

Short light–dark cycles affect sleep in mice

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Abstract

Environmental light has a strong impact on human physiology and behaviour, including cognitive functioning and alertness. Previous studies have shown that short light–dark (LD) cycles influence sleep in the albino rat. Rapid eye movement (REM) sleep increases after the onset of darkness and increases after light onset. In the present study, we investigated whether light affects sleep in mice. To this purpose the electroencephalogram and electromyogram of nine adult male C57BL/6 mice was recorded under 12 : 12 h baseline LD conditions, followed by 24 h continuous darkness (DD) and 6 days with LD cycles of different durations (2 h, 30 min, 14 min, 10 min, 4 min and 2 min), presented in a randomized order. NREM sleep was evenly distributed over the light and dark intervals of all short LD cycles. REM sleep, however, was increased during the dark intervals of short (10–30 min) LD cycles. Analysis showed that in these LD cycles, the increment in REM sleep was maximal in the second minute after dark onset, where the percentage of epochs with REM sleep increased significantly to 175% of baseline values. This increase was attributable to an increase in REM sleep episode duration. The recorded responses show that sleep in mice is affected by photic stimulation. The results demonstrate that pigmented animals can show REM sleep induction after dark onset and indicate that light has significant effects on the regulation of sleep.

Introduction

Light is important for life on earth, not only for the production of oxygen by means of photosynthesis in plants but also for regulation of a number of functions in animal species. In the first place, light provides a direct and detailed representation of the spatial environment. This is the reason that most research on light perception in mammals has concentrated on the processing of visual information in brain areas involved in pattern recognition. However, several functions in animals depend on photoreception that does not involve pattern recognition, such as synchronization to the light–dark (LD) cycle or regulation of pupillary reflexes.

Recently it has become clear that a subset of retinal ganglion cells (RGCs) is intrinsically photoreceptive and does not require rod or cone stimulation to become excited. These cells forward information about ambient light conditions to areas of the brain involved in tasks, including entrainment of the circadian clock (Gooley *et al.*, 2003), pupillary light reflexes and melatonin synthesis (Berson *et al.*, 2002; Berson, 2003), but not vision. Some of the brain areas that receive information from the RGCs are involved in sleep regulation, suggesting that light may have widespread effects in the central nervous system. These brain areas include the ventrolateral preoptic area and the ventral subparaventricular zone (Gooley *et al.*, 2003).

Light has an indirect effect on sleep via its influence on the circadian clock, but light also has a direct effect on the ultradian expression of sleep in albino rats. In these animals, short LD cycles of 1 h modulate vigilance states and the electroencephalogram (EEG) whereas circadian aspects of sleep are little affected (Alfoldi *et al.*, 1991). Nonrapid eye movement (NREM) sleep was selectively enhanced during the short light periods, whereas the amount of rapid

eye movement (REM) sleep was elevated during the short dark periods. The LD-induced changes in NREM sleep were largest during the active period of the 24-h cycle, whereas the changes in REM sleep were largest during the resting period.

When the LD periods became shorter (in the minutes range), clear effects on REM sleep were observed in the rat. REM sleep was enhanced in the period that followed immediately after dark onset, and these effects were maximal in LD cycles of 10 min (Borbely, 1976). The mechanism and the biological significance of the phenomenon are still unknown, but it has been reported that REM sleep triggering does not occur in pigmented rats such as Brown Norway or Long–Evans (Benca *et al.*, 1991, 1993). These results bring into question whether the effects of short LD cycles on REM sleep are caused by anomalous visual pathways specific for albino animals (Miller *et al.*, 1999).

The goal of the present study was to investigate whether short LD cycles affect sleep in mice. The C57BL/6 mouse was chosen because most retinal-targeted mutants and other mutants are backcrossed on this strain, allowing continuation of those studies on underlying pathways and neurotransmitter systems. Significant results with this mouse strain would indicate that light can have a direct influence on the distribution of sleep in mammals other than the albino rat.

Materials and methods

All experiments were performed under the approval of the Animal Experiments Ethical Committee of the Leiden University Medical Center according to the Dutch law on animal experiments. Nine adult male C57BL/6 mice with a mean \pm SEM body weight of 26.8 ± 0.8 g were kept in standard plexiglas cages. Food and water were available *ad libitum*. The animals were maintained under a LD cycle of 12 : 12 h (light from 08.00 to 20.00 h, 30–50 lux at the bottom of the cage, daylight fluorescent type of light).

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Under deep anaesthesia (Hypnorm and Dormicum i.p.), two stainless steel electrodes (MS333/3; Plastics One, Inc. Roanoke, VA, USA) were placed over the right parietal cortex (3 mm posterior to bregma, 2 mm lateral to midline) and over the cerebellum. Two electromyogram (EMG) electrodes (E363/70 Electrode SS subcut; Plastics One) were inserted subcutaneously and placed on the neck muscle. The electrodes were connected to stainless steel wires that were fixed to the skull with dental cement. At least 12 days were allowed for recovery after surgery and adaptation to the recording condition.

Eight consecutive 24-h recordings of EEG and EMG were obtained. The first day was under 12 : 12 h LD conditions and the second in DD. Those 2 days were then followed by six experimental days with different short LD cycles. The durations of the cycles were 2 h (i.e. 1 : 1 h) and 30, 14, 10, 4 and 2 min, and were applied in a Latin square design.

The EEG and EMG were recorded with a portable system (Institute of Pharmacology and Toxicology, University of Zurich, Zurich, Switzerland) as described previously (Vyazovskiy *et al.*, 2006). Before each recording, a calibration signal (10 Hz sine wave, 300 μ V peak-to-peak) was recorded on the EEG and EMG channel. Both signals were amplified (amplification factor \sim 2000), conditioned by analogue filters (high-pass filter -3 dB at 0.16 Hz) and sampled at 512 Hz. The signals were filtered through a digital Finite Impulse Response filter: EEG low-pass filter at 30 Hz and EMG band-pass filter between 20 and 40 Hz. EEG power spectra were computed for 4-s epochs as described previously (Deboer *et al.*, 2002).

The vigilance states (waking, NREM sleep and REM sleep) were scored off-line in 4-s epochs by visual inspection of the raw EEG and EMG signal as well as EEG power density in the slow-wave range (0.75–4.0 Hz; for details see Deboer *et al.*, 2002). Epochs with artifacts were not used for the spectral analysis, but vigilance states could always be determined. The duration and frequency of the different vigilance state episodes were computed as previously in the Djungarian hamster (Deboer & Tobler, 1996) and the mouse (Huber *et al.*, 2000).

Corresponding 24-h and light and dark mean values were analysed with an ANOVA and *post hoc* Duncan's test. For the comparison between LD and DD, overall effects on the 1-h mean values were analysed by two-way ANOVA with the factors 'time of day' and 'day'. When significant effects were present, differences were assessed with a paired *t*-test.

Results

Short LD cycles had a clear influence on the distribution of sleep and wakefulness (Fig. 1A). In all animals, the circadian distribution of the vigilance states disappeared, regardless of the duration of the short LD cycle. On the first day after the transition from DD to the short LD cycles, some remnant of a circadian rhythm in the vigilance state distribution could be observed (14 min LD cycle in the example in Fig. 1A). Because it was not possible to recognize the rest–activity cycle in the short LD conditions, no separate analysis of the two phases could be performed.

The overall amount of the different vigilance states over 24 h did not change under influence of either DD or the short LD cycles, despite the clear change in the distribution of the vigilance states (Fig. 1B). There was also no significant effect of DD on the distribution of the vigilance states or the time course in EEG slow-wave activity (SWA; mean EEG power density between 0.75 and 4.0 Hz) during NREM sleep as compared to the baseline LD 12 : 12 h (Fig. 2).

