Cold exposure and/or fasting modulate the relationship between sleep and body temperature rhythms in mice

Physiology & Behavior 149 (2015) 69–75

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Cold exposure and/or fasting modulate the relationship between sleep and body temperature rhythms in mice

Nobuo Sato [a,b], Shuri Marui [b], Makoto Ozaki [a], Kei Nagashima [b,c,*]

[a] Department of Anesthesiology, Tokyo Women's Medical University, Tokyo, Japan
[b] Body Temperature and Fluid Laboratory (Laboratory of Integrative Physiology), Faculty of Human Sciences, Waseda University, Tokorozawa, Saitama, Japan
[c] Institute of Applied Brain Sciences, Waseda University, Tokorozawa, Saitama, Japan

HIGHLIGHTS

- The relationship between sleep and core temperature (T_c) was assessed in mice.
- The sleep period correlated with T_c when mice were exposed to cold or fasted.
- REM sleep and T_c rhythms dissociated when both cold and fasting were combined.

ARTICLE INFO

Article history:
Received 6 February 2015
Received in revised form 19 May 2015
Accepted 21 May 2015
Available online 27 May 2015

Keywords:
Sleep–wake rhythm
Core temperature
Circadian rhythm
Cold exposure
Feeding condition

ABSTRACT

We assessed the relationship between core temperature (T_c) and sleep rhythms in mice, and examined the effects of ambient temperature and fasting. T_c, electroencephalograms (EEG), electromyograms (EMG), and spontaneous activity in male ICR mice ($n = 9$) were measured by telemetry for 3 days under a 12:12 h dark–light cycle. Mice were fed or fasted at an ambient temperature (T_a) of 27°C or 20°C for the final 30 h of the experiment. The vigilance state was categorized into a wake state, rapid-eye movement (REM) sleep, and non-REM (NREM) sleep, and the total sleep time (TST) was assessed. Relationships between T_c and TST, NREM periods, and REM sleep were estimated using Pearson's correlation coefficient. During cold exposure, T_c decreased during the dark and light phases, and TST and the periods of NREM and REM sleep decreased during the dark phase. Throughout the fasting period, T_c also decreased during the dark and light phases. Furthermore, the decrease in T_c was augmented when fasting and cold were combined. TST and NREM sleep periods decreased in the light and dark phases, respectively, whereas REM sleep periods decreased in both phases. Negative linear correlations ($r = -0.884$ to -0.987) were observed between T_c and TST, NREM sleep periods, and REM sleep periods, except for T_c and REM sleep periods where fasting and cold conditions were combined. The correlations between sleep and T_c rhythms were well maintained during cold exposure and fasting. However, when cold and fasting were combined, REM sleep and T_c rhythms were desynchronized.

1. Introduction

Sleep disorders are prevalent among people living in the modern era [1]. Sleep is influenced by several factors, and the disturbance of any one of these factors could trigger a sleep disorder. Fasting and/or cold are well-known factors that affect sleep [2–4]. Although interactions among sleep, thermoregulation, and metabolism are speculated upon [5], no clear evidence has been presented.

It has long been assumed that core temperature (T_c) and sleep rhythms are linked to each other [6–8], although the evidence remains limited. In humans, T_c decreases at the onset of sleep [9–11], and this reduction is largely due to skin vasodilation (i.e., heat loss) [12]. Regarding circadian T_c and sleep rhythms, sleep periods reach a peak when T_c is lowest [2,8,13]. A negative correlation between T_c and the amount of non-rapid-eye-movement (NREM) sleep during the day was also reported [14]. McGinty et al. reported that in cats, local heating of the hypothalamus increased sleep depth [15], which suggests that the T_c change modulates sleep rhythms.

At an ambient temperature (T_a) of 21–29°C, rats can control their T_c within ±1.3°C, although sleep changes even within this T_a range [4]. For instance, sleep was increased at a T_a of 29°C than at 21°C. This indicates that T_a may alter the relationship between T_c and sleep rhythms. However, the influence of T_c and T_a on sleep has only been individually assessed [2,3,16]. Therefore, it is unclear if the influence of T_a on sleep is modulated during circadian T_c changes.

