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Research report

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

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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.

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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.

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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

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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

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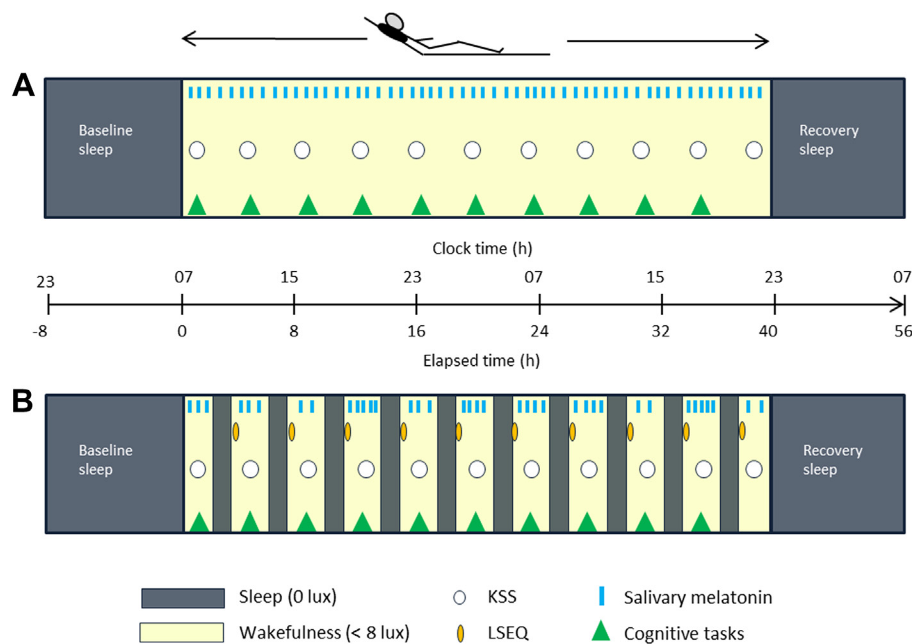

Fig. 1 – Schematic overview of the laboratory part. After baseline night (8 h), both a 40-h SD (A) and a 40-h NP paradigm (B, ten 80/160-min sleep/wake cycles) under controlled posture conditions in a within-subject design was carried out, followed by a recovery night (8 h). Gray bars in B indicate scheduled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

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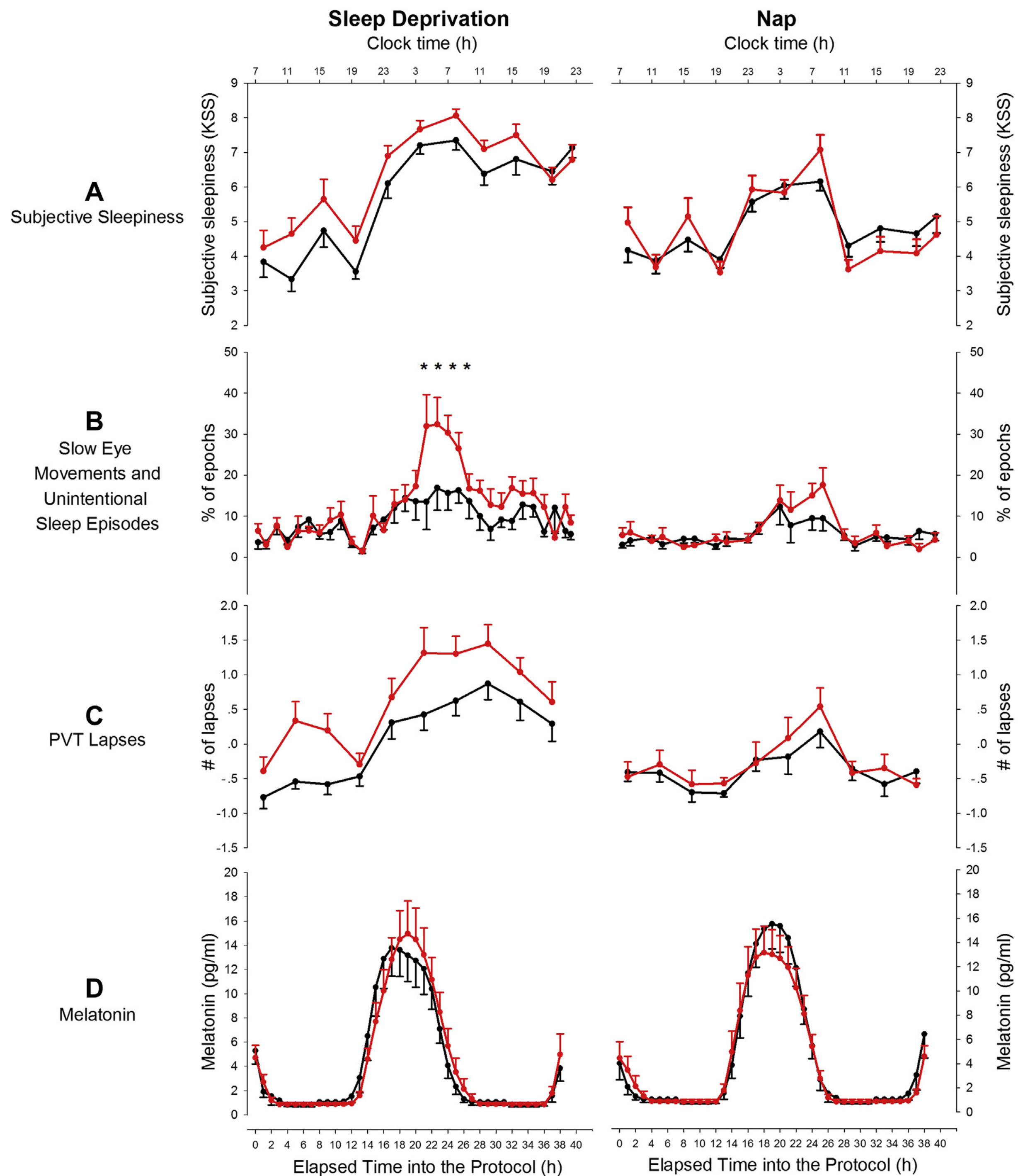