For additional analysis, the data were separated into the new light and dark intervals (Fig. 3). Both sleep states showed a significant

decrease in the new light intervals and an increase in the new dark intervals. The changes in REM sleep were proportionately larger than the changes in NREM sleep, resulting in a significant decrease in REM sleep per total sleep time (TST) in the light period and an increase in REM sleep per TST in the dark period in all short LD cycles, compared to 12 : 12 h LD ($P < 0.05$, Duncan, after significant ANOVA factor 'day'). The changes in REM sleep were largest in the cycles lasting 10, 14 and 30 min (Fig. 3). The effects on REM sleep per TST were a mirror image of the effects on REM sleep (data not shown). In shorter (< 10 min) and longer (2 h) LD cycles the changes in REM sleep were less pronounced, particularly during the light period.

In the 12 : 12 h LD cycles there was significantly more NREM sleep, REM sleep and REM sleep per TST during the light than the dark period. Under influence of the short LD cycles, the difference between light and dark in the amount of NREM sleep disappeared. The amount of REM sleep reversed in the LD cycles lasting between 10 and 30 min, with more REM sleep and REM sleep per TST in the dark period than in the light period ($P < 0.05$, two-tailed paired *t*-test after significant ANOVA factor 'LD'). With shorter LD cycles (2–4 min) this difference also disappeared.

The significant changes in REM sleep and REM sleep per TST in LD cycles lasting 10–30 min suggest that REM sleep induction may take place in the dark periods of these short LD cycles. To analyse this further, the time course of the amount of REM sleep was plotted for the 4-, 10- and 14-min cycles in the corresponding minutes before and after a LD transition (Fig. 4). A clear increase in REM sleep was seen in the second minute after the start of the dark period in short LD cycles lasting 14 and 10 min. This increase was less pronounced in LD cycles lasting 4 min. The percentage of epochs in REM sleep increased from $6.1 \pm 0.4\%$, $5.3 \pm 0.3\%$ and $6.0 \pm 0.4\%$ during the last minute of light presentation to $7.4 \pm 0.7\%$, $9.3 \pm 0.7\%$ and $8.8 \pm 0.8\%$ during the second minute of darkness in the 4-, 10- and 14-min cycles, respectively ($P < 0.05$ for 10- and 14-min cycles; two-tailed paired *t*-test). The difference in the increase in REM sleep resulted in 25% more REM sleep in the second minute after dark onset in the 10-min LD cycle compared to the 4-min LD cycle condition ($P < 0.05$ two-tailed paired *t*-test). The time course of REM sleep per TST was a mirror image of the time course of REM sleep in all short LD conditions (data not shown).

Under LD 12 : 12 h, episode frequencies of all vigilance states were higher in the light period than the dark period ($P < 0.05$, two-tailed paired *t*-test). This difference disappeared in all short LD conditions except for the 2-min LD condition where REM sleep episodes were more frequent in the dark than the light ($4.7 \pm 0.3/h$ in the light, $5.5 \pm 0.5/h$ in the dark; $P < 0.05$, two-tailed paired *t*-test). In the 12 : 12 h LD condition, the waking episodes were longer in the dark than the light period ($P < 0.05$, two tailed paired *t*-test), indicating increased waking consolidation in prolonged darkness. This difference disappeared in the short LD conditions. NREM and REM sleep episode duration did not differ between the light and dark period. The short LD conditions did not influence NREM sleep episode duration. In the 10-min LD cycle, REM sleep episode duration in the dark period increased above the duration in the light (0.86 ± 0.06 min in the light, 1.07 ± 0.03 min in the dark; $P < 0.05$, two-tailed paired *t*-test).

