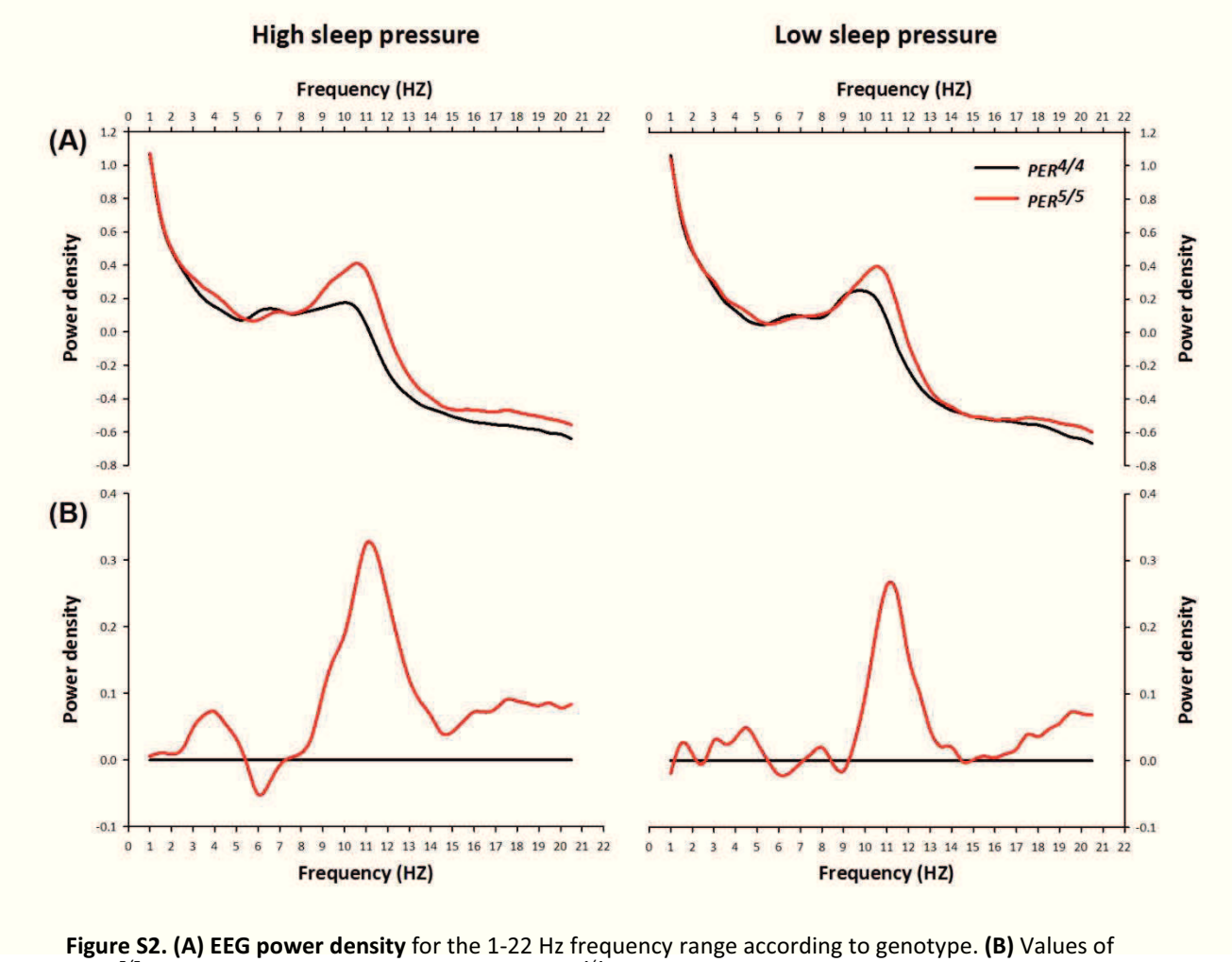

Figure S2. (A) EEG power density for the 1-22 Hz frequency range according to genotype. **(B)** Values of *PER3*$^{5/5}$ (n = 15) carriers as percentage of the *PER3*$^{4/4}$ carriers (n = 14). Log-transformed values averaged over time for each participant in each protocol.

Declaration by candidate

I hereby declare that I have independently carried out the PhD-thesis entitled "Behavioral, electrophysiological, and cerebral correlates of vulnerability to sleep loss: The impact of sleep pressure, circadian phase, and a *PER3* polymorphism". 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 and were cited accordingly, and only indicated resources have been used.

Date:

Signature: