Loss of circadian organization of sleep and wakefulness during hibernation

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The purpose of this study was to determine if there is a loss of circadian organization of sleep and wakefulness during hibernation. Am J Physiol Regulatory Integrative Comp Physiol 282: R1086–R1095, 2002; 10.1152/ajpregu.00771.2000.—We investigated circadian and homeostatic regulation of nonrapid eye movement (NREM) sleep in golden-mantled ground squirrels during euthermic intervals between torpor bouts. Slow-wave activity (SWA; 1–4 Hz) and sigma activity (10–15 Hz) represent the two dominant electroencephalographic (EEG) frequency components of NREM sleep. EEG sigma activity has a strong circadian component in addition to a sleep homeostatic component, whereas SWA mainly reflects sleep homeostasis [Dijk DJ and Czeisler CA. J Neurosci 15: 3526–3538, 1995; Dijk DJ, Shanahan TL, Duffy JF, Ronda JM, and Czeisler CA. J Physiol (Lond) 505: 851–858, 1997]. Animals maintained under constant conditions continued to display circadian rhythms in both sigma activity and brain temperature throughout euthermic intervals, whereas sleep and wakefulness showed no circadian organization. Instead, sleep and wakefulness were distributed according to a 6-h ultradian rhythm. SWA, NREM sleep bout length, and sigma activity responded homeostatically to the ultradian sleep-wake pattern. We suggest that the loss of sleep-wake consolidation in ground squirrels during the hibernation season may be related to the greatly decreased locomotor activity during the hibernation season and may be necessary for maintenance of multiday torpor bouts characteristic of hibernating species.

slow-wave activity; sigma activity; circadian rhythms; circannual rhythms; Spermophilus lateralis

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debt accumulated during torpor and was responsible for the high SWA in NREM sleep observed following periodic arousals (4, 51). In support of this hypothesis, it was found that peak euthermic SWA following periodic arousals (4, 51). However, a homeostatic interpretation of high SWA following torpor bouts was challenged by subsequent findings showing that SWA during the first few hours of euthermia following arousal in ground squirrels was not homeostatically regulated as at other times (30, 31, 46). Although cogent hypotheses have been proposed to explain these results, little is understood about the interactions between hibernation and sleep regulation.

As noted above, sleep is under strong circadian control. Hibernation in ground squirrels is also strongly controlled by another biological timing mechanism, the circannual rhythm. There have been a number of studies of the relationships between circadian and circannual rhythms in hibernators. At a minimum, we can say that there are circannual changes in the circadian system (24, 33); the circadian system continues to function during bouts of torpor (24, 37), and the circadian system plays a role in the timing of torpor (3, 24, 58). A question that has not been addressed is whether there are changes in the circadian control of sleep during the hibernation season. Given the importance of the circadian system in controlling sleep in nonhibernators and the homology between sleep and hibernation, this is an important question if we are to understand the control of hibernation. One study showed that there are significant changes in time spent asleep between summer and winter in golden-mantled ground squirrels (57), but we lack more detailed information about sleep structure and regulation in hibernators.

In this study, we investigated circadian and ultradian organization of NREM sleep during euthermic intervals between torpor bouts and circadian rhythmicity of Tbr during torpor and euthermia in squirrels maintained in constant conditions.

MATERIALS AND METHODS

Animals. Cortical EEG was recorded from five animals during their hibernation season, between November and March. Animals were captured in the Sierra Nevada of California at least 1 yr before they were involved in this study. The animals were housed individually in an environmental chamber (5°C, 12:12-h light-dark cycle) year round. They were inspected daily in their home cages to record their torpor patterns. Under pentobarbital sodium anesthesia, each animal received four stainless steel EEG screws, two subdermal stainless steel electromyogram (EMG) electrodes, and a thermocouple reentrant tube to measure Tbr. Two EEG screws were placed over the left and right cortex (2.0 mm lateral to midline and 2.0 mm posterior to bregma), and two reference EEG electrodes were placed over the cerebellum. Animals were allowed a minimum of 1 wk recovery before initiation of recording. EEG recordings during the hibernation season were taken from animals that had cycled through torpor bouts consistently for a minimum of 1 mo in their home cages. To reconcile the needs to record during long euthermic intervals at high (21°C) air temperature (T0) and for animals to establish stable hibernation at low T0, all squirrels entered torpor in their home cages at T0 of 5°C before recordings.