Discussion

The present results show that, in C57BL/6 mice, light and darkness can influence the occurrence and distribution of vigilance states. The most prominent influence was the increase in REM sleep after lights-

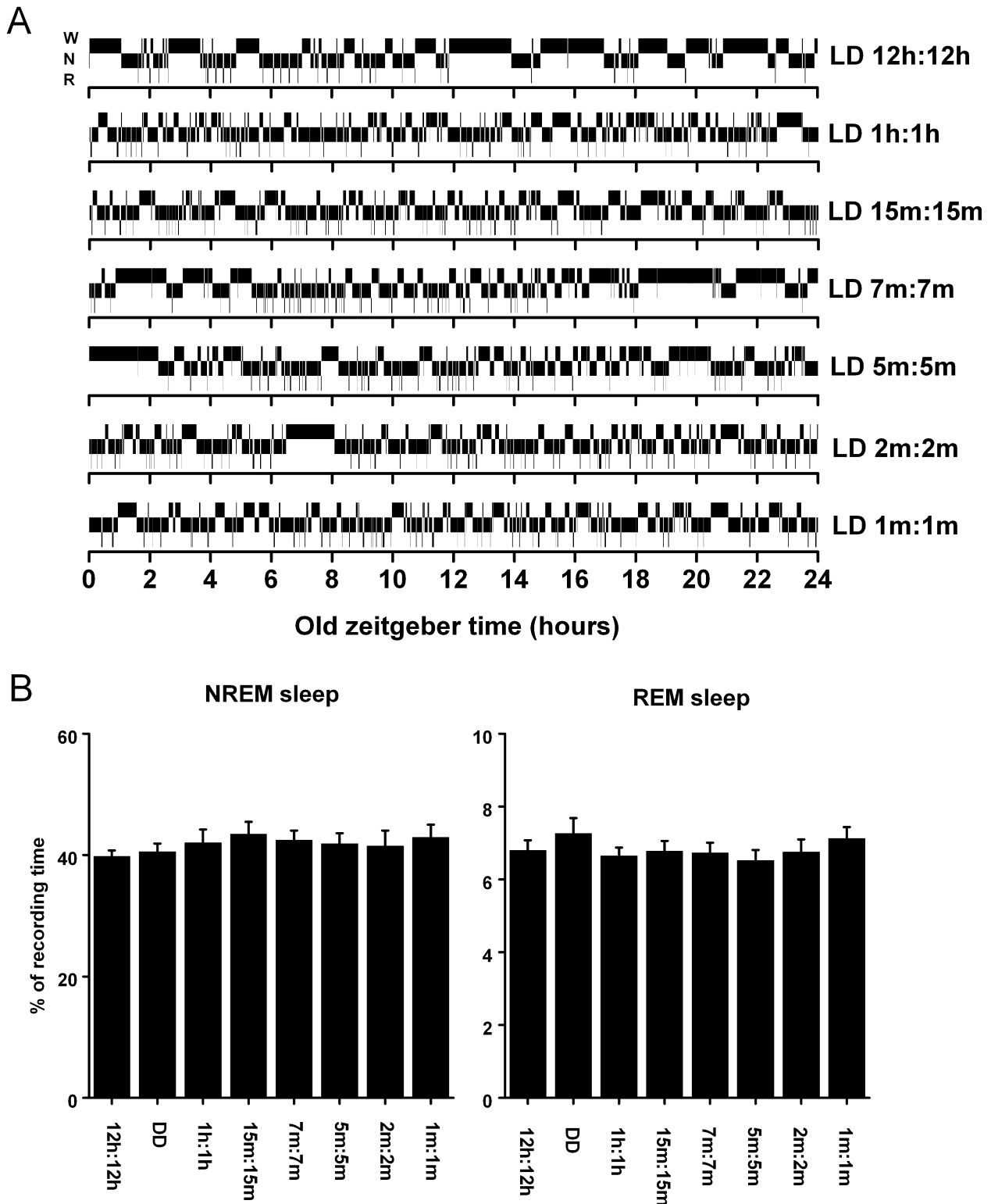


FIG. 1. (A) Twenty-four hour records of waking (W), nonrapid eye movement sleep (N) and rapid eye movement sleep (R) of a representative mouse under different LD cycle durations. Each data point is the mean of 15 4-s epochs (1 min). The data show an absence of clear 24-h rhythms in sleep-wake distribution after the animals were released in the short LD conditions. (B) Amount of nonrapid eye movement (NREM) and REM sleep plotted for each 24-h day. The duration of the LD cycle is indicated at the bottom of the graph with DD being 24 h constant darkness. No significant differences were found between the days (analysed with ANOVA).