* Corresponding author at: Body Temperature and Fluid Laboratory (Laboratory of Integrative Physiology), Faculty of Human Sciences, Waseda University, 2-579-15 Mikajima, Tokorozawa, Saitama 359-1192, Japan.
E-mail address: k-nagashima@waseda.jp (K. Nagashima).

http://dx.doi.org/10.1016/j.physbeh.2015.05.024
0031-9384/

Rats and mice become hypothermic during fasting, especially in the inactive phase (i.e., daily torpor) [17–21]. Furthermore, despite the reduction in T_c, the amount of sleep decreases in mice [22–24]. Although the results may indicate that the link between T_c and sleep rhythms is lost during fasting, Szentirmai et al. showed a negative correlation between T_c and the relative amount of NREM sleep during fasting [14]. They analyzed the relationship at a T_a of 17°C, which did not exclude the influence of cold temperatures. Moreover, the relationship between T_c and total sleep time (TST) or rapid-eye movement (REM) sleep should have been assessed. In the present study, to investigate the link between T_c and sleep rhythms, we analyzed the relationship between T_c and sleep (i.e., TST, REM sleep, and NREM sleep) during a 24-h period in mice. Furthermore, we compared the influence of two different ambient conditions (a thermoneutral condition: $T_a = 27$°C [25], and a cold condition: $T_a = 20$°C) on the correlation, and the analyses were repeated in fasted mice. We hypothesized that a linear correlation between T_c and the sleep components exists, which is modulated by T_a, and that fasting weakens this correlation.

2. Materials and methods

2.1. Animals

Mice obtained from the Institute of Cancer Research (ICR) (male: $n = 9$; age: 8–36 wk; body weight: 35–50 g) were used. They were individually housed in plastic cages (25 × 18 × 13 cm) with ad libitum access to water and food. The T_a was maintained at 27 ± 0.5°C, and the lighting cycle was 12 h of light (300 lx at eye level, on at 0700 h) and 12 h of complete darkness. The Institutional Animal Care and Use Committee of Waseda University approved all of the experimental procedures used in this study.

2.2. Surgery

Mice were anesthetized with 2% isoflurane in air (Abbott Japan, Tokyo, Japan). Using sterile techniques, a radio transmitter (TL11M2-F20-EET; Data Sciences International, St. Paul, MN) was placed in the abdominal cavity to record electroencephalograms (EEG), electromyograms (EMG), and T_c. Two EEG electrodes were placed on the surface of the right frontal and left parietal cortex, and were then anchored to the skull with dental cement. Penicillin-G (800 U, Meiji Seika Pharma, Tokyo, Japan) was injected intramuscularly to minimize postsurgical infection, and the mice were allowed to recover for at least 14 days. Recovery was verified when a mouse exhibited clear circadian T_c changes, spontaneous activity, and sleep–wake patterns.

2.3. Experimental protocols

T_c, EEG, EMG, and spontaneous activity were measured for 3 days starting at 1900 h. Signals from the transmitter were collected via a receiver board underneath the cage, and the spontaneous activity was estimated by the change in the signal strength. Data were stored on a personal computer using an acquisition program (Dataquest ART; Data Sciences International, St. Paul, MN). T_c and spontaneous activity data were collected every 10 s, and EEG and EMG data were digitized at a sampling rate of 500 Hz.

For the first 42 h of the 3 days, mice had ad libitum access to food at a T_a of 27°C. At 1300 h on Day 2, the condition was changed to either (1) ad libitum access to food at a T_a of 27°C (control), (2) ad libitum access to food at a T_a of 20°C, (3) fasting at a T_a of 27°C, or (4) fasting at a T_a of 20°C. Each condition was maintained for 30 h, and water was freely available. At 1900 h on Day 3, all mice were provided food, and the T_a was set at 27°C. Each mouse was exposed to the four different conditions in random order. Mice were considered to have recovered from the protocol when they exhibited similar circadian T_c and sleep rhythms to those observed during the first 42 h period. The interval between the protocols was at least 1 wk.

