Effects of light on cognitive brain responses depend on circadian phase and sleep homeostasis

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ABSTRACT

Light is a powerful modulator of cognition through its long term effects on circadian rhythmicity and direct effects on brain function as identified by neuroimaging. How the direct impact of light on brain function varies with wavelength of light, circadian phase and sleep-homeostasis, and how this differs between individuals, is a largely unexplored area. Using functional MRI, we compared the effects of 1-min of low intensity blue (473nm) and green light (527nm) exposures on brain responses to an auditory working memory task, while varying circadian phase and status of the sleep homeostat. Data were collected in 27 subjects genotyped for the PER3 VNTR (12 PER3<sup>5/5</sup> and 15 PER3<sup>4/4</sup>) in whom it was previously shown that the brain responses to this task, when conducted in darkness, depend on circadian phase, sleep homeostasis and genotype. In the morning after sleep, blue light, relative to green light, increased brain responses primarily in the ventro- and dorso-lateral prefrontal cortex and in the intraparietal sulcus, but only in PER3<sup>4/4</sup> individuals. By contrast, in the morning after sleep loss, blue light increased brain responses in a left thalamo-fronto-parietal circuit to a larger extent than green light, and only so in PER3<sup>5/5</sup> individuals. In the evening wake-maintenance zone following a normal waking day, no differential effect of 1-min of blue vs. green light were observed in either genotype. Comparison of the current results with the findings observed in darkness indicates that light acts as an activating agent particularly under those circumstances in which and in those individuals in whom brain function is jeopardized by an adverse circadian phase and high homeostatic sleep pressure.
INTRODUCTION

Light modulates brain function through its impact on the timing of circadian rhythms, but also through direct effects on physiology and behavior, including modulation of alertness and performance (Cajochen 2007). These responses to light are mediated by a non-classical photoreception system, which is, in part, distinct from the visual photoreception system. Melanopsin-expressing, intrinsically photosensitive ganglion cells (ipRGC), in addition to rods and cones (Hatori and Panda 2010), contribute to these responses. Melanopsin is maximally sensitive to blue light (460-480nm) and confers a shorter-wavelength maximal sensitivity to non-classical photoreception, as compared with the photopic visual system, which is maximally sensitive to longer wavelengths (~550nm).

Some of the brain areas involved in the effects of light on brain function have been elucidated in neuroimaging studies using protocols that exploit the differential sensitivities of the non-classical photoreceptive and visual systems. These studies have shown that, under rested condition during the daytime, light exposure affects alertness-related, wake-promoting subcortical structures in the brainstem, hypothalamus and thalamus, and limbic and cortical areas involved in the ongoing cognitive process (Vandewalle et al. 2006; Vandewalle et al. 2007a; Vandewalle et al. 2009b; Vandewalle et al. 2007b).

Several factors may modulate the non-classical responses to light. It is well established that the phase-shifting effects of light, as well as the direct effects of light on physiological responses such as electroencephalogram (EEG) and electrocardiogram
(ECG), depend on the circadian phase at which light is administered (Badia et al. 1991; Duffy and Czeisler 2009; Ruger et al. 2006). Furthermore, it was recently reported that sleep restriction, which will leads to an increased sleep homeostatic pressure, reduces the circadian phase-shifting effects of light (Burgess 2010). Animal data also suggest that the melanopsin-mediated impact of light on sleep and brain function during wakefulness, as assessed by EEG, not only varies with circadian phase, but is furthermore affected by sleep pressure (Tsai et al. 2009).

Waking performance and cognition assessed at the behavioral level are modulated by an interaction of circadian and sleep homeostatic processes, such that behavioral deficits are most pronounced in the morning after sleep loss (Dijk et al. 1992; Schmidt et al. 2007; Wyatt et al. 1999). The impairment of cognition following increases in homeostatic sleep pressure induced by sleep deprivation differs widely between individuals (Van Dongen et al. 2004). The brain correlates of the effects of sleep loss, and individual differences therein, have been in part elucidated (Chee and Chuah 2008; Mu et al. 2005).

A primate-specific, variable-number (4 or 5) tandem-repeat (VNTR) polymorphism in *PERIOD3 (PER3)* predicts individual differences in EEG slow wave activity (SWA), a marker of sleep homeostasis, and the extent of cognitive decline in the morning hours following sleep-loss (Dijk and Archer 2010; Groeger et al. 2008; Viola et al. 2007). We previously reported that, in the absence of light, the brain responses to a cognitive task depend on circadian phase, homeostatic sleep pressure and *PER3* genotype, such that the genotype-dependent differences were much more pronounced
in the morning after sleep loss than in the morning after sleep, or in the evening after a normal waking day (Vandewalle et al. 2009a). In the morning hours after 25h of wakefulness, the sleep-loss-vulnerable genotype \((PER3^{5/5})\) showed considerable reductions in the responses to an auditory working memory task, notably in higher associative parietal and frontal areas. By contrast, the less-vulnerable genotype \((PER3^{4/4})\) did not show such reductions, but rather recruited supplemental brain areas, including higher order frontal areas and the thalamus.

Whether the acute impact of light on brain activity in humans, as assessed by fMRI, is also modulated by sleep pressure, circadian phase and genotype, is currently not known. We investigated this question by comparing the fMRI-assessed effects of very short blue and green light exposures, while varying circadian phase and homeostatic sleep pressure in subjects homozygous for the \(PER3\) VNTR polymorphism.
MATERIALS AND METHODS

The data presented in this manuscript were collected in the same protocol as previously described (Vandewalle et al. 2009a). Full details of the protocol, subject selection and fMRI assessments can be found in that publication. Here, we only provide a summary of generic aspects of the protocol and focus on details relevant to the current manuscript.

Participants

The study was approved by the Ethics Committee of the University of Liege and participants gave their written informed consent. Fifteen PER3⁴/⁴ and 13 PER3⁵/⁵ individuals were selected from a sample of 254 right-handed individuals, aged between 18 and 30 years old who were genotyped for the PER3 VNTR, as previously described (Archer et al. 2003; Vandewalle et al. 2009a). Exclusion criteria included a body mass index >27, working night shifts during the last year or travel through more than one time zone during the last 2 months, smoking, medication, or use of psychoactive drugs, excessive caffeine and alcohol (i.e. >4 caffeine units/day; >14 alcohol unit/week). The absence of medical, traumatic, psychiatric or sleep disorders was established in a semi-structured interview. The two genotype groups were matched for age and gender. No thorough ophthalmological examination was performed but none of the volunteers reported a history of ophthalmic disorder and color blindness was ruled out by the 38 plate edition of Ishihara’s Test (Kanehara Shupman Co., Tokyo, Japan). 15 PER3⁴/⁴ and 12 PER3⁵/⁵ were included in the analyses because one PER3⁵/⁵ individual fell asleep during the fMRI session (See Supplemental Table S1 for complete subject characteristics).

Protocol - Circadian Phase and Homeostatic Sleep Pressure
Circadian phase was varied by scheduling fMRI acquisitions in the evening, 2h before habitual bedtime, i.e. close to the crest of the circadian wake promoting signal, and in the morning, 1.5h after wake time, close to the nadir of the circadian wake-promoting signal. Circadian phase was assessed from melatonin profiles measured in saliva and there were no differences between the genotypes with respect to the circadian phase (as well as clock times) at which fMRI acquisitions were scheduled [see supplemental results and tables of (Vandewalle et al. 2009a)].