For recording sessions, animals were placed in 30-cm-diameter Plexiglas cages provided with wood chips and cotton nesting material. Food (Purina Rat Chow and sunflower seeds) and water were available ad libitum, and animals were maintained in continuous (24:0-h light-dark cycle) low ambient light (20 lx). Animals were connected to a Grass model 7 polygraph by a commutator, allowing full range of movement. During recordings, squirrels were held at 10 ± 1°C and allowed to cycle through several undisturbed bouts of torpor and euthermia, to ensure that torpor patterns were well established in the recording chamber, before T0 was raised to 21°C. Animals were not disturbed during the recording sessions, and all arousals were spontaneous. This procedure ensured that all squirrels had entered torpor before recordings at the higher T0.

Data acquisition and analysis. The EEG signal was calibrated to a 200-μV direct current signal at the start of the recording, was low-pass filtered at 35.0 Hz, and high-pass filtered at 0.3 Hz, and then was digitized at 100 Hz. The integrated EMG signal was band-pass filtered between 3 and 75 Hz. The EEG signal, integrated EMG, Tbr, and T0 were recorded every 10 s on computer. T0 was measured by a fine-grade thermocouple placed in the environmental chamber in close proximity to the animals’ cages. Vigilance states were determined by visual inspection of each 10-s epoch based on the EEG and EMG signals. Vigilance state was scored as either wakefulness (high-integrated EMG and low-amplitude, high-frequency EEG), NREM sleep (low-integrated EMG and high-amplitude, low-frequency EEG characterized by sleep spindles and slow waves), or rapid eye movement (REM) sleep (low-integrated EMG and low-amplitude, high-frequency EEG). Epochs containing artifacts in the EEG were not included in subsequent spectral analysis, although vigilance state could be assessed. The spectral analysis was performed by fast Fourier transformation, yielding power density values between 0.5 and 21 Hz.

Mean T0, percent vigilance states (expressed as percentage of total recording time), SWA, and sigma activity in NREM sleep (mean EEG power density in the 1- to 4-Hz and 10- to 15-Hz ranges, respectively) were calculated for each hour of recording. SWA and sigma activity were not calculated for a given hour if <5% of that hour was spent in NREM sleep. SWA and sigma activity are expressed as a percentage of their mean values in NREM sleep during the euthermic intervals (Tbr > 34°C) between torpor bouts. NREM and REM sleep bout durations were calculated by computer routine for all NREM and REM sleep bouts longer than 1 min. A NREM sleep bout was determined to have ended if it was followed by three consecutive epochs of either wakefulness or REM sleep. A REM sleep bout was determined to have ended if it was followed by three consecutive epochs of either NREM sleep or wakefulness. The number of brief interruptions (nBI), defined as one or two consecutive epochs of wakefulness or REM sleep, during a NREM sleep bout was used to assess the degree of NREM sleep fragmentation. T0 was also calculated as T0, the deviation in °C of Tbr from the mean Tbr for the euthermic interval. To determine whether there were consistent trends in SWA and sigma activity during ultradian sleep periods during the 21°C euthermic intervals, mean values of SWA and sigma activity were calculated in 20-min intervals for ultradian sleep periods lasting up to 4.33 h for each animal.

To investigate circadian and ultradian rhythmicity during the extended euthermic intervals at 21°C, periodogram anal-
ysis was performed on the percent wakefulness, SWA, sigma activity, and Tbr calculated in 20-min intervals following the procedures of Dorrscheidt and Beek (13). As this analysis cannot accept missing data, which occurred for SWA and sigma activity for intervals in which the animals were awake, null values were replaced with minimal SWA and sigma activity values. Periodogram analysis was performed on the recordings from euthermic animals at 21°C but not on the euthermic animals held at 11°C, because the euthermic intervals at 11°C were too short (>24 h vs. 10–15 h, respectively). The duration of the euthermic intervals precluded detection of circadian rhythms through the periodogram analysis, which requires at least three cycles, but the periodogram analysis was robust in detection of ultradian (<12 h) rhythms. Circadian rhythmicity was investigated by ANOVA analysis, by a significant effect of time in the ANOVA, and by peak-to-peak period length calculation.