2.4. Sleep analysis

Noise and signal outliers above 100 Hz were removed using a digital high-cut filter. EEG (0.75–30 Hz) and EMG (20–50 Hz) signals were picked up using a band-pass filter and were then divided into 10 s epochs. The vigilance state was categorized into 3 stages (wake, REM, or NREM sleep) using commercial software (SLEEPSIGN, Kissei Comtec, Nagano, Japan). The wake stage was defined by a low-amplitude EEG with components of various frequencies and an EMG consisting of high and irregular signals. The REM sleep stage included a low-amplitude EEG with dominant 6–10 Hz theta waves and low EMG activity. The NREM sleep stage was defined by a high-amplitude EEG with low EMG activity. The sleep stages were confirmed by visual inspection and were corrected if necessary. The EEG delta frequency band during NREM sleep epochs was set at 0.75–4 Hz, and the delta power was expressed as a percentage of the total power (0.75–30 Hz). The number and duration of NREM and REM episodes were also estimated.

2.5. Statistics

Differences in mean T_c, TST, periods of NREM and REM sleep, number and duration of NREM and REM episodes, and EEG delta power during NREM sleep were all analyzed using a three-way analysis of variance (ANOVA). The factors analyzed were phase, feeding condition, and T_a. A Bonferroni post hoc-test was conducted to identify differences between specific conditions or phases. The correlations between T_c and TST, NREM sleep, and REM sleep were estimated using Pearson's correlation coefficient. Null hypotheses were rejected when $p < 0.05$, and the values presented are means ± SEM.

3. Results

In each trial, T_c showed clear circadian changes. The average T_c for the dark and light phases on Day 3 is illustrated in Fig. 1B (corresponding to the last 24 h of the shaded area of Fig. 1A). In each trial, T_c was lower in the light phase than in the dark phase. Under fed conditions, T_c was lower at a T_a of 20°C than at 27°C in either phase (37.2 ± 0.2°C and 37.5 ± 0.1°C in the dark phase and 35.9 ± 0.1°C and 36.1 ± 0.1°C in the light phase, respectively). The T_c values in the fasted condition were also lower at a T_a of 20°C than at 27°C in both phases (35.3 ± 0.1°C and 36.6 ± 0.1°C in the dark phase and 34.4 ± 0.2°C and 35.2 ± 0.1°C in the light phase, respectively). The T_c in the fasted condition was lower than that in the fed condition at a T_a of 27°C or 20°C in both phases [three-way ANOVA for repeated measures: phase: $F(1, 8) = 274.21$, $p = 0.000$; feeding condition: $F(1, 8) = 675.96$, $p = 0.000$; T_a: $F(1, 8) = 76.50$, $p = 0.000$; phase × feeding condition interaction: $F(1, 8) = 1.92$, $p = 0.203$; phase × T_a interaction: $F(1, 8) = 9.72$, $p = 0.014$; feeding conditions × T_a interaction: $F(1, 8) = 86.35$, $p = 0.000$; phase × feeding condition × T_a interaction: $F(1, 8) = 12.71$, $p = 0.007$].

Fig. 2A shows the TST during the last 24 h of the test period, and the data are shown in 3-h bins. Fig. 2B illustrates the TST in the dark and light phases. In each trial, the TST was longer in the light phase than in the dark phase. In the fed condition, the TST at a T_a of 20°C was less than that at a T_a of 27°C in the dark phase. In the fasted condition, the TST at a T_a of 20°C was less than that at a T_a of 27°C in the light phase. Furthermore, in the light phase, the TST in the fasted condition was less than that in the fed condition at a T_a of 20°C [three-way ANOVA for repeated measures: phase: $F(1, 8) = 112.28$, $p = 0.000$; feeding condition: $F(1, 8) = 0.77$, $p = 0.407$; T_a: $F(1, 8) = 18.33$, $p = 0.003$; phase × feeding condition interaction, $F(1, 8) = 8.44$, $p = 0.020$; phase × T_a interaction: $F(1, 8) = 1.98$, $p = 0.197$; feeding

