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Research report

Light modulation of human sleep depends on a polymorphism in the clock gene *Period3*

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HIGHLIGHTS

- Non-image-forming (NIF) responses to light on sleep show individual differences.
- "Blue" light increased occipital NREM slow-wave activity in *PER3*$^{5/5}$ individuals.
- *PER3*$^{5/5}$ individuals perceive "blue" light as being brighter.
- Humans homozygous for the *PER3*$^{5/5}$ allele are more sensitive to NIF light effects.

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ABSTRACT

Non-image-forming (NIF) responses to light powerfully modulate human physiology. However, it remains scarcely understood how NIF responses to light modulate human sleep and its EEG hallmarks, and if there are differences across individuals. Here we investigated NIF responses to light on sleep in individuals genotyped for the *PERIOD3 (PER3)* variable-number tandem-repeat (VNTR) polymorphism. Eighteen healthy young men (20–28 years; mean ± SEM: 25.9 ± 1.2) homozygous for the *PER3* polymorphism were matched by age, body-mass index, and ethnicity. The study protocol comprised a balanced cross-over design during the winter, during which participants were exposed to either light of 40 lx at 6500 K (blue-enriched) or light at 2500 K (non-blue enriched), during 2 h in the evening. Compared to light at 2500 K, light at 6500 K induced a significant increase in all-night NREM sleep slow-wave activity (SWA: 1.0–4.5 Hz) in the occipital cortex for *PER3*$^{5/5}$ individuals, but not for *PER3*$^{4/4}$ volunteers. Dynamics of SWA across sleep cycles revealed increased occipital NREM sleep SWA for virtuallyall sleep episode only for *PER3*$^{5/5}$ individuals. Furthermore, they experienced light at 6500 K as significantly brighter. Intriguingly, this subjective perception of brightness significantly predicted their increased occipital SWA throughout the sleep episode. Our data indicate that humans homozygous for the *PER3*$^{5/5}$ allele are more sensitive to NIF light effects, as indexed by specific changes in sleep EEG activity. Ultimately, individual differences in NIF light responses on sleep may depend on a clock gene polymorphism involved in sleep–wake regulation.

1. Introduction

Retinal photoreception encompasses not only rods and cones, but also a small subset of intrinsically photosensitive retinal ganglion cells (ipRGCs) expressing the photopigment melanopsin [1–3]. The ipRGCs play a major role to circadian entrainment, subjective and objective alertness, cognitive brain function, and numerous non-image-forming (NIF) responses [4–6], with peak sensitivity at the short-wavelength range (ca. 460–480 nm) [1,7]. A direct retinal pathway originating from ipRGCs to the ventrolateral preoptic nucleus (VLPO) provides morphological support that light modulates sleep [2]. In mice, light induces sleep during their active phase, through rod-cone and melanopsin-based pathways [3]. These short-term light effects on sleep are further described in two studies [3,8], where mice lacking melanopsin fail

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to sleep when light is presented during their active period. Furthermore, melanopsin knockout mice (Opn4−/−) showed attenuated NREM sleep delta power, a reliable marker of sleep homeostasis, during light exposure at their active period, indicating that melanopsin pathways modulate sleep homeostasis [9]. In humans, light's wavelength-dependency on sleep is such that monochromatic blue (460 nm), relative to green light (550 nm), impacts on the dynamics of NREM sleep EEG activity, with less SWA in the first sleep cycle and a rebound in the third sleep cycle [10]. In a study using polychromatic light settings, morning light reduces subsequent sleep duration by ca. 1 h (particularly REM sleep duration), with no impact on EEG activity from 0.25 to 15 Hz for NREM sleep and REM sleep [11]. Conversely, evening polychromatic light exposure has been shown to increase NREM stage 2 latency [11,12]. These numerous light effects on sleep point to individual differences that may modulate the impact of light. Novel data indicate that a variable-number tandem-repeat (VNTR) polymorphism in the clock gene *PERIOD3* (*PER3*) impacts on cognitive brain responses to light [13], and on melatonin suppression and subjective/objective alerting action of light [14]. However, it is currently unknown if a differential short-term responses to light on human sleep phenotypes are modulated by the *PER3* VNTR polymorphism. Here we investigated if individual differences in non-image-forming responses to light on sleep depend on a *PER3* polymorphism directly involved in sleep–wake regulation [15].

2. Methods

2.1. Participants

Detailed description of study participants, selection criteria and study protocol is provided elsewhere [14]. Eighteen healthy male volunteers (20–28 years; mean ± SEM: 25.9 ± 1.2) homozygous for the *PER3* polymorphism (9 $PER3^{4/4}$, 9 $PER3^{5/5}$) were matched by age, body-mass index (BMI), and ethnicity. No significant differences were observed between the two groups for age, BMI, and ethnicity (all Caucasians). All participants gave written informed consent. The study was approved by the local ethics committee (EKBB/Ethikkommission beider Basel, Switzerland) and conformed to the Declaration of Helsinki.