Homeostatic sleep pressure was varied by sleep deprivation. Each subject participated in two experimental segments (Sleep and Sleep deprivation) separated by at least one week and in counter-balanced order. Both segments were identical, except for the presence or absence of sleep between the evening and morning fMRI recordings. In the Sleep segment, subjects slept in darkness for 7.5h. EEG was recorded during this sleep episode and EEG SWA was analyzed to confirm that the two genotypes differ with respect to the homeostatic process (See Supplemental Methods and Results). A staff member ensured they were awake at all times during the sleep deprivation night.

Thus, in each subject 4 fMRI sessions were conducted: a morning session after sleep (after ~1.5h of wakefulness, at ~08:30h on average), a morning session after sleep deprivation (after ~25h of wakefulness, at ~08:30h on average), an evening session before sleep (after ~14h of wakefulness, at ~21:30h on average), and an evening session before sleep deprivation (after ~14h of wakefulness, at ~21:30h on average) (Figure 1a).
circadian phase. By contrast, the two morning sessions were scheduled at the same circadian phase and differed only with respect to time awake prior to the session.

**Light exposures, measurements and description of MRI runs**

While in the laboratory, subjects were maintained in dim light at all times (<5 lux), except for the sleep episodes (0 lux) and fMRI sessions (<0.01 lux), which were conducted in darkness with the exception of the light exposures in fMRI (see below). During sleep deprivation, only quiet activities were authorized (quiet games, video [<5 lux], and reading) and saliva samples were collected hourly until the morning fMRI session for the determination of the melatonin rhythm. Subjective alertness scores were collected every 30 min upon arrival and until the end of the protocol the next day, when the participants were awake (i.e. not during sleep in the sleep segment). Activity was strictly controlled for 60 min before the fMRI session, during which only social interactions were allowed. Three drops of tropicamidum 0.5% (Tropicol®) were administered in the eyes 20 min before entering the scanner to inhibit pupillary constriction.

- **Insert figure 1 about here (1.5 column, height: ~5 inches)** –

In the MR scanner, subjects completed two consecutive runs, during which they performed an auditory 3-back task. In this task, stimuli consisted of nine French monosyllabic consonants presented every 2.5s. For each letter, the volunteers had to state whether or not it was identical to the consonant presented 3 stimuli earlier, using an MR-compatible keypad. We published the results of the first run (10 min), which was conducted in complete darkness (Vandewalle et al. 2009a). In the second run,
comparison of the responses to the task under blue and green light exposure served as a probe to identify brain structures involved in the non-classical impact of light (Cajochen et al. 2005; Vandewalle et al. 2009b). Participants were exposed to alternating 1-min blue (473nm) and green (527nm) monochromatic light of equal photon density (photon density was $7 \times 10^{12}$ during half of the exposures and $3 \times 10^{13}$ photons/cm²/s for the other half; this is respectively equivalent to less than 0.5 and 4 lux for blue light, and to less than 20 lux and 50 lux for green light), while performing the task (Figure 1b). The run lasted 13 to 14 minutes and included 12 blocks of task, half of which were performed under blue light, while green light was administered in the other half. Darkness periods (< 0.01 lux) separated all 1-min illuminations [see (Vandewalle et al. 2010) and Supplemental Methods for more details on irradiance choice].

As for the data acquired in darkness (Vandewalle et al. 2009a), the working memory task duration was kept relatively short (14 min), to prevent differences between genotypes in the sleep-deprivation-induced deterioration in performance, which has been reported when this task was embedded in a longer duration test battery (Groeger et al. 2008). Similarly, light exposures were kept short (1-min) to prevent light-induced modification in performance induced by longer duration exposures (Cajochen et al. 2005; Lockley et al. 2006). As a result, behavioral differences could not significantly bias the fMRI data.

**fMRI data acquisition**

Functional MRI data were acquired with a 3T MR scanner (Allegra, Siemens, Germany) using multislice T2*-weighted fMRI images, which were obtained with a gradient echo-
planar sequence (EPI) using axial slice orientation (32 slices; voxel size: 3.4x3.4x3 mm$^3$ with 30% of gap; matrix size 64x64x32; repetition time = 2130ms; echo time = 40ms; flip angle = 90°). Structural brain images consisted of a T1-weighted 3D MDEFT (repetition time = 7.92ms, echo time = 2.4ms, time of inversion = 910 ms, flip angle = 15°, field of view 230x173 cm$^2$, matrix size = 256x224x173, voxel size = 1x1x1 mm$^3$).

**fMRI data analysis**

Functional volumes were analyzed using Statistical Parametric Mapping (SPM5 - http://www.fil.ion.ucl.ac.uk/spm). They were corrected for head motion, spatially normalized (standard SPM5 parameters, with voxel resampling to 2x2x2 mm$^3$ – this procedure has no impact on the validity of the analyses but improves estimation of the smoothness of statistical maps) and smoothed. The analysis of fMRI data was conducted in two steps, accounting, respectively, for fixed and random effects. For each subject, changes in brain regional responses were estimated using a general linear model, in which the different parts of the experimental design were modeled using either boxcar or stick functions, convolved with a canonical haemodynamic response function. Boxcar functions modeled the 30s illumination periods with rest, the 30s illumination periods including the 3-back task, and the darkness periods during which the task was performed. Stick functions modeled light onsets and light offsets and subject errors (false positives, false negatives and omissions, separately). Melanopsin-expressing retinal ipRGCs do not cease firing at light offset (Hatori and Panda 2010), so transient brain responses to light offsets modeled by stick function (‘events’) are unlikely to represent a non-classical response to light. Furthermore, each run included only 6 light
onsets per wavelength, which provide a limited statistical power. The regressors modeling onsets, offsets and errors were, therefore, considered as covariates of no interest, together with movement parameters derived from realignment of the functional volumes. High-pass filtering was implemented in the matrix design using a cut-off period of 256 seconds to remove low frequency drifts from the time series. Serial correlations in the fMRI signal were estimated using an autoregressive (order 1) plus white noise model and a restricted maximum likelihood algorithm.

It cannot be excluded that baseline brain activity differed between genotypes (Vandewalle et al. 2009a) and, therefore, only differences between light conditions can be reliably compared between genotypes. In addition, data acquired in darkness are too sparse to be validly compared to the data acquired under blue or green light exposure (Supplemental Methods). Therefore, in each session and in each subject, we only computed contrasts consisting of the differences between the brain responses to the task recorded under blue and green illumination (Blue>Green and Green>Blue). The resulting summary statistic images were then entered in a second-level random effects analysis. We first computed one-sample t-tests in each genotype separately and then computed conjunction analyses, based on the conjunction null hypothesis, on these one-sample t-tests to identify differences between the light conditions that were common to both genotypes. We then computed two-sample t-tests to show whether the differences observed between the genotypes separately were statistically significant across genotypes. For the sake of completeness, we pooled both genotypes together and computed one-sample t-tests to identify brain areas that were affected by the light
condition, irrespective of the genotype. All contrasts were first computed irrespective of the irradiance level and then including irradiance level as factor. Analyses including irradiance as a factor were inconclusive and are not presented [see Supplemental Methods and (Vandewalle et al. 2010) for complete details].

The resulting t-statistics maps were thresholded at $p_{uncorrected}=0.001$ and statistical inferences were performed after correction for multiple comparisons at a threshold of $p=0.05$. Corrections for multiple comparisons (Family Wise Error) were based on the Gaussian random field theory and computed on the entire brain volume, or on small spherical volumes (10mm radius) around locations identified a priori from the relevant literature. Significant effects of the light condition were expected in structures involved in the n-back tasks, working memory, arousal regulation, or reported in previous investigations of the effects of sleep deprivation in fMRI or PET (see Supplemental Methods). We also expected significant effects in areas showing non-classical responses to light exposure in our own fMRI and PET work, and in brain areas to which the melanopsin-expressing ipRGCs project, or those that are functionally linked to the SCN.
RESULTS

Differential impact of blue and green light on ongoing brain activity in the morning after 1.5h of wakefulness.