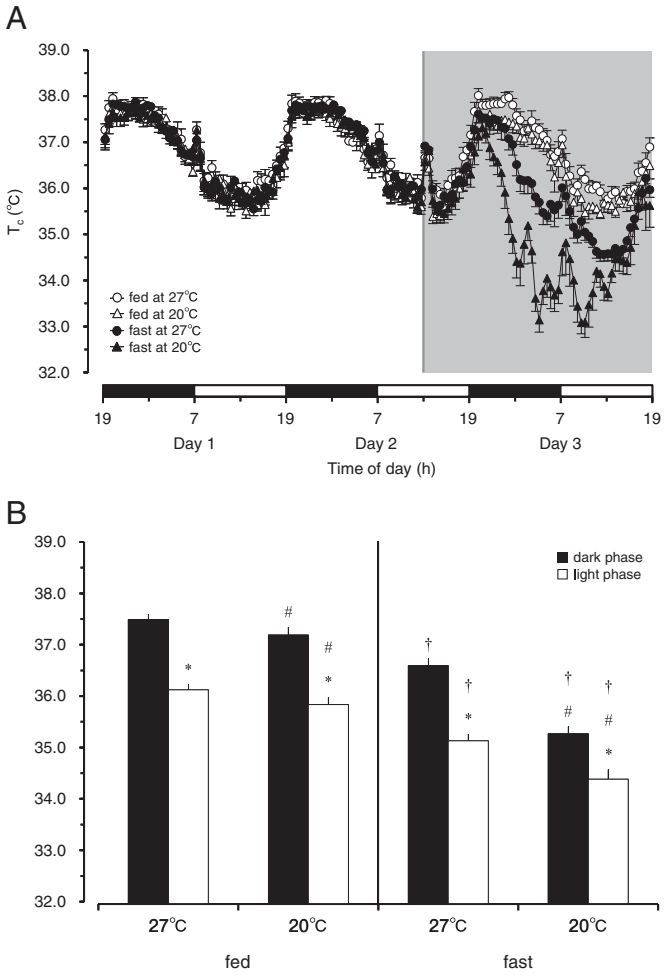

Fig. 1. The 3-day change in core temperature (T_c; A) and the average T_c in the dark and light phases (B) for the four different conditions (ambient temperature and feeding conditions). In the first 42 h, mice were fed at an ambient temperature (T_a) of 27°C. They were then fed or fasted at a T_a = 27°C or T_a = 20°C for 30 h (shaded area in A). Each data point in A denotes a 30-min average. The black and white stripes indicate the dark and light phases, respectively. Data for each phase (B) were obtained on Day 3 of the experiment. Values are means ± SEM (n = 9). *Significant differences between the dark and light phases in each trial ($p < 0.05$). #Significant differences between T_a = 27°C and T_a = 20°C under each feeding condition and in each phase ($p < 0.05$). †Significant differences between mice examined under fed and fasted conditions at each ambient temperature and during each phase ($p < 0.05$).

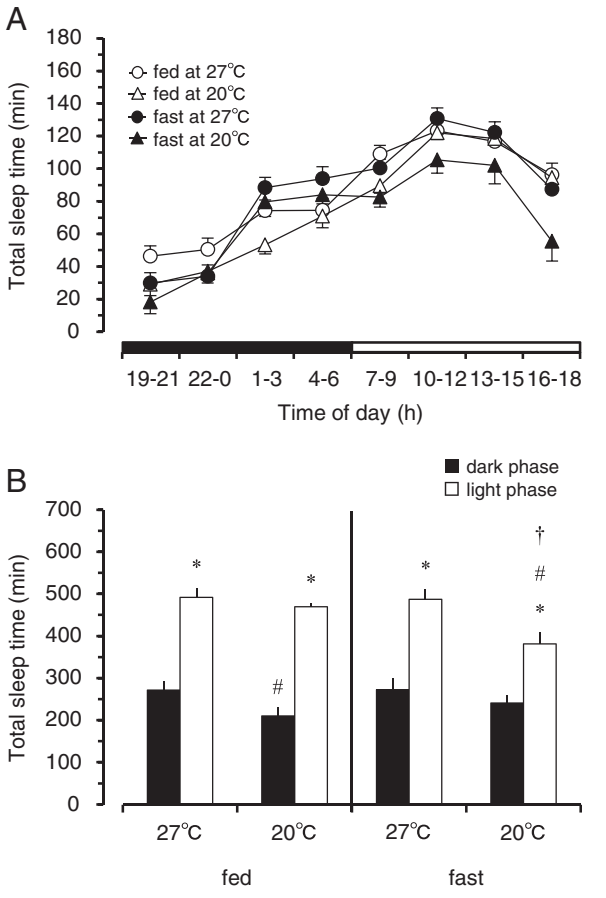

Fig. 2. Daily change in total sleep time (A) and the sum during the dark and light phases (B). Total sleep time within a 3-h bin was analyzed on Day 3 of each trial (A). Values are the means ± SEM (n = 9). *Significant differences between the dark and light phases in each trial ($p < 0.05$). #Significant differences between T_a = 27°C and T_a = 20 °C under each feeding condition and in each phase ($p < 0.05$). †Significant differences between mice examined under the fed and fasted conditions at each ambient temperature and during each phase ($p < 0.05$).

condition × T_a interaction: $F(1, 8) = 0.62$, $p = 0.452$; phase × feeding condition × T_a interaction: $F(1, 8) = 10.77$, $p = 0.011$].