We determined the relative impact of vigilance state and the circadian system on Tbr for squirrels during euthermic intervals at 21°C following the technique used by Franken et al. (21). Hourly values of percentage of recording time spent awake were regressed against hourly means of Tbr to determine the impact of vigilance state on Tbr, and the $R^2$ was taken as a measure of variance explained by vigilance state (26). The residuals of this analysis were regressed against time (using polynomial regression to model the circadian system) to calculate the relative influence of the circadian system on the remaining variation in Tbr. Vigilance state screening was not used in assessing circadian Tbr fluctuations during torpor, because torpor is a homogenous state, consisting almost entirely of NREM sleep (28). Therefore, mean Tbr and Tact in each 10-min interval during the torpor bout were plotted to assess circadian rhythms in Tbr during torpor and whether variations in Tbr could be driving Tact. The period of the circadian rhythm in Tbr during torpor and euthermia was determined by the interval from peak to peak Tbr.

During euthermic intervals at Tact of 21°C, animals were maintained in constant conditions. The circadian rhythm in Tbr was used to provide the circadian time (CT) to synchronize sleep recordings between animals. Hourly means of Tbr showed clear circadian rhythmicity, and CT 0 was established as the first hour of the rising phase of the Tbr rhythm. CT 0 did not necessarily coincide with the first hour of euthermia following arousal from torpor; therefore, CT, rather than hour of euthermia, was used to synchronize recordings between animals during euthermic intervals at 21°C. Data from the first 5 h of euthermia following arousal from torpor were not used in this analysis, as sleep characteristics during this time are strongly influenced by the Tbr the animal experienced during the preceding torpor bout (32). One-way repeated-measures ANOVAs were performed to determine whether sleep parameters varied significantly during the course of the recording. If the overall ANOVA was significant ($P < 0.05$), then post hoc tests (Fisher’s paired least significant difference) were performed. Partial correlation analysis of sleep parameters is reported as $r_{PC}$, with positive values reflecting positive correlations and negative values reflecting negative correlations (Statview 5.0, SAS Institute, Cary, NC).

**RESULTS**

There were circadian fluctuations in Tbr independent of Tact during euthermic intervals at Tact of 21°C and during torpor bouts at Tact of 9 and 21°C (Fig. 1). Tact remained constant and showed no circadian variability (Fig. 1, F and M, for 21°C; data not shown for 9°C), and thus it did not account for the observed changes in Tbr. Circadian rhythmicity in Tbr could be seen during euthermia most clearly when the effect of vigilance state on Tbr was removed by analyzing mean hourly values of Tbr during sustained (>60 s) NREM sleep bouts (Fig. 1, A-C). Recordings of Tbr in which the influence of vigilance state was not removed showed both circadian modulation of Tbr and periodic spikes in Tbr associated with intervals of wakefulness (Fig. 1, D and E). Circadian rhythmicity in Tbr occurred in torpid animals at Tact of 9 and 21°C (Fig. 1, G-L). The period of the Tbr rhythm was $21.7 \pm 1.1$ h during euthermic intervals at 21°C and $21.7 \pm 1.8$ h during torpor at 21°C, but was longer ($27.2 \pm 1.2$ h) during torpor at Tact of 9–11°C.