We first analyzed data acquired in the morning after a night of sleep, i.e. when sleep and circadian rhythms are aligned and homeostatic sleep pressure is low. As intended (see Methods), subjects felt equally alert (p=0.74), performance did not differ between the light conditions and the genotypes (p>0.05), and the interaction between the genotypes and light conditions was also not significant (p>0.5) (Figure S1b-c; Supplemental Results).

We first considered PER3^{4/4} data in our fMRI analyses because they constitute 45-50% of the general population, compared to 10% for the PER3^{5/5} genotype (Dijk and Archer 2010). Thus, the results of the PER3^{4/4} subjects in the present analysis are more likely to be comparable to previous results, which were obtained in random samples not stratified by genotype.

- Insert table 1 about here (1 column) -

Blue light, as compared with green light, significantly increased brain activity in PER3^{4/4} individuals in the left dorso-lateral prefrontal cortex (DLPFC), and in the right intraparietal sulcus (IPS) and cerebellum (CER) (Figure 2; Table 1a), while in the left fronto-polar/ventro-lateral prefrontal cortex (FPC/VLPFC), blue light seemed to rather prevent the decline observed under green light exposure (Figure 2c). No brain responses were significantly increased under green (vs. blue) light exposure in PER3^{4/4}. Surprisingly, analyses of PER3^{5/5} fMRI data revealed no significant modulation of the
brain responses to the task by the light condition (blue>green or green>blue). The impact of the wavelength of the light exposure on \( \text{PER3}^{3/4} \) brain activity was, however, not significantly different from \( \text{PER3}^{5/5} \) individuals. In addition, we did not detect impacts of the light condition that were common to both genotypes (conjunctions).

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**No impact of light exposure on brain function in the evening wake maintenance zone after 14h of wakefulness.**

In the evening wake maintenance zone, subjects of both genotypes felt equally alert (\( p \geq 0.7 \)) and performance was not affected by the light condition and genotype (\( p > 0.15 \)), both in the evening before sleep was allowed, and before sleep deprivation (Figure S2; Supplemental Results).

We did not detect any significant impact of 1 minute of light (blue>green and green>blue) on the brain responses to the task in either genotype when considering fMRI data acquired in the evening before sleep or before sleep deprivation and there were no activations common to both genotypes (conjunctions) (Table 1b). Even when considering data irrespective of genotype by pooling all 27 subjects, no significant impact of the light condition on ongoing brain activity could be detected.

**Differential impact of blue and green light on ongoing brain activity in the morning after 25h of wakefulness: differential response in \( \text{PER3}^{5/5} \) vs. \( \text{PER3}^{3/4} \)**

Subjects of both genotypes felt equally sleepy, before and during the fMRI experiment (\( p > 0.1 \); Figure S3a-b; Supplemental Results), under the conditions of high homeostatic sleep pressure in the morning. Performance to the task did not differ between the
genotypes and between the light conditions (p>0.1), and there was no interaction between genotype and light condition (p>0.45) (Figure S3c-d; Supplemental Results).

Analyses of the fMRI data revealed no significant impact of the light condition on the brain activity related to the task (blue>green or blue<green) in the PER34/4 genotype (Table 1c). By sharp contrast, as compared with green light, blue light significantly increased task-related brain activity in the PER35/5 genotype in the right fronto-polar cortex (FPC) and dorso-lateral prefrontal cortex (DLPFC), the left premotor cortex (PMOT), the bilateral intraparietal sulcus (IPS), the bilateral insula (INS), the cerebellum (CER), and an area of the left dorso-posterior thalamus (THAL) compatible with the dorsal pulvinar, while in the left fronto-polar cortex blue light rather maintained brain responses compared with the decline in activation observed under green illumination (Figure 3 central panels). The majority of these effects were significantly different from PER34/4 (significant interaction between genotype and light condition – * bold Table 1c). Importantly, no brain responses were significantly increased under green (vs. blue) light exposure in PER35/5 and no impact of the light condition was detected when considering the population as a whole (i.e. irrespective of the genotype), or when trying to identify effects of the light condition common to both genotypes (conjunctions).

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DISCUSSION

This study confirms our previous findings (Vandewalle et al. 2007a; Vandewalle et al. 2007b) that, as compared to longer wavelength light, shorter wavelength light exposure increases ongoing non-visual cognitive activity in subcortical and cortical brain areas. Compared with green light, 1-min of low-intensity blue light was able to significantly increase brain activity in a widespread set of higher order cortical areas, including the fronto-polar, lateral prefrontal, and premotor cortex, the intraparietal sulcus, insula, cerebellum and thalamus, which are all known to be involved in executive control and working memory (Cabeza and Nyberg 2000). The present data do also show that the effects of light depend on circadian phase and homeostatic sleep pressure and also differ between the PER3 genotypes. The differences between the genotypes were most prominent in the morning after sleep loss, in accordance with our results in darkness (Vandewalle et al. 2009a). Thus, in the morning during sleep loss, effects of light were only observed in $\text{PER3}^{5/5}$, whereas in the morning after a night of sleep, similar significant effects of light were found only in $\text{PER3}^{4/4}$. In the evening after a normal waking day, no impact of 1-min light exposure on brain function was detected in either genotype.

When interpreting these data and comparing them to other studies, we need to consider that in most previous studies the duration of light exposures was in the range of hours rather than minutes, and that in previous studies individual differences and genotypes were not considered (Cajochen et al. 2005; Lockley et al. 2006). In other words, we assume that an effect of light would have been detected in both genotypes in
all sessions had we used longer or more intense light exposures. The parameters of the light exposure used in our protocol allowed for the detection of differences between sessions and individuals, and thereby provide insights into the mechanisms underlying the effects of light.

All fMRI data were collected before differences in performance between conditions and genotypes emerged, and we can thereby be confident that the effects we observed are not secondary to behavioral affects but in fact precede and herald significant behavioral changes. Future research is, however, required to confirm that the observed significant differences in brain responses will ultimately lead to behavioral differences. Finally, when interpreting these data, one needs to appreciate that we can only assess the difference in the response between blue and green light (see Methods and Supplemental Methods) and cannot assess the separate impact of blue or green light exposures, which are likely to exist (Gooley et al. 2010).

**Photoreception involved in the impact of light exposure on cognitive brain activity.**

As in our previous studies (Vandewalle et al. 2007a; Vandewalle et al. 2007b), the present results show that, compared to green light, shorter wavelength blue light induced sustained increases in the brain responses to an auditory task in the absence of any spatially-structured visual stimulus. This supports the involvement of non-classical photoreception, and we favor this interpretation. However, color preference (Palmer and Schloss 2010) or color opponency mechanisms (Conway 2009) could also be involved [(Vandewalle et al. 2010) for a full discussion of this aspect].
The effects we observe are likely to arise from several retinal photoreceptors (Lall et al. 2010), and we cannot isolate their respective contribution. The maximal sensitivity (460 – 480 nm) of melanopsin ipRGCs is close to the peak wavelength of the blue light we used (473nm), and the light levels we used are compatible with its activation (Lall et al. 2010). In rodents, rods may contribute to the impact of light at irradiance levels higher than previously expected (Altimus et al. 2010; Lall et al. 2010). However, the maximal sensitivity of rods (505 nm) is intermediate between the blue and green (527 nm) light we administered, reducing their potential influence. Finally, short-wavelength sensitive cones (S-cones), which are maximally sensitive to light around 420 nm could also have contributed to our effects [as well as M- or L-cones (Vandewalle et al. 2010)].