Fig. 3A and B shows the NREM sleep periods, which were analyzed in the same manner as the TST. NREM sleep periods were longer in the light phase than in the dark phase for each trial. In the fed condition, the NREM sleep period during the dark phase at a T_a of 20°C was shorter than that at a T_a of 27°C. In the fasted condition, the NREM sleep period during the light phase at a T_a of 20°C was shorter than that at a T_a of 27°C. Furthermore, the NREM sleep period in the light phase was shorter in the fasted condition than in the fed condition at a T_a of 20 °C [three-way ANOVA for repeated measures: phase: $F(1, 8) = 119.20$, $p = 0.000$; feeding condition: $F(1, 8) = 0.09$, $p = 0.771$; T_a: $F(1, 8) = 12.12$, $p = 0.008$; phase × feeding condition interaction, $F(1, 8) = 6.47$, $p = 0.035$; phase × T_a interaction: $F(1, 8) = 3.95$, $p = 0.082$; feeding condition × T_a interaction: $F(1, 8) = 0.43$, $p = 0.531$; phase × feeding condition × T_a interaction: $F(1, 8) = 14.01$, $p = 0.006$].

Fig. 3C illustrates the episode duration for NREM sleep. The episode duration for NREM sleep in the fed condition was longer in the light phase than in the dark phase. In both phases in the fasted condition, the episode duration was shorter at a T_a of 20°C than at a T_a of 27°C. In the dark phase, the episode duration was longer in the fasted condition than in the fed condition for both ambient temperatures [three-way ANOVA for repeated measures: phase: $F(1, 8) = 8.17$, $p = 0.021$; feeding condition: $F(1, 8) = 6.13$, $p = 0.038$; T_a: $F(1, 8) = 51.36$, $p = 0.000$; phase × feeding condition interaction: $F(1, 8) = 7.63$, $p = 0.025$; phase × T_a interaction: $F(1, 8) = 0.01$, $p = 0.912$; feeding conditions × T_a interaction: $F(1, 8) = 1.45$, $p = 0.262$; phase × feeding condition × T_a interaction: $F(1, 8) = 2.35$, $p = 0.164$].

Fig. 3D illustrates the number of NREM sleep episodes. There were more episodes during the light phase than the dark phase, except at a T_a of 27°C in the fed condition. However, no differences were observed in the number of NREM sleep episodes between the two ambient temperatures. In the dark phase, fewer NREM sleep episodes occurred in the fasted condition than in the fed condition at a T_a of 27°C [three-way ANOVA for repeated measures: phase: $F(1, 8) = 59.87$, $p = 0.000$; feeding condition: $F(1, 8) = 5.99$, $p = 0.040$; T_a: $F(1, 8) = 4.37$, $p = 0.070$; phase × feeding condition interaction: $F(1, 8) = 1.11$, $p = 0.323$; phase × T_a: $F(1, 8) = 1.52$, $p = 0.253$; feeding conditions × T_a interaction: $F(1, 8) = 0.67$, $p = 0.436$; phase × feeding condition × T_a interaction: $F(1, 8) = 4.59$, $p = 0.064$].

Fig. 4A illustrates the REM sleep period, shown in 3-h bins. Fig. 4B illustrates the REM sleep period in the light and dark phases for each trial. The REM sleep period was longer during the light phase than during the dark phase, except in the fasted condition (T_a of 27°C). During the dark phase, for both feeding conditions, the periods were shorter at a T_a of 20°C than at a T_a of 27°C. Moreover, the period was shorter in the fasted