Although both vigilance state and the circadian system are known to influence Tbr in mammals (21), in euthermic ground squirrels during the hibernation season vigilance state did not have a significant impact on Tbr ($P = 0.08, R^2 = 0.032$; Fig. 2). Tbr was elevated during periods of wakefulness, as expected (Fig. 1, D and E). When animals were awake >50% of the recording time, $T_{deu}$ was $0.52 \pm 0.07°C$ above the daily mean (Fig. 2). However, there was wide variability in Tbr during sleep (>2°C), which was attributable to the circadian system; 61.5% of the residual variance in Tbr, following regression analysis of Tbr against percent wakefulness, was circadian ($P < 0.001$). The same Tbr value was obtained from animals awake during the circadian trough of Tbr, as in animals asleep during the peak in Tbr rhythm. The circadian rhythm in Tbr could also be seen when the effect of vigilance state was removed by plotting hourly mean values of Tbr during sustained NREM sleep episodes (Fig. 1, A-C). Because of the large variability of Tbr during sleep, vigilance state was a poor predictor of Tbr, and accounted for only 3% of total variability in Tbr, making circadian control the dominant influence on Tbr (Figs. 1 and 2).

Sleep was distributed throughout euthermic intervals, and neither sleep nor wakefulness was consolidated into a circadian phase coordinated with the Tbr rhythm. Instead, intervals of wakefulness, lasting from 45 min to 2 h, regularly followed 3 to 4 h of sleep (Fig. 3). At 11°C, squirrels typically awoke during the fourth or fifth hour of euthermia, breaking sleep during euthermia into 3- to 4-h blocks. In the longer euthermic intervals at 21°C, there were multiple periods of wakefulness interrupting sleep. At 21°C, euthermic intervals lasted over 24 h, as opposed to euthermic intervals of 10–14 h at 11°C, allowing analysis of the periodicity of wakefulness at 21°C. Periodogram analysis showed significant ($P < 0.05$) ultradian rhythmicity between 5.7 and 8.7 h in each case (Fig. 4), except for the shortest (37 h) euthermic interval (Table 1). The brevity of this 37-h euthermic interval greatly weakened the power of the periodogram analysis. The ultradian periodicity in occurrence of wakefulness of 5.7 h resulted in significant harmonic values at multiples of the ultradian period at 11.7, 16.3, and 22.8 h (Fig. 4).
Periodogram analysis found ultradian periodicity in SWA as was found in percent wakefulness in each case (Table 1). Whereas ultradian rhythmicity was detected in both percent wakefulness and SWA, significant ultradian periodicity was detected in only one instance for sigma activity and not at all for Tbr. Near-circadian (19.0 and 23.7 h) rhythmicity was detected by periodogram analysis for sigma activity and Tbr only in the longest recording (48 h).

SWA during the first 5 h of euthermia was primarily determined by Ta, with low amounts of NREM sleep and low SWA following torpor at Tbr of 21°C as reported previously (32, 45, 48). After the initial 5 to 7 h of euthermia and following the first sustained period of wakefulness, however, SWA reflected prior sleep-wake history during the 3- to 4-h ultradian sleep periods rather than the conditions of the previous torpor bout.

Within the ultradian sleep periods, SWA and sigma activity followed an inverse relationship (Fig. 5). SWA reached maximal values 20–40 min after sleep onset, and then it fell during the remainder of the sleep episode. In contrast, sigma activity was high at sleep onset, during the first 20 min of sleep, but it fell rapidly to minimal levels during most of the sleep episode when SWA remained high. Sigma activity rose again near the end of the sleep episode after SWA had fallen to low levels, after 3 h of sleep. NREM sleep accounted for 78.1% of total recording time during these ultradian sleep periods, REM sleep accounted for 17.7 ± 0.7%, and brief awakenings accounted for 4.1 ± 0.5%. NREM sleep bout length averaged 6.4 ± 0.3 min, and REM sleep bout lengths averaged 2.1 ± 0.1 min.