off in the short LD cycles of 10–30 min. This is a remarkable result as experiments in the rat and other species suggest that this feature is present in albino rats only. Similar experiments in non-albino rats

(Benca *et al.*, 1991; Leung *et al.*, 1992; Benca *et al.*, 1993; Benca *et al.*, 1998), cats (Chamblin & Drew, 1971) and ground squirrels (Satinoff *et al.*, 1975) did not show a redistribution of REM sleep

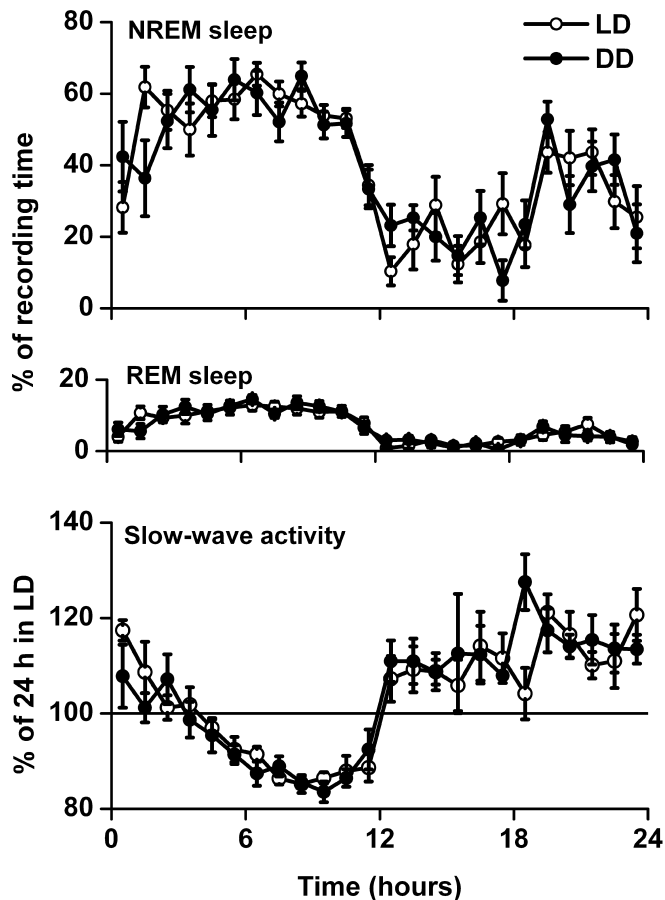


FIG. 2. Time course of 1-h values of NREM sleep, REM sleep and EEG SWA (EEG power density between 0.75 and 4.0 Hz) during the baseline day (LD) and the day in constant darkness (DD). No differences were observed between the two conditions, indicating that the LD cycle had no direct effect on sleep in C57BL/6 mice (analysed with ANOVA).

whereas, in the golden hamster, REM sleep predominated during the light period of short LD cycles (Tobler & Borbely, 1977).

During the baseline day, the distribution of sleep and wakefulness corresponds to the results of previous studies, with ~40% NREM and 6–7% REM sleep over 24 h (Huber *et al.*, 2000; Deboer *et al.*, 2002). The short LD cycles did not affect the relative contribution of the different vigilance states over the 24-h period; this is in accordance with the notion that the total amount of sleep and waking over 24 h is homeostatically regulated (Aschoff, 1993; Franken *et al.*, 1995; Deboer & Tobler, 1996).

