Biological Rhythms and Non-Classical Photoreception

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Attenuated circadian rhythms in mice lacking the prokineticin 2 gene

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Circadian clocks drive daily rhythms in virtually all organisms. In mammals, the suprachiasmatic nucleus (SCN) is recognized as the master clock that synchronizes other central and peripheral oscillators to evoke circadian rhythms of diverse physiology and behaviour. How the timing information is transmitted from the SCN clock to generate overt circadian rhythms is largely unknown. Prokineticin 2 (PK2), a clock-controlled gene that encodes a secreted protein, has been indicated as a candidate SCN clock output signal that regulates circadian locomotor rhythm. A powerful approach to gain insight into the physiological function of PK2 in circadian rhythmicity is inactivation of the PK2 gene. Here we report the generation and analysis of PK2-null mice. The majority of PK2-null mice was rhythmic and exhibited identical free-run period as wild-type mice. However, PK2-null mice exhibited significantly reduced rhythmicity for a variety of physiological and behavioral parameters, including locomotor activity, sleep-wake cycle, body temperature, food intake, circulating glucocorticoid and glucose levels as well as the expression of peripheral clock genes. In addition, PK2-null mice showed accelerated acquisition of food anticipatory activity (FAA) during a day-time food restriction, further confirming the weaker control of circadian rhythm from the SCN. We conclude that PK2 is a SCN output factor and is critical for the maintenance of robust circadian rhythms.

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Polymorphism in the clock gene PER3 predicts sleep structure and EEG power spectra

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Morningness-eveningness as assessed by the Horne-Östberg scale is associated with a variable number tandem repeat (VNTR) polymorphism in the clock gene PER3. The objective of the current investigation was to characterise sleep timing and sleep structure in subjects homozygous for this polymorphism. Healthy volunteers were selected on the basis of their genotype, independent of diurnal preference or any other characteristic related to sleep. More than 250 volunteers (age 20–35) were genotyped for the PER3 polymorphism. Individuals homozygous for the rarer 5-repeat VNTR (PER3-5/5) were identified and matched for age, gender, ethnicity and body mass index with individuals homozygous for the 4-repeat VNTR (PER3-4/4). Subjects ($n = 28$) wore actigraphs and completed daily sleep diaries for three weeks prior to a five-day laboratory study. The laboratory study was completed by 24 subjects and consisted of two baseline sleep episodes followed by an approximately 40-h constant routine and a recovery sleep episode. The questionnaires and sleep diary analyses indicate that the two groups did not differ with respect to either their Horne-Östberg score or habitual sleep-wake timing and sleep duration. ($P > 0.05$). Preliminary analyses of the PSG data revealed that the PER3-5/5 and PER3-4/4 subjects differ in sleep architecture. During baseline sleep the duration and percentage of slow wave sleep and stage 4 sleep was greater in PER3-5/5 subjects than in PER3-4/4 subjects ($P < 0.05$). This difference was confirmed by power spectral analysis of the EEG which showed differences in the time course of SWA between the genotypes. Analysis of EEG power spectra during REM sleep demonstrated robust and statistically reliable effects of genotype on power density in the theta/alpha range such that the absolute values were highest in PER3-5/5 subjects. These data show that a VNTR polymorphism in the circadian clock gene PER3 modulates sleep structure and EEG power spectra during sleep in humans.

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Circadian periodicity of the melatonin rhythm and cellular per2 oscillations in early and late human chronotypes

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Humans prefer sleeping at night, but not everybody sleeps at the same time. In the framework of the 5th European Framework Program BRAINTIME, early and late sleepers were selected by the Munich Chronotype Questionnaire on the basis of their sleep timing on free days. They participated in an experiment under time isolation to investigate functioning of their biological clock. The *in vivo* period length of the melatonin rhythm was calculated based on regularly collected saliva samples during 3 days of continuous red dim light exposure. In addition a skin biopt of each individual was obtained. Subsequent analysis by a real-time clock read out system of the cultured human skin primary fibroblasts, infected with a Per2:luc lentivirus, revealed period lengths of the cellular Per2 oscillations *in vitro*. Average period length ($\pm$SE) of the melatonin rhythm is significantly shorter in early chronotypes than in late chronotypes; 24.3 h $\pm$ 0.1 ($n = 9$) versus 24.7 h $\pm$ 0.1 ($n = 9$) respectively, $P < 0.01$ (Mann–Whitney, 1-tailed). Analysis of cellular Per2 oscillations revealed much longer period lengths. Average period length ($\pm$SE) of Per2 oscillations in early types is 27.4 h $\pm$ 0.2 ($n = 5$), in late types 28.0 h $\pm$ 0.2 ($n = 8$), $P < 0.03$ (Mann–Whitney, 1-tailed). No significant correlation is observed between the period lengths of the melatonin rhythm and the cellular Per2 oscillations in the complete data set: $r_s = 0.34$, $n = 12$, NS. The lack of correlation between *in vivo* melatonin rhythmicity and *in vitro* Per2 oscillations indicates that the analysis of cellular rhythmicity *in vitro* can, in the current state, not replace studies with humans under time isolation. The indication that both *in vitro* and *in vivo* rhythmicity of circadian clock parameters show shorter period lengths in early types than in late types, suggests that a core clock driven difference in period length underlies the existence of human chronotypes.