Fig. 2 – Time courses during SD (left panels) and NP (right panels) by genotype; *PER3*[55]: red lines, *PER3*[44]: black lines. (A) Subjective sleepiness mean values (B) SEMs and USEs % of epochs per bin (C) PVT lapses, transformed (D) Salivary melatonin. Clock time indications refer to 7 a.m. wake 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 $\pm$ 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 $\pm$ 61 (mean clock time $\pm$ SD in min) in $PER3^{4/4}$ and 07:10 h $\pm$ 43 (mean clock time $\pm$ 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 $\pm$ SE) compared to low (NP, -0.28 ± 0.05, mean KSS values $\pm$ 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 **p < .0001**	F (10, 565) = 46.44 **p < .0001**	F (1, 27) = .79 p = .38	F (1, 565) = 10.22 **p = .0015**	F (10, 565) = 1.9 **p = .0425**	F (10, 565) = 11.49 **p < .0001**	F (10, 565) = 1.11 p = .35
SEM/USE	F (1, 1061) = 68.78 **p < .0001**	F (20, 1061) = 14.57 **p < .0001**	F (1, 27.1) = 2.06 p = .16	F (1, 1061) = 7.49 **p = .0063**	F (20, 1061) = 2.7 **p < .0001**	F (20, 1061) = 2.98 **p < .0001**	F (20, 1061) = .82 p = .70
SEM/USE SD	n.a.	F (30, 774) = 8.94 **p < .0001**	F (1, 27) = 2.79 p = .11	n.a.	F (30, 774) = 1.85 **p = .0039**	n.a.	n.a.
PVT Lapses	F (1, 512) = 163.02 **p ≤ .0001**	F (9, 513) = 23.85 **p ≤ .0001**	F (1, 27) = 2.72 p = .11	F (1, 513) = 18.45 **p < .0001**	F (9, 513) = 1.18 p = .30	F (9, 513) = 8.99 **p ≤ .0001**	F (9, 513) = .45 p = .91
PVT RT	F (1, 512) = 119.5 **p ≤ .0001**	F (9, 512) = 31.33 **p ≤ .0001**	F (1, 27) = 1.83 p = .19	F (1, 512) = 6.77 **p = .0095**	F (9, 512) = .79 p = .62	F (9, 512) = 5.03 **p < .0001**	F (9, 512) = .51 p = .87
DLMO	F (1, 27) = 6.57 **p = .0162**	n.a.	F (1, 27) = .00 p = .99	F (1, 27) = 1.14 p = .30	n.a.	n.a.	n.a.
DLMOff	F (1, 27) = .01 p = .92	n.a.	F (1, 27) = .51 p = .48	F (1, 27) = .06 p = .81	n.a.	n.a.	n.a.