Although there was no evidence of circadian organization of sleep and wakefulness during euthermic in-

**Fig. 1.** Brain temperature (Tbr) rhythmicity during the hibernation season. Left: Tbr during euthermic intervals (A-E); F is air temperature (Ta) during recording E. Circadian rhythmicity in Tbr can be seen during euthermic intervals when the effect of vigilance state is removed, by plotting mean hourly values of Tbr during sustained non-rapid eye movement (NREM) sleep bouts (A-C). In unprocessed Tbr data, plotted in 10-s bins, both circadian and vigilance state effects on Tbr can be seen (D and E). Baseline Tbr has an overt circadian rhythm in which Tbr spikes, associated with periods of wakefulness indicated by *, are superimposed. Right: Tbr during hibernation bouts (G-L); M is Ta during recording L. Ta during the hibernation recordings was 9°C for G-I and 20–21°C for J-L.
intervals, both Tbr and sigma activity retained circadian organization during eutherma. CT was determined from the circadian rhythm in Tbr, which was independent of vigilance state distribution (Figs. 1, 2, and 6). When sleep was analyzed in reference to CT, there was a significant change in sigma activity over time (ANOVA, P < 0.001); sigma activity rose above the daily mean at CT 0-8, fell below the mean at CT 10, 14-18, and 20-22, and then rose above the mean again at CT 23-24 (Fig. 6). In contrast, no corresponding circadian changes in vigilance state distribution, NREM sleep bout length, NREM sleep fragmentation rate (nBI/min NREM sleep), or SWA could be detected in this analysis. Individual analysis of each euthermic interval also showed no circadian rhythm in these parameters. High levels of percent wakefulness, NREM sleep bout length, NREM sleep fragmentation rate, and SWA were associated with the ultradian sleep-wake cycle, not with the circadian rhythm in Tbr and sigma activity (Fig. 6). Neither NREM sleep bout length nor SWA remained high throughout the period of low Tbr or remained low during the period of high Tbr. Regression analysis indicated that percent wakefulness accounted for 24% of the variability seen in SWA and 40% of the variability in sigma activity. Analysis of the residuals of these regression analyses against CT showed no significant circadian fluctuations in SWA (P > 0.35), but a significant circadian component remained in sigma activity (P < 0.0001, data not shown), substantiating the other analyses indicating maintenance of circadian rhythmicity in sigma activity and Tbr, but not the other measured sleep parameters.

The free-running circadian rhythms in Tbr and sigma activity were out of phase (Fig. 6). The rhythm in sigma activity was phase advanced by 3–4 h compared with the Tbr rhythm, rising above the daily mean at CT 23 vs. CT 2 for Tbr. Sigma activity fell below the daily mean at CT 10, whereas Tbr fell 4 h later at CT 14. Despite this apparent phase shift, partial correlation analysis indicated that the variable most highly correlated with Tbr was sigma activity ($r_{PC} = 0.25$). In contrast, SWA was not correlated to Tbr ($r_{PC} = 0.06$). Instead, SWA was positively correlated with NREM sleep bout length ($r_{PC} = 0.41$) and negatively correlated with NREM sleep fragmentation rate and percent wakefulness ($r_{PC} = -0.39$ and -0.36, respectively). These parameters followed the 6-h ultradian rhythm in wakefulness. Sigma activity was most highly correlated with percent wakefulness, NREM sleep fragmentation rate, and Tbr ($r_{PC} = 0.38$, 0.29, and 0.25, respectively).

Fig. 3. Hypnogram, Tbr, and slow-wave activity (SWA) profiles (μV²/Hz) for a 48-h euthermia interval following arousal from hibernation at Ta of 21°C. Except for the first several hours of euthermia, wakefulness occurs periodically between sleep bouts, which are characterized by clear NREM-rapid eye movement (REM) sleep cycling and declining SWA within each sleep bout.
DISCUSSION

Under constant conditions during the hibernation season, golden-mantled ground squirrels retained circadian rhythmicity in T_br, both during torpor and euthermia, as had been shown previously for T_b (24). In contrast, no circadian organization could be detected in the occurrence of sleep and wakefulness, in SWA in NREM sleep, or NREM sleep bout length, all of which followed ~6-h ultradian rhythm. The absence of detectable circadian rhythmicity in these parameters reflects either absence or severe diminution of circadian organization of sleep and wakefulness during the hibernation season. In contrast to the lack of circadian organization in most other sleep parameters, sigma activity retained significant circadian rhythmicity during the hibernation season and also displayed typical inverse relation to SWA during ultradian sleep bouts. Although SWA has been shown to primarily reflect sleep homeostasis and prior sleep-wake history (9), sigma activity is regulated by both circadian and sleep homeostatic systems (11, 50, 53, 54).