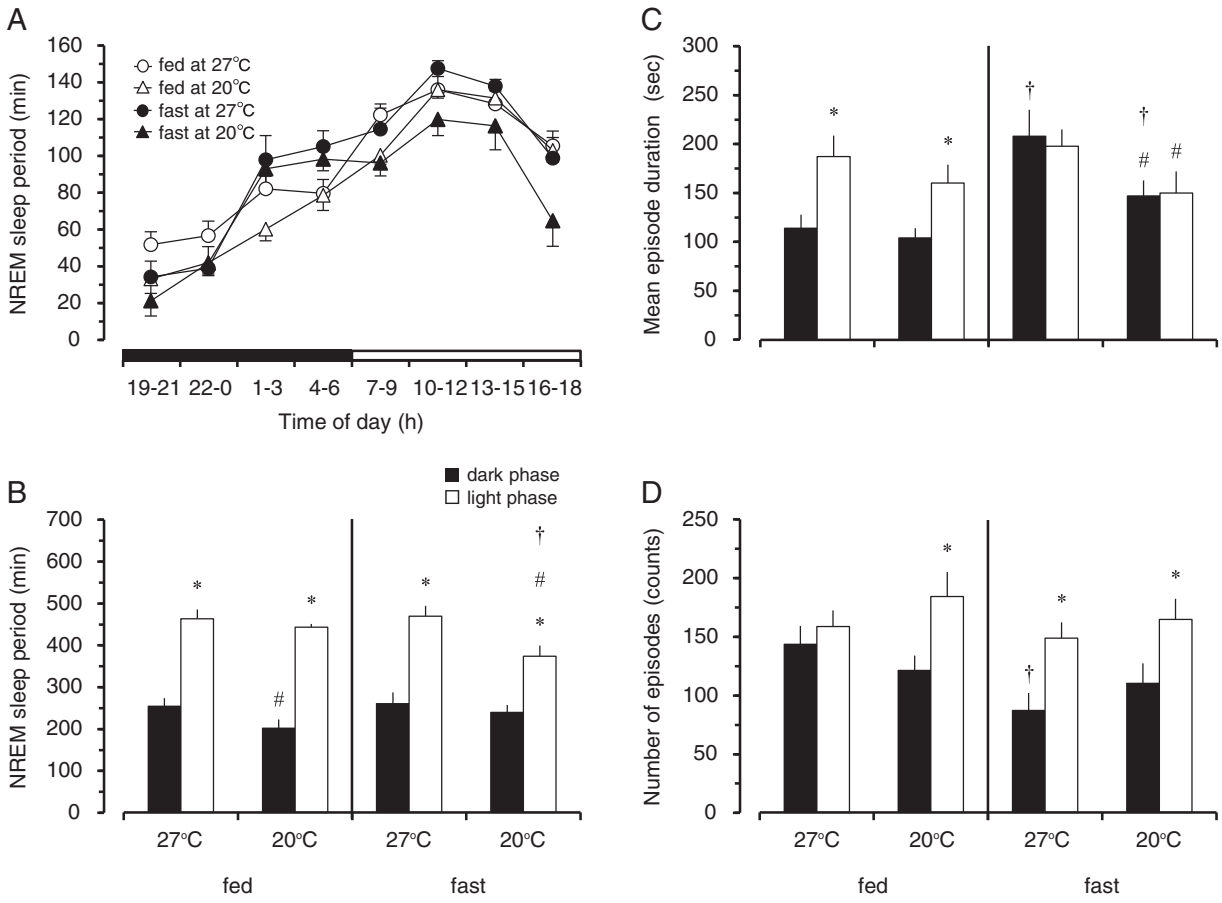

Fig. 3. Daily changes in the NREM sleep period (A), and the sum (B), average episode duration (C), and number (D) of NREM sleep periods during the light and dark phases. The analyses were conducted in the same manner as the total sleep time analyses. Values are means ± SEM ($n = 9$). *Significant differences between the dark and light phases in each trial ($p < 0.05$). #Significant differences between $T_a = 27°C$ and $T_a = 20°C$ under each feeding condition and in each phase ($p < 0.05$). †Significant differences between mice examined under fed and fasted conditions at each ambient temperature and during each phase ($p < 0.05$).

condition than in the fed condition for both ambient temperatures. At a T_a of 27°C, the period was also shorter during the light phase but not during the dark phase [three-way ANOVA for repeated measures: phase: $F(1, 8) = 19.77$, $p = 0.002$; feeding condition: $F(1, 8) = 25.45$, $p = 0.001$; T_a: $F(1, 8) = 20.85$, $p = 0.002$; phase × feeding condition interaction: $F(1, 8) = 25.30$, $p = 0.001$; phase × T_a interaction: $F(1, 8) = 0.98$, $p = 0.352$; feeding conditions × T_a interaction: $F(1, 8) = 2.38$, $p = 0.162$; phase × feeding condition × T_a interaction: $F(1, 8) = 0.55$, $p = 0.479$].