Recent data implied the involvement of melanopsin, and of ipRGCs expressing this photopigment, in the acute regulation of sleep by light exposure in nocturnal rodents (Altimus et al. 2008; Lupi et al. 2008; Tsai et al. 2009). The role of the melanopsin-intrinsic response to light also appeared to vary according to time of day, i.e. changes in circadian phase or homeostatic sleep pressure (Altimus et al. 2008; Tsai et al. 2009), which could be related to our present findings.

**The impact of light on cognitive brain responses is determined by differences in sleep homeostasis and in its interaction with circadian phase**

It is well established that homeostatic sleep pressure increases with time awake and affects brain function (Chee and Chuah 2008; Drummond and Brown 2001). Based on EEG SWA data, we previously interpreted the differential response of the two genotypes
to sleep deprivation in the context of the homeostatic and circadian regulation of performance and posited that these differences were related to a faster build up of homeostatic sleep pressure in $PER3^{5/5}$, or higher amplitude oscillation of the sleep-homeostat, rather than differences in the circadian process (Dijk and Archer 2010; Viola et al. 2007). In accordance with our previous observations, in the present protocol the genotypes did not differ with respect to circadian phase of the melatonin rhythm (Vandewalle et al. 2009a), and SWA at the beginning of baseline was higher in $PER3^{5/5}$ than in $PER3^{4/4}$, and dissipated more rapidly during the night (see Supplemental Results and Figure S4). Thus, the most challenging conditions for maintaining cognitive performance are encountered by the $PER3^{5/5}$ individuals in the morning after sleep loss. This is when the effects of light and the differences between the genotypes are most pronounced, implying that light especially affects cognitive brain function under challenging conditions in vulnerable individuals.

We cannot exclude that the differential light response between the genotypes is related to retinal processes. Rodent Per3 knock-out data imply a role for Per3 in the light sensitivity of the non-classical photoreception system (van der Veen and Archer 2010). However, in the current protocol the differences between the genotypes varied with changes in circadian phase and homeostatic sleep pressure. Our results are, therefore, unlikely to be directly related to differences in light sensitivity, unless we assume that homeostatic sleep pressure and circadian phase affects retinal function in a genotype-dependent manner. We also cannot exclude that genotype-dependent differential response to certain aspects of the sleep deprivation protocol, such as the prolonged
exposure to dim light, underlie the observed differences in response to light, rather than differences in sleep homeostasis.

Comparisons of the impact of sleep loss on brain activity in darkness and under blue and green light exposures

1. Exposure to blue light maintains cognitive brain function during sleep loss?

Sleep loss is associated with deficits in sensory processing, attention and decision making (Chee and Chuah 2008). Accordingly, as summarized in Figure 4, we reported that in PER35/5 individuals kept in darkness, sleep loss led to widespread reductions in activations in higher-order lateral prefrontal and parietal areas, as well as in lower-order temporal and occipital sensory areas (Vandewalle et al. 2009a) (Figure 4, red dots).

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In sleep deprived PER35/5 individuals, blue light exposure increased activation in the intraparietal sulcus (Figure 4, white-red dots), which is a key associative area involved in the top-down regulation of attention (Corbetta and Shulman 2002). These increases were found in the vicinity of the deactivations observed in darkness, which could suggest that exposure to light helps in restoring diminished attention resources. Blue light administration to PER35/5 during sleep loss also increased responses in the prefrontal cortex, not only in the dorso-lateral prefrontal areas, which showed decreased activation in darkness, but also in the fronto-polar cortex (Figure 4, white-red dots). This is remarkable because, according to a recent model, the fronto-polar cortex is at the top of executive control, establishing optimal response strategies in tasks involving multiple cognitive processes (Koechlin and Hyafil 2007). These results support
the hypothesis that under challenging conditions of high sleep pressure and circadian misalignment, light promotes higher-order processes, including attention, thereby maintaining optimal cognitive performance.

During sleep loss, increased activations were also found in the thalamus under blue light exposure in PER3^{5/5} in a location compatible with the dorsal pulvinar, which is a key area in the regulation of alertness and cognition and in mediating the non-classical of light on brain function (Vandewalle et al. 2009b). The impact of light in brain function could, therefore, be mediated by increasing or facilitating information flow within thalamo-frontal and thalamo-parietal loops (Shipp 2004).

2. Genetically-determined endogenous drive for wakefulness sets the impact of light exposure on cognition?

A ventro-lateral prefrontal and a thalamic area showed compensatory increased activation in PER3^{4/4} during sleep loss in darkness (Figure 4, blue dots, thalamus not shown) (Vandewalle et al. 2009a) and we detected an impact of light exposure in similar locations in PER3^{5/5}. One could hypothesize that the compensatory mechanisms already in place in PER3^{4/4} prevented exposure to light from having an activating impact.

This assumption is supported by the remarkable absence of impact of 1-min light exposure on the brain responses to the task in both genotypes in the evening wake maintenance zone. In that portion of the circadian cycle, increasing sleep pressure seems to have a minimal impact on brain function (Cohen et al. 2010), suggesting that sleep-homeostasis-challenge is efficiently countered by the endogenous maximal
circadian drive for wakefulness. Light would, therefore, act as a more potent external activating agent if endogenous mechanisms are not already taking place.

Even though not significantly different between genotypes, the fact that, in the morning after sleep, exposure to blue and green light did not differentially modulate brain responses in PER3<sup>5/5</sup> is somewhat puzzling. PER3<sup>4/4</sup> and PER3<sup>5/5</sup> genotypes have been linked to evening and morning chronotype, respectively (Archer et al. 2003). In comparison to evening types, morning people find it easier to perform in the morning and this maybe related to the steeper decline of SWA in the course of the nocturnal sleep episode, which was also observed in PER3<sup>5/5</sup> individuals in the current study (Figure S4). Neural populations in PER3<sup>5/5</sup> might, therefore, be recruited to the working memory in the context of a lower level of sleep homeostatic pressure, which is assumed to be associated with high signal-to-noise-ratio synaptic transmission (Hill et al. 2008). We speculatively propose that these optimal functional conditions would prevent light from having an activating impact on brain activity in the morning immediately after a night of sleep in PER3<sup>5/5</sup>. Interestingly, in a prior investigation, we could not detect an impact of light in the few individuals who reported optimal alertness (Vandewalle et al. 2006).

**Conclusion**

As a whole, our results are compatible, with a melanopsin-driven light impact on cognitive brain function that is dependent on the genetically-determined susceptibility to homeostatic and circadian changes. However, future work is required to separate the
impact of the visual or non-classical photoreception systems and of the different retinal photoreceptors in the modulation of cognitive brain activity.
REFERENCES


Mu Q, Mishory A, Johnson KA, Nahas Z, Kozel FA, Yamanaka K, Bohning DE and George MS (2005) Decreased brain activation during a working memory task at rested baseline is associated with vulnerability to sleep deprivation. *Sleep* 28:433-446.


FIGURE LEGENDS

Figure 1. Experimental design.

a. General overview. Sleep and sleep deprivation segments were counter-balanced.


T1: task 1. Subjects performed an auditory 3-back task in complete darkness. Results from these sessions are published (Vandewalle et al. 2009a).

T2: task 2. Subjects performed an auditory 3-back task while being alternatively exposed to 1-min blue (473 nm) and green (527 nm) monochromatic light exposures.

Figure 2. Significant non-classical (blue>green) light-induced modulation of brain activity after 1.5h of wakefulness in PER34/4.