The most surprising result in this study was the absence of circadian sleep-wake consolidation, even though circadian rhythms in T_br and sigma activity were maintained. Loss of daily SWA rhythms and circadian consolidation of sleep and wakefulness has also been seen in winter-acclimated Siberian hamsters maintained in short days at 15°C (6). It was not known, however, whether these hamsters maintained circadian rhythms in sigma activity or T_br, as observed in the present study. Circadian rhythms in sleep and T_b are independently regulated by the ventral and dorsal subparaventricular zone (SPZ) of the hypothalamus, which is a major efferent target of the suprachiasmatic nucleus (SCN). Lesioning the ventral SPZ results in almost complete loss of circadian rhythms in sleep but retention of circadian rhythms in T_b, whereas lesioning the dorsal SPZ results in loss of circadian T_b rhythms but complete retention of circadian rhythms in sleep (35). It would be of interest to compare the ventral SPZ of summer-active and hibernating ground squirrels to determine whether the observed loss of circadian sleep rhythms with retention of circadian T_br rhythms is due to changes in the ventral SPZ during the hibernation season.

One possible explanation for the loss of circadian sleep-wake consolidation seen in the present study is that the constant conditions caused desynchrony be...

Table 1. Results of ultradian periodogram analysis of sleep parameters during extended euthermic intervals between hibernation bouts at T_a of 21°C

<table>
<thead>
<tr>
<th>Duration of Euthermic Recording</th>
<th>Periodicity of % Wakefulness</th>
<th>Periodicity of SWA</th>
<th>Periodicity of Sigma Activity</th>
<th>Periodicity of T_br</th>
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<tr>
<td>44.3</td>
<td>5.7</td>
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<tr>
<td>37.0</td>
<td>11.0</td>
<td>11.3</td>
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<td>48.0</td>
<td>8.7</td>
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<td>37.0</td>
<td>6.3</td>
<td>7.3</td>
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<tr>
<td>46.0</td>
<td>5.7</td>
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Duration of each euthermic interval (h) is given, as is the significant (P < 0.05) periodicity (h) during each euthermic interval of wakefulness, slow-wave activity (SWA), sigma activity, and brain temperature (T_br), which were calculated in 20-min bins. ns Indicates no significant peak in the periodogram analysis (1–12 h). Harmonic multiples of the ultradian peak are not listed. T_a, air temperature.

Fig. 5. Profiles of SWA (thick line) and sigma activity (thin line) for ultradian sleep periods during euthermia at T_a of 21°C (means from 5 euthermic intervals, 9–13 data points/euthermic interval). Both SWA and sigma activity had a significant effect of time in the sleep period and are inversely related (ANOVA, P < 0.0001). EEG, electroencephalogram.
between the rhythms and loss of coherence within each rhythm, with sleep-wake consolidation being the most sensitive to disruption by constant conditions. Substantiating this possibility is the finding that the circadian rhythms in Tb and sigma activity were out of phase by 4 h. However, inducement of arrhythmia by the constant conditions seems unlikely as exposure to bright, constant light (150–200 lx) does not cause loss of circadian rhythmicity in diurnal species (8, 34, 63) as it does in nocturnal species (8, 41). Additionally, circadian locomotor activity rhythms are maintained even in nocturnal species at the low light intensity (20 lx) and constant light used in the present study (41), as has also been shown previously for golden-mantled ground squirrels (34). Furthermore, the loss of daily rhythms in SWA and sleep-wake consolidation was also observed in Siberian hamsters entrained to a short-day photoperiod (6), suggesting that a seasonal loss of sleep consolidation may be part of winter adaptation in these species.