Despite the stability of the different vigilance states over 24 h, a clear redistribution of sleep and waking in the short LD conditions occurred. This resulted in an even distribution of the vigilance states over 24 h and prohibited the separate determination of an active and a rest phase. Under baseline LD conditions, NREM and REM sleep are most prominent in the light phase (Huber *et al.*, 2000; Deboer *et al.*, 2002). Under the influence of short LD cycles, NREM sleep became evenly distributed across 24 h with no difference between the light and dark periods. In contrast, in short LD cycles lasting 10–30 min, most REM sleep and REM sleep per TST occurred in the dark period. Albino rats double the amount of REM sleep in the dark as compared to the light period under short LD cycles (Tobler & Borbely, 1978). In our mice REM sleep in the short dark periods was ~30% higher than the amount in the short light period. This difference is similar to the

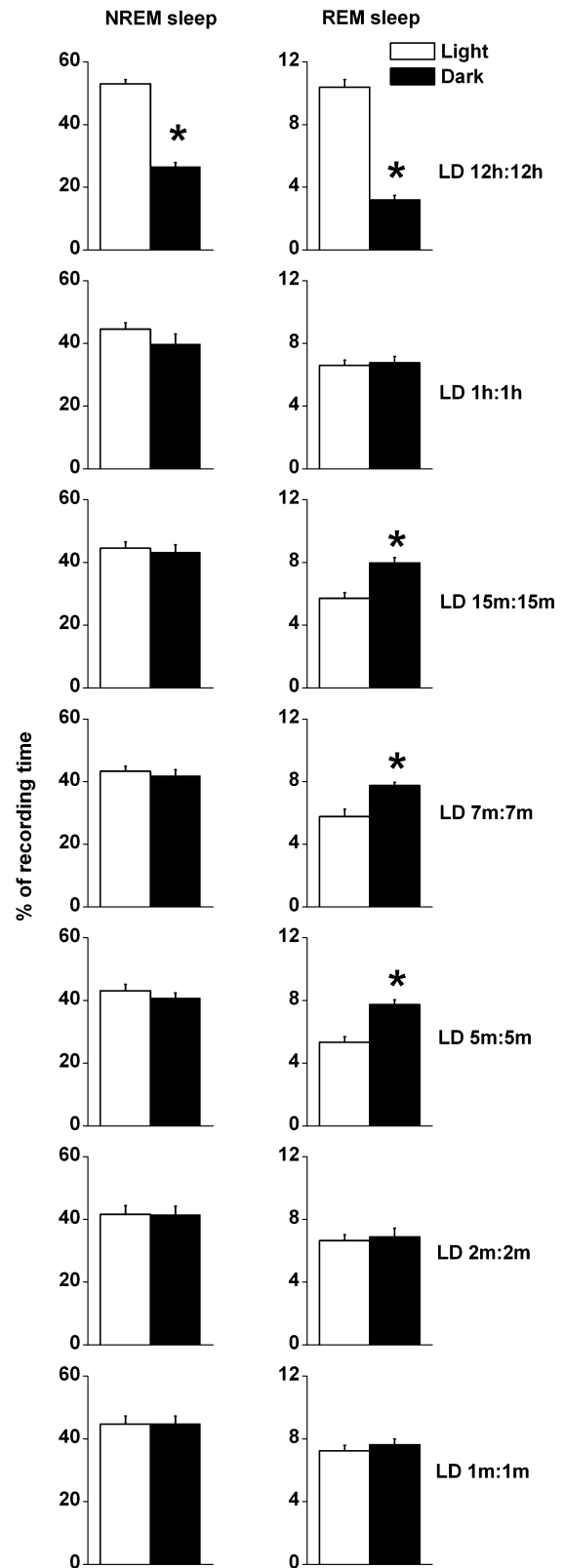


FIG. 3. Amount of NREM sleep and REM sleep plotted for the baseline day (LD 12 h:12 h) and each experimental day with short LD cycles. Days where light and dark values differ significantly are indicated by stars ($P < 0.005$, two-tailed paired *t*-test after significant ANOVA factor 'light condition'). For all days, NREM and REM sleep values were increased above baseline during the dark period and decreased below baseline during the light period ($P < 0.05$, Duncan's test after significant ANOVA factor 'day').