Central panels. Statistical results overlaid on the population mean structural image ($p_{uncorrected} < 0.001$). Lateral panels. Activity estimates (mean arbitrary units – a.u.) under blue and green light exposures.

a. Right cerebellum (CER); b. Left cerebellum (CER); c. Left fronto-polar cortex (FPC); d. Right intraparietal sulcus (IPS); e. Left ventro-lateral prefrontal cortex (VLPFC); f. Left ventro-lateral prefrontal cortex (VLPFC).

(*) Significant differences between blue and green light exposure (only in PER34/4; $p_{corrected} < 0.05$); (ns) Not significantly different between genotypes ($p_{corrected} > 0.05$).

Figure 3. Significant non-classical (blue>green) light-induced modulation of brain activity after 25h of wakefulness in PER35/5.
Central panels. Statistical results overlaid on the population mean structural image \((p_{uncorrected} < 0.001)\). Lateral panels. Activity estimates under blue and green light exposure.

- a. Left cerebellum (CER); b. Right fronto-polar cortex (FPC); c. Left fronto-polar cortex (FPC); d. Left insula (INS); e. Right insula (INS); f. Left ventro- and ventro/dorso-lateral prefrontal cortex (VLPFC); g. Right ventro-lateral prefrontal cortex; h. Left premotor cortex (PMOT); i. Left thalamus (dorsal pulvinar) (THAL) [inset: enlarged view in a representative subject]; j. Right intraparietal sulcus (IPS); k. Left intraparietal sulcus (IPS).

(*) Significant differences between blue and green light exposure (only in \(PER3^{5/5}\); \(p_{corrected} < 0.05\)); (#) Significant difference between genotypes [(blue>green) x \((PER3^{5/5}>PER3^{4/4})]\) \((p_{corrected} < 0.05)\); (ns) Not significantly different between genotypes \((p_{corrected} > 0.05)\).

Figure 4. Schematic representation of the impact of sleep loss on cognitive cortical activity in \(PER3^{4/4}\) and \(PER3^{5/5}\) individuals in darkness (Vandewalle et al. 2009a) and under (blue) light exposures.

- Compensatory increase in activation in the morning hours after 25h of wakefulness in \(PER3^{4/4}\), found notably in the ventro-lateral prefrontal cortex, temporal cortex, cerebellum and thalamus (thalamus not shown).
- Decrease in activation in the morning hours after 25h of wakefulness in \(PER3^{5/5}\), observed notably in the occipital, temporal, parietal and lateral prefrontal cortices.
Blue-light-induced increase in activity after 25h of wakefulness in PER3\textsuperscript{5/5} (thalamus not shown). See Table 1c for locations.

Blue-light-induced increase in activity after 1.5h of wakefulness in PER3\textsuperscript{4/4}. See Table 1a for locations.

DLPFC: ventro/dorso-lateral prefrontal cortex; FPC/VLPFC: fronto-polar/ventro-lateral prefrontal cortex; IPS: intraparietal sulcus; PMOT: premotor cortex.
Table 1: Significant differences between brain responses to the 3-back task under blue and green light exposures after 1.5h, 14h and 25h of wakefulness, in each PER3 genotype separately, and in the whole population (i.e. irrespective of genotype).

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Side</th>
<th>X, Y, Z</th>
<th>Z</th>
<th>(P_{\text{corrected}}) Value</th>
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<tr>
<td><strong>a. MORNING SESSION AFTER SLEEP (1.5h of wakefulness)</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Blue &gt; Green (\times) PER3(^{4/4})</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Fronto-polar/ventro-lateral prefrontal cortex (c)</td>
<td>L</td>
<td>-38 40 -10</td>
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</tr>
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<td></td>
<td>L</td>
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<tr>
<td>Intraparietal sulcus (d)</td>
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<td>0.010</td>
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<tr>
<td></td>
<td>R</td>
<td>42 -22 36</td>
<td>3.28</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>34 -38 36</td>
<td>3.14</td>
<td>0.028</td>
</tr>
<tr>
<td>Cerebellum (a,b)</td>
<td>R</td>
<td>38 -58 -36</td>
<td>3.31</td>
<td>0.018</td>
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<tr>
<td></td>
<td>L</td>
<td>-44 -60 -36</td>
<td>3.14</td>
<td>0.028</td>
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<td><strong>Green &gt; Blue (\times) PER3(^{4/4})</strong></td>
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<td>No significant voxel</td>
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</tr>
<tr>
<td><strong>Blue &gt; Green OR Green &gt; Blue (\times) PER3(^{5/5})</strong></td>
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<tr>
<td>No significant voxel</td>
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<td><strong>Blue &gt; Green (\times) whole population (irrespective of genotype)</strong></td>
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<tr>
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<td></td>
<td>R</td>
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<td><strong>Green &gt; Blue (\times) whole population (irrespective of genotype)</strong></td>
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<tr>
<td><strong>Blue &gt; Green OR Green &gt; Blue (\times) conjunction between PER3(^{5/5}) and PER3(^{4/4})</strong></td>
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<td><strong>b. EVENING SESSION BEFORE SLEEP OR SLEEP LOSS (14h of wakefulness)</strong></td>
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<tr>
<td>No significant voxel</td>
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</tr>
<tr>
<td><strong>c. MORNING SESSION AFTER SLEEP LOSS (25h of wakefulness)</strong></td>
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<td></td>
</tr>
<tr>
<td>Blue &gt; Green (\times) PER3(^{5/5})</td>
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<tr>
<td>Fronto-polar cortex (b,c)</td>
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<td>3.65</td>
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</tr>
<tr>
<td></td>
<td>R</td>
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<td>0.018</td>
</tr>
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<td>Hemisphere</td>
<td>MNI Coordinates</td>
<td>t Value</td>
<td>p Value</td>
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<tr>
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<td>------------</td>
<td>-----------------</td>
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<td>---------</td>
</tr>
<tr>
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<td></td>
<td>L</td>
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</tr>
<tr>
<td></td>
<td>R</td>
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<td></td>
<td>R</td>
<td>38 -48 40</td>
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<td>Anterior insula (d,e)</td>
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<td>0.011</td>
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<td></td>
<td>R</td>
<td>38 28 -6</td>
<td>3.28</td>
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<td>Cerebellum (a)</td>
<td>L</td>
<td>-44 -68 -40</td>
<td>3.20</td>
<td>0.027</td>
</tr>
</tbody>
</table>

**Green > Blue x PER3<sup>5/5</sup>**

No significant voxel

**Blue > Green OR Green > Blue x PER3<sup>4/4</sup>**

No significant voxel

**Blue > Green OR Green > Blue x whole population (irrespective of genotype)**

No significant voxel

**Blue > Green OR Green > Blue x conjunction between PER3<sup>5/5</sup> and PER3<sup>4/4</sup>**

No significant voxel

* Significant wavelength-by-genotype interaction ([Blue > Green x PER3<sup>5/5</sup> x PER3<sup>4/4</sup>])

( ) Letters between brackets correspond to labels of figure 2 and 3.
**Figure 1**

### Wakefulness prior to the recording (hrs)

- **a**
  - Prior wake: 14h
  - Sleep: 1.5h
  - Sleep deprivation: 25h

### Time relative to sleep midpoint of scheduled wake time (hrs)

- **fMRI (<0.01 lux)**
- Strictly controlled period (<5 lux)
- Sleep deprivation period (<5 lux)
- Controlled period (<5 lux)

### Wakefulness prior to the recording (hrs)

- **b**
  - 3-back task: DARKNESS
  - 3-back task: LIGHT
  - Morning: +4
  - Evening: -7.5

**Figure 1**
Figure 2

Activity estimates (a.u. ± SEM)

a. CER
b. CER
c. FPC/VLPFC
d. IPS
e. VLPFC
f. DLPFC

* ns

Z = -12
Z = -34
Z = -12
Z = 26
Y = -26

PER3^4/4
PER3^5/5
Figure 3

Activity estimates (a.u. ± SEM)

- a. CER
- b. FPC
- c. FPC
- d. INS
- e. INS
- f. DLPFC
- h. PMOT
- i. THAL
- j. IPS
- k. IPS

PER3^4/4
PER3^5/5

Z = -40
Z = 40
Z = -8
Z = 22

X = -18
Y = -48

ns
* #
Figure 4

RIGHT

LEFT

IPS

FPC / VLPFC

DLPFC

DLPFC

PMOT

IPS
The effects of light on cognitive brain responses depend on circadian phase and sleep pressure

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2. Surrey Sleep Research Centre, University of Surrey, Guildford, UK.