The ultradian sleep patterns recorded during the euthermic intervals in this study strongly resemble those of animals rendered arrhythmic by ablation of the SCN (SCNx) (14, 43, 49, 52). SCNx results in a loss of the circadian consolidation of sleep and wakefulness. Sleep in SCNx animals is dominated by the ultradian sleep-wake cycle (14, 23, 43). However, loss of circadian sleep-wake consolidation does not affect sleep homeostasis; homeostatic responsiveness of SWA to sleep deprivation remains intact in SCNx animals (49, 52), as it does in hibernators during their euthermic intervals, with the exception of the first several hours of euthermia (30, 31, 46). The homeostatic responses of increased SWA following wakefulness, a decreasing trend in SWA during NREM sleep, and the inverse relationship between SWA and sigma activity in NREM sleep were also seen in the present study (Figs. 3 and 5). Thus homeostatic regulation of sleep is maintained during the hibernation season, although circadian sleep-wake consolidation is lost.
The absence of circadian consolidation of wakefulness reported in the present study at first may appear to contradict previous reports of maintenance of circadian locomotor activity throughout the hibernation season (5, 22, 63). One possible explanation for the apparent discrepancy in the results is that the use of the running wheels in the earlier studies may have increased the coherence and amplitude of the locomotor activity rhythms they recorded. The effects of running wheels on circadian rhythms have been well documented (38, 61). However, it is unlikely that increased circadian consolidation of rhythms can be attributed to the light use of running wheels of ground squirrels during the hibernation season. The use of running wheels is reduced 90% during the hibernation season in this species, even in animals that are main- running wheels on circadian rhythms have been well doc- umented (38, 61). However, it is unlikely that increased circadian consolidation of rhythms can be attributed to the light use of running wheels of ground squirrels during the hibernation season. The use of running wheels is reduced 90% during the hibernation season in this species, even in animals that are main-

Moreover, the circadian running wheel activity rhythms reported previously and ultradian sleep- wake rhythms reported in this study may not be mutually exclusive. Daily locomotor activity patterns are substantially different when recorded by running wheel use and by passive motion detectors (1), with the former method providing more striking differences in activity between light and dark period activity levels than the latter. Circadian rhythms in sleep-wake distribution and running wheel use are not strictly analogous, and the apparent contradic-
tion between the results of the present study and previous studies on running wheel use emphasizes the importance of using alternate measures of circadian rhythmicity.

The great reduction in locomotor activity level during the hibernation season may itself play a role in the seasonal weakening of circadian sleep-wake consolidation (15, 16, 20, 59). Mice with high levels of locomotor activity, due to higher intrinsic activity levels in some strains or to access to running wheels, have stronger circadian sleep-wake consolidation, whereas less active mice have decreased sleep-wake consolidation and in- creased sleep fragmentation, paralleling our findings with ground squirrels. Increased locomotor activity level during the summer is associated with increased circadian sleep-wake consolidation, as assessed by the ratio of total sleep time in night vs. daytime in 12:12-h light-dark photoperiod in sciurid rodents. In Sper- 
mophilus tridecemlineatus, Eutamias dorsalis, and Tamias striatus, the ratio of nighttime sleep to daytime sleep ranged from 1.04 to 1.20 when animals were not provided with running wheels (17, 55), whereas the sleep ratio was 2.83 when E. sibiricus were provided with running wheels (10). Thus increased locomotor activity level increases circadian sleep-wake consolidation in these diurnal rodents, as has been demonstrated in nocturnal rodents (20, 59). Definitive assessment of the relative influence of constant conditions, decreased locomotor activity, and changes in circadian drive in causing the loss of circadian sleep-wake con- solidation during the hibernation season must await sleep recordings of squirrels during the summer under constant conditions. It must be noted, however, that although locomotor activity may modulate circadian sleep-wake organization, even the most inactive mice and rats retain circadian rhythms in sleep and waking (15, 16, 20, 59). Lesions of the ventral and dorsal SPZ have similar effect on circadian rhythms in locomotor activity and sleep cycles (35), indicating common reg- ulatory pathways for these two parameters. It may be fruitful to investigate the SPZ regarding the changes in sleep organization and in locomotor activity during the hibernation season.

