Long Photoperiod Restores the 24-h Rhythm of Sleep and EEG Slow-Wave Activity in the Djungarian Hamster (*Phodopus sungorus*)

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Abstract Photoperiod influences the distribution of sleep and waking and electroencephalogram (EEG) power density in the Djungarian hamster. In an experimental procedure combining short photoperiod (SP) and low ambient temperature, the light-dark difference in the amount of sleep was decreased, and the changes in slow-wave activity (SWA) (mean EEG power density between 0.75 and 4.0 Hz) in nonrapid eye movement (NREM) sleep within 24 h were abolished. These findings, obtained in three different groups of animals, suggested that at the lower ambient temperature, the influence of the circadian clock on sleep-wake behavior was diminished. However, it remained unclear whether the changes were due to the photoperiod, ambient temperature, or both. Here, the authors show that EEG and electromyogram recordings in a single group of animals sequentially adapted to a short and long photoperiod (LP) at low ambient temperature (~15 °C) confirm that EEG power is reduced in SP. Moreover, the nocturnal sleep-wake behavior and the changes in SWA in NREM sleep over 24 h were restored by returning the animals to LP and retaining ambient temperature at 15 °C. Therefore, the effects cannot be attributed to ambient temperature alone but are due to a combined effect of temperature and photoperiod. When the Djungarian hamster adapts to winter conditions, it appears to uncouple sleep regulation from the circadian clock.

Key words electroencephalogram, Djungarian hamster, photoperiod, sleep, spectral analysis

Changes in photoperiod are reliable predictors of seasonal variation and trigger physiological and behavioral adaptations in many mammalian species (reviewed in Bartness and Goldman, 1989; Reiter, 1993). The responses are mediated by the circadian pacemaker located in the suprachiasmatic nucleus (SCN) (Bittman et al., 1991; Pittendrigh and Daan, 1976) and the pineal gland by means of changes in the duration of melatonin secretion (Arendt et al., 1981; Bartness and Goldman, 1989; Reiter, 1993; Tamarkin et al., 1985). Even in species that do not show profound seasonal physiological changes, the duration of melatonin secretion is a function of photoperiod (reviewed in Reiter, 1993). Moreover, seasonal fluctuations in the human SCN have been reported (Hofman and Swaab, 1992, 1993). Although photoperiod affects circadian physiology and properties of the circadian pacemaker, there is no consensus on seasonal aspects of sleep. In humans (Wehr, 1991; Wehr et al., 1993; Wirz-Justice et al., 1984) and elephants (Tobler, 1992), sleep duration is increased in a shortened photoperiod. However, in the rat (Borbély and Neuhaus, 1978; Franken et al., 1995), Siberian chipmunk (Dijk and Daan, 1989), and Djungarian hamster (Deboer...
and Tobler, 1996, 1997a), a change in photoperiod merely induced a redistribution of sleep across 24 h.

Sleep regulation has been investigated within the framework of the two-process model of sleep regulation (Borbély, 1982; Daan et al., 1984). Both diurnal and nocturnal species exhibit a high initial value in EEG slow-wave activity (SWA; mean electroencephalogram [EEG] power density between 0.75 and 4.0 Hz) in nonrapid eye movement (NREM) sleep, and a subsequent gradual decline during the main rest period. Invariably, prolonged waking is followed by an increase in SWA, and in several species, a dose response relation between the increase in SWA and prior waking duration was found (rat: Tobler and Borbély, 1986; human: Dijk et al., 1987; cat: Lancel et al., 1991; ground squirrel: Larkin and Heller, 1998; Strijkstra and Daan, 1998; mouse: Huber et al., 2000; Djungarian hamster: Deboer and Tobler, 2000).