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 $PER3^{4/4}$

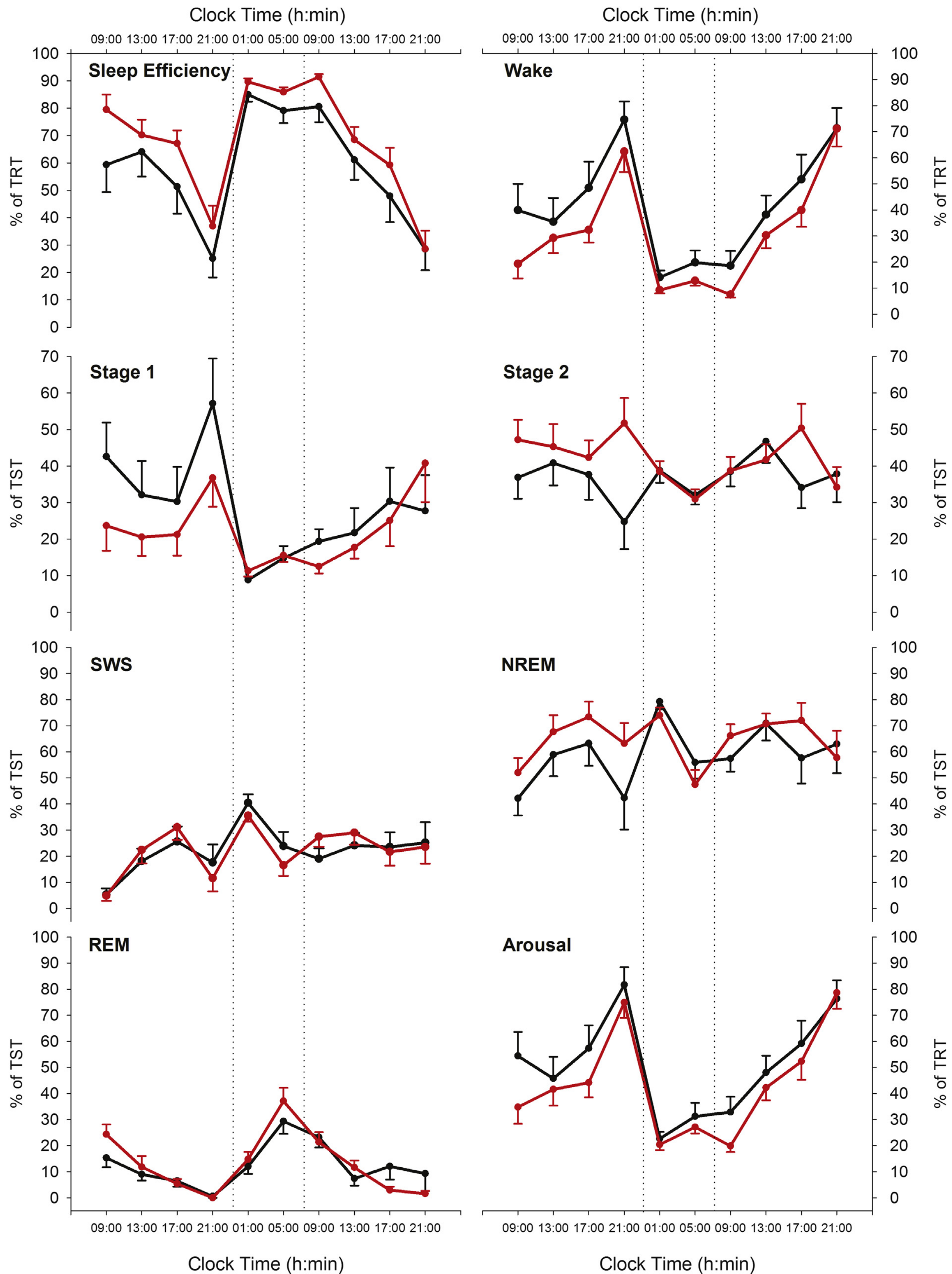

Fig. 3 – Visually scored nap sleep by genotype (*PER3*$^{5/5}$: red lines, *PER3*$^{4/4}$: black lines). SWS: Slow wave sleep. TST, SE, wake and arousal: % of TRT. Stage 1, 2, SWS, NREM and REM: % of TST. Time of day indicates start of the nap sleep episode (80 min duration) and refer to a 7 a.m. wake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

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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

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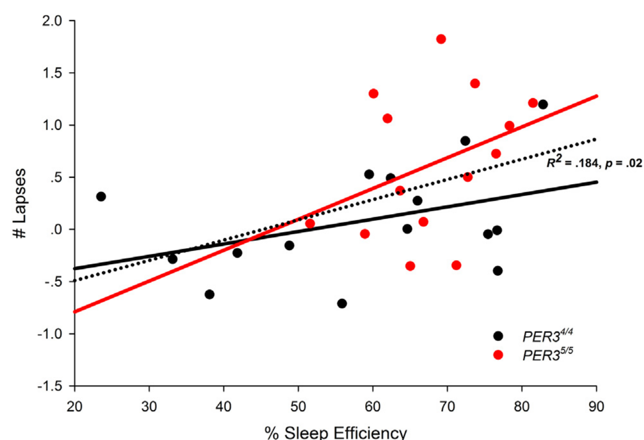

Fig. 4 – Mean SE during naps in relation to overall mean number of PVT lapses during SD. Individual values and regression lines; black line and dots represent *PER3*$^{4/4}$ carriers, red line and dots represent *PER3*$^{5/5}$ carriers. Dashed line represents regression for the whole group. R^2 and *p*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

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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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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cortex.2013.11.008.

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