ONLINE SUPPLEMENTAL INFORMATION
Supplemental Methods

The complete details of the procedures followed for genotyping and melatonin assays as well as the complete analyses of subjective sleepiness and performance during training can be found in (Vandewalle et al. 2009).

Sleep-wake cycles and timing of fMRI sessions

Volunteers were requested to refrain from all caffeine and alcohol-containing beverages and intense physical activity for 3 days before participating in the study. After both segments of the experiment, actigraphy data were visually inspected and statistical analyses revealed no significant difference between genotypes in sleep onset and wake times, sleep midpoint and sleep durations during the 7 days of scheduled sleep preceding each experimental segment (p>0.1). Clock times of fMRI sessions, time awake before each fMRI session, time of fMRI sessions relative to actigraphy-assessed sleep midpoint were also not significantly different between the genotype (p>0.1) [See Supplemental Results and Tables of (Vandewalle et al. 2009) for full details].

Light exposure

This design was based on our previous study which tried to separated S- and M- cone and melanopsin ipRGCs contribution to the non-classical impact of light on brain function using 430nm (violet), 527nm (green) and 473nm (blue) monochromatic light, respectively (Vandewalle et al. 2007). For the present study, we used the same blue and green monochromatic light (473 and 527nm, respectively), although the maximal sensitivity of the classical photopic pathway is situated around 550nm. The green light we used remains much more visually effective and therefore our design still allows for
the detection of a non-classical impact of light. In addition, comparing blue and green light of these wavelengths has the advantage of reducing the potential confound of a contribution of rods to the observed effects, because maximal sensitivity of rods (~505nm) is roughly intermediate between 473 and 527nm.

Technical limitations at the time of the experiment did not allow for the administration of higher irradiance levels than those used (3x10^{13}\text{ph/cm}^2/\text{s}). In addition, at the time the research was planned and undertaken (2006-2007), the involvement of rods in non-classical responses to light had not yet been demonstrated. Because the sensitivity of melanopsin expressing ganglion cells is relatively low (Lall et al. 2010), care was taken not to use irradiances that would be too low for detecting a significant impact of only 1 min of light exposure. Therefore, a lower irradiance level of 7x10^{12}\text{ph/cm}^2/\text{s} was chosen. Analyses including irradiance as a factor were un-conclusive mainly due to a sensitivity issue. Increasing irradiance differences and the number of trials or task blocks by lengthening the protocol are required to investigate the impact of irradiance on our results [see supplemental results of (Vandewalle et al. 2010) for a more complete discussion of this issue].

Narrow interference band-pass filters (Full Width at Half Maximum [FWHM]: 10nm; Edmund Optic, UK) were used to produce the three monochromatic illuminations. A filter wheel (AB301-T, Spectral Products, NM) was computer controlled to switch band-pass filters. The light was transmitted by a metal-free optic fiber from a source (PL900, Dolan-Jenner Industries, MA) to small diffusers that ensured a uniform illumination over most of the visual field. Light was administered through a 4 x 5.5 cm
frame placed 3 cm away from the eye. Spectra of each monochromatic light were checked at the level of the diffusers (AvaSpec-2048, Avantes, The Netherlands), and the 480-nm and 532-nm band-pass filters used produced light with a maximum radiance at respectively 472.8 nm and 527.3 nm. Irradiance could not be measured directly in the magnet, but the light source was calibrated (840-C power meter, Newport, Irvine, CA) after correction for prereceptoral lens absorption for the different wavelengths (Stockman and Sharpe 2000).

In order to un-correlate task and light onsets, the auditory task was performed during 30 s of the 60-s illumination periods. Half of the illuminations started with 30 s of rest, the other half terminated with 30-s rest periods. Darkness periods (< 0.01 lux) separated all 60-s illuminations. The auditory task was performed during half of the darkness periods, the duration of which were then 5 to 12.5 s. Rest was requested during the other half; in which case darkness was lasting 9 to 14 s. Illuminations with one color were always followed by darkness periods and then by illuminations in the other color of the session.

Note that we chose to have a constant order for both recording sessions, T1 (darkness) and T2 (light exposures). Before investigating the non-classical impact of light on brain function we had to demonstrate that sleep-loss had a differential impact on brain function in the two PER3 VNTR homozygous genotypes in the absence of light. Performing the task for 14 min and being exposed to blue and green light exposure in T2 could have affected brain activity in T1 if it were to be placed first. Comparison between T2 and T1 to isolate the separate non-classical impact of blue and green light (Gooley et
al. 2010) were therefore biased by an order effect and were not computed. Isolating the contribution of each wavelength was also not possible because of the absence of a valid estimation of activation in darkness during T2 (data in darkness are too sparse).

3-back task
The n-back task requires that subjects state whether or not each presented item is identical to the item presented n stimuli earlier (Cohen et al. 1997). Our stimuli consisted of nine French monosyllabic consonants, which were 500 ms long and the inter-stimulus-interval was 2500 ms. For each consonant, volunteers were requested to state whether or not it was identical to the consonant presented 3 stimuli earlier, by pressing a button on an MR compatible keypad. Series of stimuli were constructed with ~30 % positive answers. Twelve consonants were presented in each illumination period for a total of 30 s, and 2 to 5 consonants were presented in half of the darkness periods, for a total of 5 to 12.5 s. Series could, therefore, be 29 consonant long if a darkness period with the task was placed between 2 consecutive illumination periods during which the task was performed. Series were presented only once and were randomly assigned to one of the scanning sessions. Rest periods could last up to 64 s if a rest period in darkness was placed between two consecutive illumination rest periods. Stimuli were produced using COGENT 2000 (http://www.vislab.ucl.ac.uk/Cogent/) implemented in MATLAB (Mathworks Inc., MA) and were transmitted to the subjects using MR CONTROL amplifier and headphones (MR Confon, Germany). The first session
was preceded by a short session during which volunteers had to set the volume level to ensure an optimal auditory perception.

**Sleep EEG recordings and analysis**

Polysomnography was recorded during the sleep segment of the experiment. Data were recorded at Fz, Cz, Pz and Oz (reference: left mastoid), bipolar vertical and horizontal EOG, chin EMG, at 500 Hz, from DC to Nyquist frequency (Vamp, BrainProducts; Synamp, Neuroscan). Sleep was scored following standard criteria (Rechtstchaffen and Kales 1968), sleep cycles were visually determined. After manual exclusion of period of artifacts (eye or body movements, arousals, etc), EEGs were subjected to spectral analysis on Fz using a Fast Fourier Transform (4-s Welch window, 2-s steps, Hanning window) resulting in a 0.25 Hz spectral resolution. Relative spectral densities were obtained by normalizing power density in any given frequency band by the total power from 0.5 to 20 Hz. ANOVA with EEG power spectral density from 0.5Hz to 4Hz (known as slow wave activity [SWA]) as within subject factor and genotype as between subject factor, were followed when necessary by post hoc tests.