The Djungarian hamster (*Phodopus sungorus*) is a nocturnal rodent, which displays a large spectrum of behavioral and physiological adaptations to changes in photoperiod. The effect of photoperiod on sleep in this species has been investigated extensively, but never in the same individuals (Deboer and Tobler, 1996, 1997a). Sleep was redistributed over 24 h, but the total amount in a long photoperiod (LP) and short photoperiod (SP) was the same. Furthermore, EEG power density was lower in SP compared to LP (Deboer and Tobler, 1996). Exposing the hamsters to 16 °C ambient temperature (Ta), that is, a few degrees below thermoneutrality (Heldmaier and Steinlechner, 1981b), reduced the light-dark amplitude of the vigilance states and abolished the light-dark difference in brain temperature and the changes of SWA in NREM sleep within a 24-h day (Deboer and Tobler, 1997a). This effect could be related to the reduction of nocturnal activity observed in the cold (Ruf, 1991), or the negative correlation between the amount of nocturnal activity and torpor episode frequency (Ruf et al., 1991). The results resembled the sleep and SWA pattern obtained in guinea pigs (Tobler et al., 1993) and in rats after bilateral lesion of the SCN (Mistlberger et al., 1983; Tobler et al., 1983; Trachsel et al., 1992), suggesting that lowering *T*<sub>a</sub> below thermoneutrality may have reduced the influence of the circadian clock on the sleep-wake behavior in the hamster.

However, it was unclear whether all the differences between LP and SP and SP with low *T*<sub>a</sub> in the hamsters could be specifically attributed to photoperiod and *T*<sub>a</sub> or whether differences between animals in the different groups (e.g., electrode placement) contributed to the effects. The second aim was to investigate whether the normal sleep-wake distribution would be restored when hamsters were brought back to LP (LD 16:8) after recordings had been obtained in SP (LD 8:16) keeping *T*<sub>a</sub> at 14 to 15 °C in both conditions.

**MATERIALS AND METHODS**

**Animals**

Adult male Djungarian hamsters (*Phodopus sungorus*), raised in summer under natural photoperiod (breeding stock of the Philipps Universität, Marburg, Germany), were transferred to macrolon cages (36 × 20 × 35 cm) in October and kept at 14.5 °C *T*<sub>a</sub> in SP (lights from 0900 to 1700 h MET; 60-130 lux) with food and water available ad libitum. As previously, animals (n = 8) were selected for implantation of EEG and electromyogram (EMG) electrodes when episodes of daily torpor were recognized and the change in weight and fur color indicated a strong adaptation to SP (Deboer and Tobler, 1997a).

At the age of 5.4 (±0.3) months, the selected individuals (mean weight 26.4 ± 0.8 g, n = 8) were implanted with electrodes for EEG and EMG recordings and a thermistor to measure cortical temperature (TCRT). The electrodes and the thermistor were soldered to a plug and attached to a cable that was fixed to the skull and anchored to the three screws with dental cement (Deboer et al., 1994). For recording, the animals were transferred to a sound-attenuated chamber where they were exposed to similar conditions as before (LD 8:16; daylight-type fluorescent tubes, 18 W, 10-100 lux; 14.5 °C *T*<sub>a</sub>).

**Experimental Protocol**

The experiments were performed in winter (December and January). EEG, EMG, and TCRT were recorded continuously for 1 day. At the end of the winter season (beginning of March), the photoperiod was reversed (LD 16:8), but *T*<sub>a</sub> was maintained at 14.5 °C. At least 2 months were allowed for adaptation to the new photoperiod. All animals restored their LP physiology (fur color change from white-gray to brown-gray and regrowth of the gonads). Subsequently, another 24-h record was obtained in May.
Vigilance states in the long photoperiod (LP) and short photoperiod (SP).