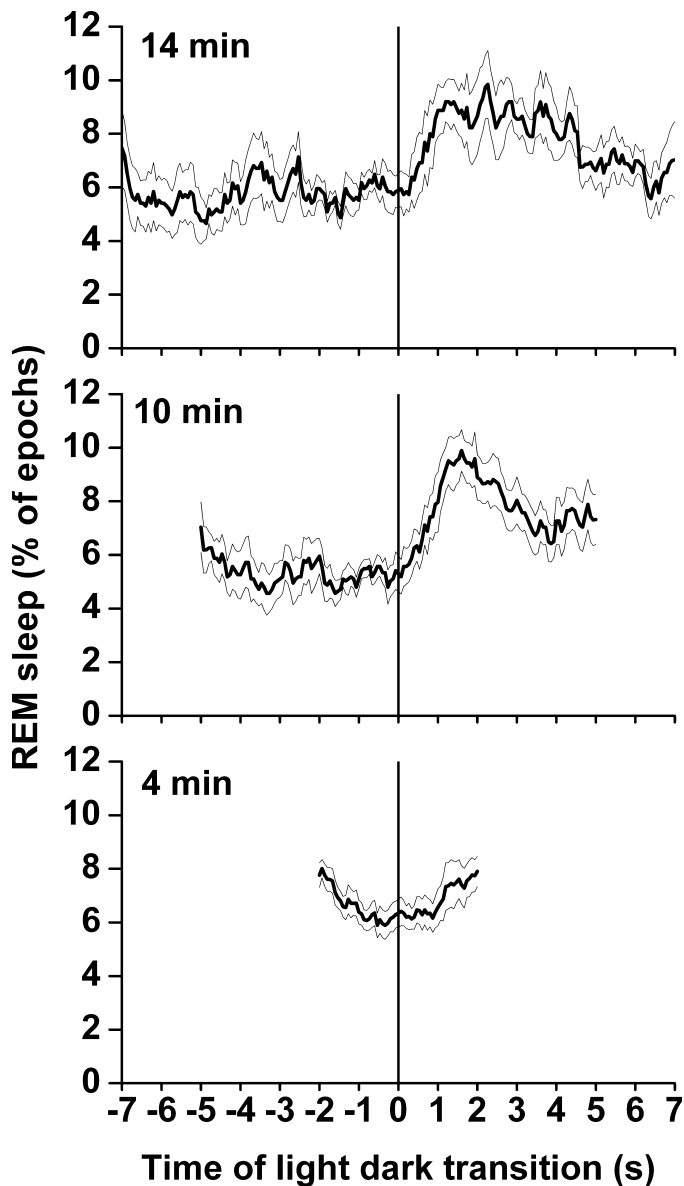


FIG. 4. Time course of the percentage of epochs scored as REM sleep at the LD transition. Lines indicate the mean time course (thick solid line) and SEM (thin lines). Epochs were obtained over the entire 24-h period. A significant increase in REM sleep was obtained in the 2nd min after dark onset in the 10-min LD cycle compared to the 4-min LD cycle ($P < 0.05$, paired t -test).

effects in nonalbino AD rats in a 20-min LD cycle, but no effects were observed in AD rats under 10-min LD cycles (Tobler & Borbely, 1978). Our data indicate that LD cycles lasting 10–30 min were most effective in inducing the occurrence of REM sleep in the dark period. Analysis of vigilance state episode frequencies and duration suggested that in the 10-min LD cycle the increase in REM sleep is achieved by an increase in REM sleep episode duration. This is different from the rat, where the increase in REM sleep was due to an increase in REM sleep episode frequency (Tobler & Borbely, 1978).

Detailed analysis of the time course of sleep and waking in the course of a short LD cycle showed that, during LD cycles of 14 and 10 min duration, REM sleep and REM sleep per TST increased after dark onset, reaching peak values in the second minute of the short dark period. For 10-min LD cycles, the percentage of epochs with REM sleep almost doubled in the second minute of darkness. A similar time

course, with a peak in REM sleep in the second minute after the onset of darkness, was previously observed in the albino rat under 10-min LD cycles (Borbely, 1976; Leung *et al.*, 1992; Miller *et al.*, 1999; Tsai, 2002).