2.2. Protocol

A balanced cross-over design study was carried out during the winter season (January to March), with three segments separated by one week. The protocol started 10 h after volunteers' habitual wake-up time and ended the next day after usual wake-up time. Sleep–wake schedules were assessed by wrist actigraphy (actiwatch L, Cambridge Neurotechnology Ltd., Cambridge, UK) and self-reported sleep logs. During each protocol, participants underwent successively 1.5 h under dim light (<8 lx), 2 h under complete darkness, 2 h light exposure (compact fluorescent lamps with 6500 K or 2500 K or incandescent light bulbs at 3000 K), and a post-light period of ca. 45 min under dim light (<8 lx) until habitual sleep time. In our study, prior light exposure was controlled for such that participants were under 1.5 h of dim light and 2 h of darkness before light exposure, since it impacts on subsequent light sensitivity [16]. As a result, photoreceptor systems achieve a stable state of photo-equilibrium, through a reduction on the 'bleaching effect' of previous light exposure [17]. During the "pre-light exposure" (dim and dark), participants continuously performed waking EEGs, a cognitive test battery, salivary melatonin and cortisol samples, questionnaires for visual comfort, visual analogue scales, subjective sleepiness scales, and mental effort scales, under the same conditions as for the light exposure. In other words, they performed exactly the same tasks as for the light exposure, under virtually identical settings. The post-light exposure was used to slightly minimize the possibility of longer time to fall asleep (sleep latency to NREM stage 2), due to the immediately preceding light exposure. Each protocol was conducted at the same time-of-day (evening), and light intensity (ca. 40 lx) for each participant. Light at 40 lx was used since it is a typical indoor environmental intensity in naturalistic settings, during the evening hours. The treatment order (6500 vs. 2500 K vs. 3000 K) was counter-balanced to avoid possible order effects of the light conditions. Detailed information of light settings and study rationale are provided in [18]. Here we report data on light exposure to 6500 K and 2500 K, since exposure to 2500 K and 3000 K resulted in very similar effects.

2.3. Genotyping

DNA was extracted with the NucleoSpin®Tissue Kit (Marchery-Nagel AG, Oensingen, Switzerland). All genotypes were determined with allele-specific polymerase chain reaction (PCR) on a MJ Research PTC-225 thermal cycler (MJ Research/Bio-Rad, Reno, NV, USA) using Hot FIREPol® DNA polymerase and a forward and reverse primer. The *PER3* forward primer was as follows: 5′ TTACAGGCAACAATGGCAGT 3′ and the reverse primer: 5′ CCACTACCTGATGCTGCTGA 3′ (annealing temperature: 59 °C, 25 mM $MgCl_2$).

2.4. Subjective perception of Visual comfort

To assess each participant's subjective perception of visual comfort, we used the validated visual comfort scale (VCS) [19], which consists of a visual analogue scale with a 100 mm scale that probes visual well-being, comfort, and brightness.

2.5. Salivary melatonin

Salivary melatonin was assessed every 40 min during wakefulness. A direct double-antibody radioimmunoassay was used for melatonin assays (validated by gas chromatography–mass spectroscopy with an analytical least detectable dose of 0.65 pm/mL; Bühlmann Laboratory, Schönenbuch, Switzerland) [20]. The minimum detectable dose of melatonin (analytical sensitivity) was set at 0.2 pg/ml.

2.6. Polysomnographic recordings

Sleep EEG activity was recorded continuously during the scheduled sleep period with the Vitaport Ambulatory system (Vitaport-3 digital recorder TEMEC Instruments BV, Kerkrade, the Netherlands). Eight EEG derivations (F3, F4, C3, C4, P3, P4, O1, O2, referenced against linked mastoids, A1 and A2), two electrooculograms, two submental electromyograms, and two electrocardiograms were recorded. All signals were low pass filtered at 30 Hz (fourth order Bessel type anti-aliasing, total 24 dB/Oct) at a time constant of 1.0 s. After online digitization by using a 12 bit AD converter (0.15 μV/bit) and a sampling rate at 128 Hz for the EEG, the raw signals were stored on a flash RAM card (Viking, Rancho Santa Margarita, CA, USA) and later downloaded to a PC hard drive. Sleep stages were visually scored per 20 s epochs (Vitaport Paperless Sleep Scoring Software), according to [21], by a single experienced polysomnography technician, blind to genotype and light conditions. NREM sleep was defined as the sum of NREM stages 2, 3, and 4. Slow wave sleep (SWS) was defined as the sum of NREM sleep stages 3 and 4. EEG artifacts were detected by an automated artifact algorithm (CASA, 2000 PhyVision B.V., Gemert, The Netherlands). Spectral analysis was conducted using a fast Fourier transformation (FFT; 10% cosine 4 s window), which yielded a 0.25 Hz bin resolution. EEG power spectra were calculated