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The effects of short light-dark cycles on sleep in mice

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Light input to the retina is not only used for image formation, but for synchronization of the biological clock to the external environment as well. In addition, light may affect sleep directly. Short light-dark (LD) cycles in the range of 5–10 min – and far outside the range of entrainment of the biological clock – are able to trigger rapid eye movement (REM) sleep in the albino rat (Borbely 1976). Under influence of these LD cycles REM sleep is enhanced during the dark, whereas non-REM (NREM) sleep is enhanced during the light. We have now investigated the influence of short LD cycles in mice. Under deep anesthesia C57/BL6 mice were implanted with electroencephalogram (EEG) and electromyogram (EMG) electrodes (Deboer et al 2002). After a baseline recording in 12 h light-12 h dark the animals were subjected to 24 h continuous darkness (DD). Subsequently six experimental days started, where the animals were subjected to short LD cycles of different durations. The durations were: 2 h, 30 min, 14 min, 10 min, 4 min, and 2 min and were presented to the animals in a randomized order. Each condition lasted 24 h. The amount of NREM and REM sleep was significantly reduced during DD, but EEG slow-wave activity (SWA; EEG power density between 0.75–4.0 Hz) in NREM sleep did not differ from the recordings in LD. During the different short LD cycles, sleep and waking were redistributed evenly over 24 h, but the total amount of the different vigilance states over 24 h did not differ from baseline. LD schedules of 14 and 10 min were able to induce an increase in REM sleep in the 2nd 1-min interval of the dark period. The data show that also in C57BL/6 mice sleep can be directly influenced by short LD cycles. In contrast to albino rats (Tobler et al 1994) EEG SWA during NREM sleep was not increased under DD conditions. In mice REM sleep can be induced in the dark by LD cycles of similar durations as in albino rats.

Reference:

1. Borbely AA (1976) Brain Res 114: 305–317.
2. Deboer T, et al (2002) J Neurophysiol 88: 839–846.
3.Tobler I, et al (1994) Behav Brain Res 63: 205–211.

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Superiority of blue (470 nm) light in eliciting non-image forming brain responses during auditory working memory in humans: a fMRI study

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Light exerts profound effects on physiology and behaviour in humans. Some of these effects may be mediated in part by a recently discovered, blue (470 nm) light sensitive photoreceptor system. Healthy volunteers (n = 18) participated in a fMRI experiment during two visits, 2 days apart. On both visits, they were maintained in dim-light (< 5 lux) for 3 h before performing auditory 2-back task sessions in a 3T Allegra MR scan (Siemens, Germany) (32 slices, voxel size: 3.4 x 3.4 x 3 mm, TR: 2130ms, TE: 40ms, FA: 90°). Sessions were acquired before (< 0.01 lux), during and after (< 0.01 lux) one eye was exposed to a monochromatic light (~3 x 1013 ph cm^{-2} s^{-1}). Visits were identical except for light condition (blue–470 nm or green–550 nm) and were counterbalanced. We show that, while participants perform an auditory working memory task during the day, blue monochromatic light elicits greater Non-Image Forming (NIF) responses than green monochromatic light in areas involved in working memory (left middle frontal gyrus, right insula, left intraparietal sulcus, left supramarginal gyrus), and in the left thalamus implicated in the modulation of cognition by arousal. As intended, we observe these effects without behavioral modification, excluding confounding effects of variations in alertness and performance. This is the first report of greater NIF responses to blue compared to green light exposure in brain structures involved in higher cognitive functions. The data suggest that the melanopsin photoreception system modulates subcortical structures, which in turn affect a widespread set of cortical areas. Data also have implications for lighting system design.This work was supported by FNRS, FMRE, PAI/IAP, ULg, and Wellcome Trust.
Keywords: fMRI, NIF response, blue light exposure

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Moderate intensity room light can entrain the circadian pacemaker to a non–24-h day

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While studies in humans have demonstrated that the range of entrainment of the circadian pacemaker can be expanded by use of bright light, the light intensity required to induce entrainment to a non–24-h day initiated at an adverse phase is unknown. We evaluated the efficacy of moderate intensity room light to entrain subjects to a 24.65-h day initiated at an adverse phase.