<table>
<thead>
<tr>
<th></th>
<th>Long Photoperiod</th>
<th>Short Photoperiod</th>
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</thead>
<tbody>
<tr>
<td>Waking</td>
<td>33.3 (1.5)</td>
<td>36.2 (1.7)</td>
</tr>
<tr>
<td>D</td>
<td>53.8 (4.6)</td>
<td>42.3 (1.9)</td>
</tr>
<tr>
<td>24-h</td>
<td>40.1 (1.8)</td>
<td>40.3 (1.7)</td>
</tr>
<tr>
<td>NREMS</td>
<td>56.9 (1.5)</td>
<td>55.1 (1.4)</td>
</tr>
<tr>
<td>D</td>
<td>42.7 (3.7)</td>
<td>50.2 (1.8)</td>
</tr>
<tr>
<td>24-h</td>
<td>52.2 (1.5)</td>
<td>51.8 (1.5)</td>
</tr>
<tr>
<td>REMS</td>
<td>9.7 (0.3)</td>
<td>8.7 (0.7)</td>
</tr>
<tr>
<td>D</td>
<td>3.4 (0.1)</td>
<td>7.4 (0.6)</td>
</tr>
<tr>
<td>24-h</td>
<td>7.6 (0.4)</td>
<td>7.9 (0.6)</td>
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</tbody>
</table>

NOTE: Mean values are expressed as percentage of recording time (± SEM, n = 8).

Data Acquisition and Analysis

The EEG and EMG signals were amplified (amplification factor ~2000), conditioned by analog filters (high-pass filter: ~3 dB at 0.016 Hz; low-pass filter: ~3 dB at 40 Hz; less than ~35 dB at 128 Hz), sampled with 512 Hz, digitally filtered (EEG: low-pass FIR filter 25 Hz; EMG: band-pass FIR filter 20-50 Hz), and stored with a resolution of 128 Hz. EEG power spectra were computed for consecutive 4-sec epochs by FFT routine within the frequency range of 0.25 to 25.0 Hz. Between 0.25 and 5.0 Hz, the values were collapsed into 0.5-Hz bins, and between 5.25 and 25.0 Hz, into 1-Hz bins. EMG signals were integrated over 4 sec, and TCRT and Td inside the cage were recorded at 4-sec intervals. Before the start of each recording, the EEG and EMG channels were calibrated with a 10-Hz sine wave, 300µV peak-to-peak signal. After the experiment, the vigilance states were determined for 4-sec epochs by visual scoring (Deboer et al., 1994). Epochs containing EEG artifacts were omitted from further analysis of the power spectra (3.6% ± 1.0% [SEM] of total recording time, n = 8), but vigilance states could always be determined. Td inside the cage at the level of the animal was 14.5 ± 0.1 °C and did not differ between the 2 recording days (p > 0.05, two-tailed paired t-test).

Analysis of variance (ANOVA on 2-h and 4-h mean values) served to determine effects of time-of-day within a day. Differences between the photoperiods were assessed by paired t-tests after significant ANOVA (where appropriate). The duration and frequency of vigilance state episodes were determined according to criteria described previously (Deboer et al., 1994; Deboer and Tobler, 1996).

RESULTS

The return to LP was paralleled by a remarkable increase in the light-dark difference of sleep and waking (Table 1). The light-dark difference in the percentage of time spent in waking increased from 6.1% ± 1.5% in SP to 20.5% ± 5.0% in LP (p < 0.05, two-tailed paired t-test), whereas total amount of sleep and waking within 24-h were unaffected (Table 1). The adaptation to LP considerably reduced the frequency and duration of NREM sleep episodes and increased the frequency and duration of REM sleep episodes in the light period (Table 2). In the dark period, NREM sleep episode duration and the frequency and duration of REM sleep episodes were reduced. These changes are reflected in the distribution of waking of the individuals (Fig. 1). After adaptation to LP, the longer waking episodes are restricted to the dark period, whereas in SP, they are equally distributed over the light and dark periods. In general, the waking episode duration did not differ between the photoperiods (Table 2). The mean 2-h values of the daily time course of the vigilance states in the two photoperiods is shown in Figure 2. In SP, no vigilance state showed significant changes in the course of the 24-h day, whereas after adaptation to LP, they all did (ANOVA factor “2-h interval” SP: p > 0.2 for all vigilance states, LP: p < 0.00001 for all vigilance states).