Locomotor activity level has also been shown to affect the period of circadian rhythms, with high levels of activity being associated with shorter periods of circa-
dian rhythmicity (tau) in hamsters, mice, and rats (16, 38, 61). This relationship between activity level and tau sheds light on the circannual changes in circadian period length observed in ground squirrels (5, 39, 63). Tau is shorter during the summer months when animals are highly active. During the hibernation season, activity level is greatly reduced and tau is longer, producing the characteristic cycle of cycles first noted by Mrosovsky et al. (39). In studies under light-dark cycles, the shorter summer tau is manifested by early activity onset, and the longer tau of the hibernation season leads to later activity onset (22). These changes in the circadian system do not simply result from decreased T_{br} during torpor, as they are seen even when torpor is prevented by testosterone implants (22). The disruption of circadian rhythmicity before hiber-
nation and reestablishment of rhythmicity with a longer tau are seen in both golden-mantled ground squirrels and Turkish hamsters (24, 40). Thus, low activity levels typical of the hibernation season may be responsible for the lengthening of the period of the circadian rhythm governing both T_{b} and locomotor activity.

Not all hibernating animals retain apparent circa-
dian rhythmicity during the hibernation season. Whereas some species, such as bats and golden- 
mantled ground squirrels, maintain circadian rhyth-
icity in T_{b} throughout the hibernation season when held in constant conditions (24, 37), other species, such as European hamsters, Syrian hamsters, and hedgehogs, lose circadian rhythmicity in T_{b} (18, 25, 60). One explanation for the variability in mainte-
nance of circadian T_{b} rhythmicity during the hiber-
nation season is that all hibernating species may lose circadian sleep-wake consolidation during the hibernation season. Thus circadian T_{b} rhythms may only be observed in those species with a strong role for the circadian system in determining T_{b}, such as the golden-mantled ground squirrel. In contrast, no cir-
cadian rhythmicity in T_{b} would be seen in species in which vigilance state has the dominant influence on T_{b}, such as the rat, in which 84% of all variability in T_{br} is ascribable to vigilance state (21). In a species in which T_{b} is determined primarily by vigilance state, the circadian system may remain active and
regulate the timing of arousal from and entrance into torpor (3, 24, 40, 60), but no circadian Tb rhythm would be seen because it would be masked by the ultradian sleep-wake rhythm.

Perspectives

Why do hibernators lose circadian organization of vigilance states during the hibernation season? Circadian rhythmicity is thought to confer advantages to animals by synchronizing their activity patterns with the environmental light-dark cycle (7). However, maintaining circadian rhythmicity during the hibernation season may not be advantageous, or even possible, for animals isolated from the environment in their hibernacula (27). Summer-active animals gather food, protect territories, and engage in other behaviors during particular phases of the light-dark cycle. Ground squirrels may not be adversely affected by a lack of circadian rhythmicity during the hibernation season, because they do not leave their burrows to interact with other animals or the environment during the hibernation season (62).

The absence of circadian sleep-wake consolidation may facilitate multiday bouts of hibernation. An important function of the circadian system is to consolidate wakefulness and sleep in particular phases of the circadian day (2, 14). Thus, in animals with strong circadian sleep-wake regulation, entrance into torpor is limited to their circadian rest period, whereas animals with weakened or lost circadian sleep-wake consolidation may enter torpor at any time of day (42), during one of the ultradian sleep periods, and may remain in torpor for multiple days. As up to 90% of the energy used during the hibernation season is consumed during the periodic euthemic intervals (36), it may be advantageous to minimize the duration of torpor by eliminating the endogenous circadian alerting that results in consolidated wakefulness. Weakening of the circadian alerting signal may allow animals to remain torpid for multiple days. In species that use daily torpor, the circadian system is necessary for the timing of torpor and to ensure that animals arouse before their daily activity period (42).

In conclusion, maintenance of multiday hibernation bouts may require weakening the circadian control of wakefulness. To ensure that an animal remains torpid for multiple days, it may be necessary to weaken the wake-promoting signal generated by the SCN (14), resulting in the loss of circadian sleep-wake consolidation during euthermia and, possibly, maintenance of multiday bouts of torpor.

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LOSS OF CIRCADIAN SLEEP ORGANIZATION IN HIBERNATING SQUIRRELS


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