Method: Five healthy subjects participated in a 71-day inpatient protocol consisting of 3 baseline days (24 h, 16:8 h wake:sleep) followed by 60 experimental days (24.65 h, 16.43:8.22 h). Light intensity during the experimental days was ~50 lux at a height of 137 cm in the horizontal angle. The timing of sleep on the first experimental day was shifted 12 h compared to baseline to induce circadian misalignment. Circadian phase was measured by the Dim Light Melatonin Onset (DLMO) measured six times under dim light (~1.8 lux at a height of 137 cm in the horizontal angle) and postural control (before and after the experimental days, and on experimental Days 6, 20, 34 and 48). To assess entrainment, phase angle was calculated from the difference between DLMO and scheduled bedtime.

Results: The initial (1.5 h) and final (1.4 h) phase angles measured before and after the experimental days ($P > 0.05$) showed that subjects were entrained by the experimental conditions ($P > 0.05$). The interim phase angle assessments showed that entrainment was achieved within 3 weeks. The average phase angle was 6.5 h on experimental day 6 and 1.5 h on experimental day 20 ($P < 0.01$). The average phase angle was 2.4 h on experimental day 34 and 2.2 h on experimental day 48 ($P > 0.05$), indicating they had stabilized. Furthermore, these two-phase angles were not significantly different from the final phase angle (1.4 h; $P > 0.05$).

Conclusion: Our results demonstrate that moderate intensity room light is able to entrain subjects scheduled to a 24.65-h activity-rest cycle within 3 weeks even when that schedule is initiated at an adverse circadian phase.

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Light behaviour and treatment on the night-shift during winter and spring

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The dark season during winter in Scandinavia prevents workers from daylight exposure when travelling home from the night shift. This study aimed to study the influence of bright light on night work and on recovery after night shift during a dark as well as bright season of the year.

Method: A group of permanent night workers (n = 19) worked on 12-hour shifts three days every week. The group worked at a postal service centre sorting packages. The workers had a mean age of 51 years (38–63 years) and half of them (n = 9) were women. They were exposed to either a bright light exposure (BL, 1500 lux at eye level) during breaks or normal light exposure (NL) for 2 weeks in a crossover design at winter and spring. During the study period of 4 weeks each season the workers wore actigraphs with light sensors and answered daily questions on sleep, sleepiness (every 2 h). They were also questioned on sleep disturbances and light exposure in the bedroom.

Results: The amount of light, > 500 lux, received at work was 1–3 min (NL) and 9–14 min (BL) during both seasons according to light sensors. Mean 24-h average light levels reached 76 lux during winter and 483 lux during spring. Sleepiness decreased at work in the BL condition on the second night shift (rep ANOVA, condition*night, F = 5.9, P = 0.05). Interactions with the factor season demonstrated that the reduction of sleepiness was more pronounced in winter. When questioned of the estimated time of days needed for recovery after the last night shift, the BL condition yielded less time of recovery (mean BL = 1.17; mean NL = 1.61, t = 3.4, P = 0.005). Those stating more problems with disturbed sleep took greater care in blocking outdoor light during day sleep (t-test, P = 0.010) and those not blocking the light claimed to sleep more heavily, more seldom woke up and had less difficulty to fall asleep again. Conclusion: Seasonal differences in BL-treatment appear to have rather moderate influences on sleepiness and no effects on sleep for quickly rotating night workers or shift workers.

Keywords: shiftwork, bright light, season

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Circadian rhythm in degree of sleep inertia following awakening

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Introduction: Sleep inertia is the sleepiness or impaired cognitive performance immediately upon awakening, the majority of which dissipates within an hour. We tested whether there exists a circadian rhythm in the degree of sleep inertia.

Methods: A total of 14 adult subjects were studied throughout a 10-day protocol performed in dim light during which subjects slept across all circadian phases, achieved by scheduling a recurring 28-h day. Subjects were awoken using a standardized auditory stimulus three times each sleep period and immediately performed a computerized 2-min serial addition test and reported subjective sleepiness (Karolinska Sleepiness Scale). Sleep inertia was quantified as the change within 20 min of awakening. Data are presented only following awakenings from Stage II sleep. Core body temperature (CBT) was used as a circadian phase marker, and data was binned into four circadian bins (0° ± 45°, 90° ± 45°, etc.; 0° = CBT minimum). Visual General Linear Model was used to test for significant rhythmicity.

Results: Immediately upon awakening, subjects performed an average of 23 correct additions, which improved to 27 after 20 min. There was a significant circadian rhythm in sleep inertia of cognitive performance (P = 0.004), with peak improvement during the biological night (circadian bin 270° and 0°), which was 2.5 times as large as the improvement during the biological day (180°). There also was a significant circadian rhythm in sleep inertia of subjective sleepiness, with a decrease in sleepiness in the 20 min following awakening for all circadian bins, except for bin 270°.

Conclusions: There is a clear circadian rhythm in the degree of sleep inertia of cognitive performance and in the sleep inertia of subjective sleepiness. These findings may have important implications for professions requiring decision making immediately upon awakening, e.g., on-call medical professionals.

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