Table 1. Vigilance states in the long photoperiod (LP) and short photoperiod (SP).

<table>
<thead>
<tr>
<th>Episode</th>
<th>Long Photoperiod</th>
<th>Short Photoperiod</th>
</tr>
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<tbody>
<tr>
<td>Waking</td>
<td>33.3 (1.5)</td>
<td>36.2 (1.7)</td>
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<tr>
<td>NREMS</td>
<td>56.9 (1.5)</td>
<td>55.1 (1.4)</td>
</tr>
<tr>
<td>REMS</td>
<td>9.7 (0.3)</td>
<td>8.7 (0.7)</td>
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</tbody>
</table>

NOTE: Mean values (± SEM, n = 8).

Table 2. Vigilance state episode duration (min) and frequency (/h) in long photoperiod (LP) and short photoperiod (SP).

<table>
<thead>
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<tbody>
<tr>
<td>Waking</td>
<td>3.9 (0.3)</td>
<td>7.1 (0.4)</td>
</tr>
<tr>
<td>NREMS</td>
<td>4.1 (0.3)</td>
<td>3.7 (0.2)</td>
</tr>
<tr>
<td>REMS</td>
<td>4.2 (0.5)</td>
<td>4.2 (0.1)</td>
</tr>
</tbody>
</table>

NOTE: Mean values (± SEM, n = 8).

a. p < 0.05, b. p < 0.001, light versus dark; c. p < 0.01, LP versus SP; d. p < 0.06, LP versus SP; two-tailed paired t-test.
In all vigilance states, EEG power density over 24 h increased from SP to LP in the slow-wave range, whereas above 4 Hz, power density was not affected (Fig. 3). SWA in NREM sleep did not change in the course of 24 h in SP (Fig. 2 bottom panels, ANOVA factor 2-h interval, \( p > 0.35 \)), while in LP, it decreased significantly during the light, increased during the dark, and reached a maximum in the second half of the dark period (ANOVA factor 2-h interval \( p < 0.0001 \)). SWA attained its lowest values in the second half of the light period and the first 2-h interval of the dark (Duncan multiple range test, \( p < 0.05 \)).

In addition to the changes in the slow-wave range within NREM sleep in LP, there were significant changes over 24 h in the theta range (5.25-7.0 Hz) and
DISCUSSION

Adapting the hamsters to an LP after they had been recorded in an SP, retaining a low Ta, induced a remarkable recovery of the light-dark amplitude in all sleep variables. Therefore, the disappearance of a light-dark difference in sleep previously obtained in SP where Ta had also been reduced (Deboer and Tobler, 1997a) was not due to Ta alone but resulted from the combined effect of an adaptation to SP and a lower Ta. The data show that a subsequent adaptation to LP, maintaining the same Ta, enhances the sleep-wake amplitude and restores the changes in SWA in NREM sleep over 24 h. This is consistent with the negative correlation found between the amount of nocturnal activity and torpor episode frequency (Ruf et al., 1991). The data support the notion that the time course of SWA in NREM sleep is determined by the distribution of sleep and waking (Borbély, 1982; Daan et al., 1984).

The increase in EEG power density in the slow-wave range after adaptation to LP confirms the finding we obtained in different groups of hamsters, one adapted to LP and one to SP (Deboer and Tobler, 1996). In general, prolonged recording of animals with chronically implanted electrodes is likely to be accompanied by a decrease in overall EEG power (unpublished observation). Therefore, the present increase after >16 weeks between recordings must be a specific effect of photoperiod. The mechanisms underlying the increase in EEG power could be an enhanced synchronization in neuronal firing patterns in the slow-wave range. Alternatively, it could be a consequence of an increase in the frequency of cortical postsynaptic potentials (PSPs) in individual neurones that generate specific frequencies in the EEG (Steriade et al., 1993). In the latter case, less energy would be needed to maintain neuronal membrane potentials in SP, and this could represent one of the adaptations of the Djungarian hamster to reduce metabolic rate (Heldmaier and Steinlechner, 1981a).