*A priori* locations of interest used for fMRI multiple comparison correction over small spherical volumes

*A priori* areas of interest and the publications in which they were reported.

Frontopolar cortex: -30 49 1 mm, 30 49 1 mm (Collette et al. 2007)

Lateral prefrontal cortex: 44 24 22 mm, 58 18 22 mm, -54 32 12 mm, -46 6 34 mm, -48 16 28 mm, -54 16 34 mm, -50 30 32 mm (Vandewalle et al. 2009); -40 32 20 mm, -32 -8 56 mm (Koechlin et al. 2003), -30 39 0 mm (Bedwell et al. 2005)
Intraparietal sulcus: 42 -32 36 mm, -34 -48 46 mm, -44 -40 48 mm (Vandewalle et al. 2009)

Thalamus: -18 -24 10 mm (Vandewalle et al. 2007)

Insula: -28 22 -6 mm, 28 24 -8 mm (Vandewalle et al. 2009)

Cerebellum: -48 -42 68 mm (2006), -32 -62 -38 mm, 32 -56 -34 mm, 30 -60 -38 mm (Vandewalle et al. 2009)
Supplemental Results

Demographics

Demographic results have been published previously (Vandewalle et al. 2009). PER3\(^{4/4}\) and PER3\(^{5/5}\) populations did not significantly differ for any of the measured characteristics (Table S1), except for the Epworth Sleepiness Scores (ESS), which measures the self-assessed probability of falling asleep in various non-stimulating situations (Johns 1991). It is worth emphasizing that these scores were obtained at least one week before the experiment, i.e. before being assigned to a regular sleep schedule for 7 days. It is also important to note that the significant difference between genotypes in ESS scores was not related to the difference we observed in response to light as indicated by regressions analyses performed in SPM, which did not lead to any significant results for any of the contrasts of interest.

Performance during the training session

Subjects reached on average a performance of 87 ± 13.9 % on the last task blocks and there were no significant differences between the genotypes (p=0.72).

Behavior of the morning session after sleep (1.5h of wakefulness)

Subjective sleepiness

Subjective sleepiness was assessed by the Karolinska Sleepiness Scale (KSS) (Akerstedt and Gillberg 1990) and did not differ between the genotypes (p=0.74) [Figure S1a; see (Vandewalle et al. 2009) for full details].
Accuracy to the task

Repeated-measures ANOVA with genotype (PER3\textsuperscript{4/4}, PER3\textsuperscript{5/5}) as between-subject factor, and light-condition (blue, green) as within-subject factor on performance to the task (accuracy), revealed no significant main effect of the light condition (F = 0.04; df = 1, 25; p = 0.84) and a trend for genotype (F = 3.63; df = 1, 25; p = 0.07). There was no significant light condition-by-genotype interaction (F = 0.40; df = 1, 25; p = 0.53) (Figure S1b).

Reaction times

Subjects were instructed to privilege accuracy to reaction times and to wait for the sound to be over before responding. Reaction times were nevertheless analyzed for the sake of completeness.

Repeated-measures ANOVA with genotype (PER3\textsuperscript{4/4}, PER3\textsuperscript{5/5}) as between-subject factor and light-condition (blue, green) as within-subject factor, showed a trend for light-condition (F = 3.18; df = 1, 25; p = 0.09) whereas genotype (F = 1.8; df = 1, 25; p = 0.19), and the light-condition-by-genotype interaction (F = 0.006 ; df = 1, 25; p = 0.94) were non-significant (Figure S1c).

Behavior of the evening sessions (14h of wakefulness) before sleep and before sleep loss

Subjective sleepiness
Subjective sleepiness scores collected in the evening before sleep or in the evening before sleep loss did not differ significantly between the genotypes (p≥0.70) [Figure S2a; see (Vandewalle et al. 2009) for full details].

**Accuracy to the task**

Repeated-measures ANOVA with genotype (PER34/4, PER35/5) as between-subject factor, and light-condition (blue, green) as within-subject factor on performance to the task (accuracy) in the evening before sleep, revealed no significant main effect of light-condition (F = 1.79; df = 1, 25; p = 0.19), nor genotype (F = 0.04; df = 1, 25; p = 0.84), as well as no significant light-condition-by-genotype interaction (F = 0.92 ; df = 1, 25; p = 0.35) (Figure S2b, left panel).

Repeated-measures ANOVA with genotype (PER34/4, PER35/5) as between subject factor, and light-condition (blue, green) as within-subject factor on performance to the task (accuracy) in the evening before sleep loss, revealed no significant main effect of light-condition (F = 0.10; df = 1, 25; p = 0.76), nor genotype (F = 0.13; df = 1, 25; p = 0.72), as well as no significant light-condition-by-genotype interaction (F = 0.69 ; df = 1, 25; p = 0.41) (Figure S2b, right panel).

**Reaction times**

For reaction times during the evening before sleep, repeated-measures ANOVA with genotype (PER34/4, PER35/5) as between-subject factor, and light-condition (blue, green) as within-subject factor, showed that the effect of light-condition (F = 0.96; df = 1, 25; p = 0.34), genotype (F = 2.64; df = 1, 25; p = 0.12), and the light-condition-by-genotype interaction (F = 0.09 ; df = 1, 25; p = 0.77) were all non-significant (Figure S2c, left panel).
Repeated measures ANOVA with genotype ($PER3^{4/4}$, $PER3^{5/5}$) as between-subject factor, and light-condition (blue, green) as within-subject factor on reaction times of the 

*evening before sleep-loss*, showed that the effect of light-condition ($F = 0.03; \text{df} = 1, 25; \ p = 0.86$), genotype ($F = 0.26; \text{df} = 1, 25; \ p = 0.61$), and the light-condition-by-genotype interaction ($F = 0.68 ; \text{df} = 1, 25; \ p = 0.42$) were all non-significant (Figure S2c, left panel).

**Behavior of the morning session during sleep-loss (25h of wakefulness)**

**Subjective sleepiness**

Subjective sleepiness scores of the sleep deprivation period were analyzed in 2 steps [see (Vandewalle et al. 2009) for full details]. First, we considered the KSS scores collected during the 60 minutes of strictly controlled activity that preceded each fMRI session and while subjects were in the scanner, i.e. the scores collected after the first and second runs (Figure S3a). Analysis revealed that these scores were significantly higher than the corresponding score collected during the morning-after-sleep session ($p<0.001$) but did not differ between the genotypes ($p=0.62$). We then considered the KSS scores collected during the period of total sleep deprivation (Figure S3b), before the beginning of the 60 minutes of strictly controlled activity (values already analyzed above were not included). Analysis showed that subjective sleepiness did not differ between the genotype during the night of sleep deprivation ($p = 0.97$).

**Accuracy to the task**

Repeated measure ANOVA with genotype ($PER3^{4/4}$, $PER3^{5/5}$) as between-subject factor, and light-condition (blue, green) as within-subject factor on performance to the task (accuracy), revealed no significant main effect of the light-condition ($F = 1.51; \text{df} = 1, 25$;
p = 0.23) nor genotype (F = 0.035; df = 1, 25; p = 0.85), as well as no significant light condition-by-genotype interaction (F = 2.53 ; df = 1, 25; p = 0.12) (Figure S3c).