It is well known that rodents, despite their nocturnality, prefer a dark environment for sleep (Fishman & Roffwarg, 1971). Based on the sleep patterns of albino rats in constant light and darkness (Fishman & Roffwarg, 1972; Tobler *et al.*, 1994) it has been argued that light is an aversive stimulus that disrupts sleep nonspecifically. However, this does not explain the opposite effects of light on the amount of NREM and REM sleep, and the data obtained in DD show that sleep is not affected by the absence of light. This indicates that an LD cycle with a circadian duration has no direct sleep-promoting or sleep-disrupting effects in mice.

Borbely *et al.* (1975) suggested that short LD cycle effects on REM sleep may be secondary to NREM sleep responses. Light enhancement of NREM sleep may result in decreased REM sleep, whereas dark-inhibition of NREM sleep may permit REM sleep in the rat. In our study, however, mice showed equal amounts of NREM sleep in the short light and dark periods, but increased REM sleep and REM per TST in the corresponding dark periods. This suggests that light has independent effects on NREM and REM sleep expression in C57BL/6 mice.

The present findings call into question what anatomical pathways and physiological mechanisms mediate the effects of light on sleep. It has previously been shown that the olivary pretectal nucleus (OPN) mediates the influence of light on REM sleep in the albino rat (Miller *et al.*, 1999). The OPN is known to be involved in the pupillary light response and receives photic information via a direct bilateral projection in, amongst others, mice, rats and primates (Scalia, 1972; Pierson & Carpenter, 1974; Young & Lund, 1994). Melanopsin containing retinal ganglion cells innervate the OPN (Gooley *et al.*, 2003) and animals deficient in melanopsin show a strongly diminished pupillary light response, underscoring the importance of melanopsin in mediating transmission of light information to this brain area (Hattar *et al.*, 2003; Lucas *et al.*, 2003).

Different neurotransmitters may be involved in the response of sleep to light. The level of serotonin is known to increase under the influence of light whereas melatonin, norepinephrine and acetylcholine decrease (Quay, 1968; Hery *et al.*, 1972; Roberts, 2000). Except for melatonin, which is exclusively released during the dark phase (Vivien-Roels *et al.*, 1998), each of these substances may have influenced the distribution of the different vigilance states in the present experiment. Acetylcholine and norepinephrine are increased during REM sleep, but the presence of a response to short LD cycles is unknown. Serotonin agonists induce a decrease in REM sleep (Boutrel *et al.*, 2002), which is evident from human patients who lose most of their REM sleep when treated with antidepressant drugs (Landolt *et al.*, 2001). Interestingly, serotonin responds to short LD cycles of 60 min with an increase during the light period and a decrease during the dark period (Hanselmann & Borbely, 1976), indicating that changes in serotonin levels may have contributed to our results. A direct pathway from the retina to the raphe nuclei (Foote *et al.*, 1978; Heym *et al.*, 1982) is likely to mediate these effects. As serotonin inhibits REM sleep, a decrease in serotonin during the short dark periods would lead to an increment in REM sleep, consistent with our observations.

The finding that short LD cycles in pigmented mice induce REM sleep in the dark opens up the possibility that light may have a much larger impact on sleep in other mammalian species, including humans, than previously presumed. The effects of light on different brain areas result in a number of physiological responses, including changes in sleep architecture, as observed in the present study. We expect that the

identification of effects of light will have significant meaning for health and disease, as reviewed by Navara & Nelson (2007). The high occurrence of sleep disorders, as well as the nightly use of artificial light in either children's bedrooms or clinical institutions, underscore the need for future research in this direction.

Abbreviations

DD, continuous darkness; EEG, electroencephalogram; EMG, electromyogram; LD, light–dark; NREM, nonrapid eye movement; REM, rapid eye movement; RGC, retinal ganglion cells; SWA, slow-wave activity; TST, total sleep time.

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