The time course of SWA in NREM sleep in SP was similar to that seen normally in the guinea pig and in the rat after bilateral lesion of the SCN (Tobler et al., 1993; Trachsel et al., 1992). The common feature between these animals was the even distribution of sleep and waking over 24 h. Similar recordings after lesions of the SCN in squirrel monkeys showed that sleep was increased by reducing wake bout length, increasing sleep bout length and the frequency of wake and sleep bouts to the level seen during the rest phase in intact animals (Edgar et al., 1993). It was proposed that the circadian clock opposes the sleep drive by lengthening waking bout duration during the active phase (Edgar et al., 1993).

In the Djungarian hamster, the redistribution of sleep and waking from SP to LP is reflected in changes in the frequency and duration of vigilance state episodes (Table 2). In SP, wake episode duration was increased, and NREM sleep episode duration and frequency were decreased during the rest phase compared to LP. Moreover, in the rat, wake episode duration may have increased after SCN lesions (Mistlberger et al., 1987). It is possible that in nocturnal species, such as the hamster and the rat, the SCN induces higher sleep maintenance during the rest phase, whereas in the diurnal squirrel monkey, it induces higher wake maintenance during the active phase. However, the hamsters did not increase the total amount of sleep over 24 h to the level of the light period in SP but maintained the same amount of sleep as in LP. Even the disappearance of the light-dark dif-
ference in sleep and waking induced by chronic melatonin administration in Djungarian hamsters did not affect the amount of sleep over 24 h (Deboer and Tobler, 1997b). Also in the rat, a remarkable stability of the 24-h sleep quota was found despite changes in photoperiod (Borbély and Neuhaus, 1978; Franken et al., 1995), or SCN lesion (Mistlberger et al., 1983; Tobler et al., 1983).

Some caution is necessary in the comparison between animals recorded in different photoperiods and animals with lesions of the SCN because the latter are usually recorded under constant lighting conditions. Moreover, in the Djungarian hamster, other circadian behaviors, for example, daily torpor, are still in synchrony with the light-dark cycle (Heldmaier and Steinlechner, 1981b) and show a free-running rhythm in constant darkness (Ruf et al., 1989). In the present study, EEG power density above 5 Hz retained its changes in the course of the 24-h day in SP (Fig. 4). This indicates that although the clock no longer influences the distribution of sleep and waking and the time course of EEG SWA in NREM sleep, it still exerts its influence on other physiological and behavioral parameters.

Our results suggest that in the Djungarian hamster, the preparation for the harsh environment encountered during winter entails a dissociation between sleep homeostasis and the circadian clock. Upon return to LP, the latter regains control over sleep-wake behavior. It remains unclear whether this is a direct effect of photoperiod on sleep-wake behavior or whether it is mediated via the different physiological changes that parallel the adaptation to SP. Sleep deprivation experiments in the Djungarian hamster have
previously shown that regardless of photoperiod, sleep homeostasis is unaffected (Deboer et al., 1994; Deboer and Tobler, 1996, 1997a). Also, in the guinea pig (Tobler and Franken, 1993) and SCN-lesioned rats (Trachsel et al., 1992), sleep homeostasis is intact, supporting the notion that sleep homeostasis and circadian regulation of sleep-wake behavior are mediated by two separate processes (Borbély, 1982; Daan et al., 1984).

In conclusion, the data obtained in the same animals in two different photoperiods support our previous finding that EEG power density is reduced in SP. Moreover, light-dark changes in sleep-wake behavior, and the time course of EEG SWA in NREM sleep can be recovered by returning the animals to an LP. Therefore, their absence in SP is not caused by low Ta alone but is a combined effect of photoperiod and low Ta. Sleep regulation seems to be uncoupled from the circadian clock when the Djungarian hamster adapts to winter conditions, supporting the notion that sleep homeostasis and circadian regulation of sleep and waking are regulated by two separate processes.

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