**Reaction times**

Repeated-measures ANOVA with genotype (PER34/4, PER35/5) as between-subject factor, and light-condition (blue, green) as within-subject factor on reaction times, showed that the effect of light-condition (F = 2.72; df = 1, 25; p = 0.11), genotype (F = 0.028; df = 1, 25; p = 0.60), and the light-condition-by-genotype interaction (F = 0.52 ; df = 1, 25; p = 0.48) were all non-significant (Figure S3d).

**Spectral EEG analysis**

Analyses of relative SWA data revealed a significant effect of cycle (F= 25,863; df = 3; p< 0.001) but not of genotype (F = 0.848; df = 1; p = 0.36) (Figure S4) and a trend for the cycle-by-genotype interaction (F = 2.06; df = 1,3; p = 0.11). Exploratory post-hoc Fischer tests showed that the only significant difference was a higher relative slow wave activity in PER35/5 than in PER34/4 during the first non-rapid eye movement (NREM) cycle (p< 0.05). This is in accordance with our previous data (Viola et al. 2007) and in accordance with the hypothesized faster build up of homeostatic sleep pressure in PER35/5. SWA returns to similar level in both genotypes in the fourth (last) NREM cycle suggesting that the dissipation of SWA is faster in PER35/5 and that PER35/5 individuals benefit more from sleep (Dijk and Archer 2010).
SUPPLEMENTAL REFERENCES


SUPPLEMENTAL FIGURE LEGENDS

Figure S1: Behavioral results in the morning after sleep (1.5 h of wakefulness).

Time (hrs) relative to sleep midpoint. **Blue: PER3^{4/4}. Red: PER3^{5/5}**.

a. Subjective sleepiness during the hour preceding the fMRI session after a night of sleep and while participants were in the MR scanner.

b. Accuracy to the 3-back task performed in the MR scanner under blue and green light exposure (mean ± SD).

c. Reaction times during the 3-back task performed in the MR scanner under blue and green light exposure (mean ± SD).

Figure S2: Behavioral results in the evening (14 h of wakefulness) before sleep (left panels) and before sleep-loss (right panels).

Time (h) relative to sleep midpoint. **Blue: PER3^{4/4}. Red: PER3^{5/5}**.

a. Subjective sleepiness during the hour preceding the fMRI session after a night of sleep and while participants were in the MR scanner.

b. Accuracy to the 3-back task performed in the MR scanner under blue and green light exposure (mean ± SD).

b. Reaction times during the 3-back task performed in the MR scanner under blue and green light exposure (mean ± SD).

Figure S3. Behavioral results in the morning following sleep loss (25 h of wakefulness).

Time (h) relative to sleep midpoint. **Blue: PER3^{4/4}. Red: PER3^{5/5}**.
a. Subjective sleepiness (KSS scores) during the night of total sleep deprivation.

b. Subjective sleepiness during the hour preceding the fMRI session after a night of sleep deprivation and while participants were in the MR scanner.

c. Accuracy to the 3-back task performed in the MR scanner under blue and green light exposure (mean ± SD).

d. Reaction times during the 3-back task performed in the MR scanner under blue and green light exposure (mean ± SD).

Figure S4: Relative power density of the slow wave activity band during sleep in both genotypes.

Mean (± SEM) relative power density of the 0.5-4Hz band for the 4 non-REM sleep cycles of the night of sleep of the sleep segment in both PER3 genotypes.

Blue: PER3^{4/4}. Red: PER3^{5/5}.

* significant difference between the genotypes for the first non-REM sleep cycle (p<0.05)
SUPPLEMENTAL TABLE

Table S1: Subject characteristics [mean +/- SD] [Already published in (Vandewalle et al. 2009)].

<table>
<thead>
<tr>
<th>PER3 genotype</th>
<th>PER3^{4/4}</th>
<th>PER3^{5/5}</th>
<th>P\text{_value}</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>15</td>
<td>12*</td>
<td></td>
</tr>
<tr>
<td>AGE</td>
<td>24.13 ± 0.95</td>
<td>24.17 ± 1.17</td>
<td>0.98</td>
</tr>
<tr>
<td>BODY MASS INDEX</td>
<td>21.9 ± 0.59</td>
<td>21.9 ± 0.59</td>
<td>0.49</td>
</tr>
<tr>
<td>SEX (M/F)</td>
<td>8 / 7</td>
<td>7 / 5</td>
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</tr>
<tr>
<td>CHRONOTYPE</td>
<td>49.80 ± 3.80</td>
<td>52.33 ± 2.90</td>
<td>0.59</td>
</tr>
<tr>
<td>YEARS OF EDUCATION</td>
<td>17.1 ± 0.68</td>
<td>16.5 ± 0.6</td>
<td>0.52</td>
</tr>
<tr>
<td>IQ</td>
<td>125.5 ± 2.85</td>
<td>125.9 ± 3.19</td>
<td>0.72</td>
</tr>
<tr>
<td>ANXIETY LEVEL</td>
<td>5.13 ± 1.05</td>
<td>5.33 ± 1.16</td>
<td>0.89</td>
</tr>
<tr>
<td>MOOD</td>
<td>3.6 ± 0.83</td>
<td>5.42 ± 1.33</td>
<td>0.22</td>
</tr>
<tr>
<td>SLEEP DISTURBANCE</td>
<td>3.33 ± 0.51</td>
<td>4.17 ± 0.53</td>
<td>0.24</td>
</tr>
<tr>
<td>DAYTIME PROPENSITY TO FALL ASLEEP (Epworth Sleepiness Scale)</td>
<td>3.70 ± 0.64</td>
<td>6.96 ± 1.07</td>
<td>0.008*</td>
</tr>
<tr>
<td>ORDER: Sleep segment before Sleep Deprivation segment</td>
<td>6/15</td>
<td>6/12</td>
<td>0.45</td>
</tr>
<tr>
<td>Women using oral contraceptive</td>
<td>6/7</td>
<td>5/5</td>
<td>0.58</td>
</tr>
<tr>
<td>Women in luteal phase</td>
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<td>0/5</td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td>Caucasian (all)</td>
<td>Caucasian (all)</td>
<td></td>
</tr>
<tr>
<td>Right handed participants</td>
<td>15/15</td>
<td>12/12</td>
<td></td>
</tr>
</tbody>
</table>

ANXIETY LEVEL was measured on the 21 item Beck Anxiety Inventory (BAI) (Beck et al. 1988); CHRONOTYPE was assessed by the Horne-Östberg Questionnaire (Horne and Östberg 1976); DAYTIME PROPENSITY TO FALL ASLEEP during daytime non-stimulating situations was assessed by the Epworth Sleepiness Scale at least one week prior to the first segment of the protocol (Johns 1991); IQ (intellectual quotient) was assessed by the Advanced Progressive Matrices (Raven et al. 1998); MOOD was assessed using the 21 item Beck Depression Inventory II (BDI-II) (Steer et al. 1997); SLEEP DISTURBANCE was determined by the Pittsburgh Sleep Quality Index Questionnaire (Buysse et al. 1989). The Edinburgh Inventory (Oldfield 1971) was administered to verify that the participants were right-handed.

* 13 PER3^{5/5} completed the protocol but one fell asleep during the MSD session and was removed from all analyses. Note that levels of statistical significance in this table were not affected if the subject was included.

*# this significant difference was not related to the difference we observed in response to light.
Figure S2

Accuracy to the task (%) Reaction time (ms)

PER3^{4/4} PER3^{5/5}
Figure S3

(a) Sleep loss fMRI acquisitions

(b) Overnight sleep deprivation

(c) Accuracy to the task (%)

(d) Reaction time (ms)

- PER34/4
- PER35/5

Time relative to sleep midpoint (hr)