Fig. 4C illustrates the episode duration for REM sleep, which did not differ between the light and dark phases in each trial. In the fasted condition, the episode duration in the dark phase was shorter at a T_a of 20°C than at a T_a of 27 °C. In addition, the episode duration at a T_a of 27°C was longer than that in the fed condition during the dark phase [three-way ANOVA for repeated measures: phase: $F(1, 8) = 0.25$, $p = 0.631$; feeding condition: $F(1, 8) = 0.35$, $p = 0.571$; T_a: $F(1, 8) = 24.69$, $p = 0.001$; phase × feeding condition interaction: $F(1, 8) = 0.28$, $p = 0.614$; phase × T_a interaction: $F(1, 8) = 1.79$, $p = 0.217$; feeding conditions × T_a interaction: $F(1, 8) = 1.68$, $p = 0.231$; phase × feeding condition × T_a interaction: $F(1, 8) = 0.39$, $p = 0.550$].

Fig. 4D illustrates REM sleep episodes. There were more REM sleep episodes during the light phase than during the dark phase in the fed condition. During the dark phase in both feeding conditions, there were fewer REM sleep episodes at a T_a of 20 °C than at a T_a of 27°C. Fewer REM sleep episodes were observed in the fasted condition than in the fed condition for both ambient temperatures and during both light phases, except during the dark phase at a T_a of 27°C [three-way ANOVA for repeated measures: phase: $F(1, 8) = 13.52$, $p = 0.006$; feeding condition: $F(1, 8) = 25.80$, $p = 0.001$; T_a: $F(1, 8) = 13.51$, $p = 0.006$; phase × feeding condition interaction: $F(1, 8) = 20.30$, $p = 0.002$; phase × T_a interaction: $F(1, 8) = 1.19$, $p = 0.308$;feeding conditions × T_a interaction: $F(1, 8) = 1.65$, $p = 0.234$; phase × feeding condition × T_a interaction: $F(1, 8) = 0.14$, $p = 0.719$].

The ratio of the NREM sleep period to the TST did not differ between the light and dark phases in each trial. Moreover, in the fed condition, the ratio did not differ between phases and T_a conditions (dark phase: 0.927 ± 0.012 and 0.943 ± 0.008; light phase: 0.932 ± 0.009 and 0.937 ± 0.009 at T_a of 27°C and 20°C, respectively). In the fasted condition, the ratio at a T_a of 20°C was greater than at a T_a of 27°C for the dark phase (dark phase: 0.941 ± 0.008 and 0.986 ± 0.004 at T_a of 27°C and 20°C, respectively). However, in the light phase, the ratio did not differ between the T_a conditions (light phase: 0.954 ± 0.010 and 0.972 ± 0.006 at T_a of 27 °C and 20 °C, respectively). During both light phases, the ratios were greater in the fasted condition than in the fed condition, except in the dark phase at a T_a of 27°C [three-way ANOVA for repeated measures: phase: $F(1, 8) = 0.01$, $p = 0.930$; feeding condition: $F(1, 8) = 53.65$, $p = 0.000$; T_a: $F(1, 8) = 11.50$, $p = 0.009$; phase × feeding condition interaction: $F(1, 8) = 0.00$, $p = 0.969$; phase × T_a interaction: $F(1, 8) = 12.46$, $p = 0.008$; feeding conditions × T_a interaction: $F(1, 8) = 5.13$, $p = 0.053$; phase × feeding condition × T_a interaction: $F(1, 8) = 1.14$, $p = 0.316$].

The EEG delta power in NREM sleep did not differ between light and dark phases in each trial. During both feeding conditions, the power did not differ between both ambient temperatures in either the dark (fed condition: 101.6 ± 1.3% and 101.0 ± 3.3%; fast condition: 108.0 ± 6.3% and 110.1 ± 6.7% at a T_a of 27°C and 20°C, respectively) or light

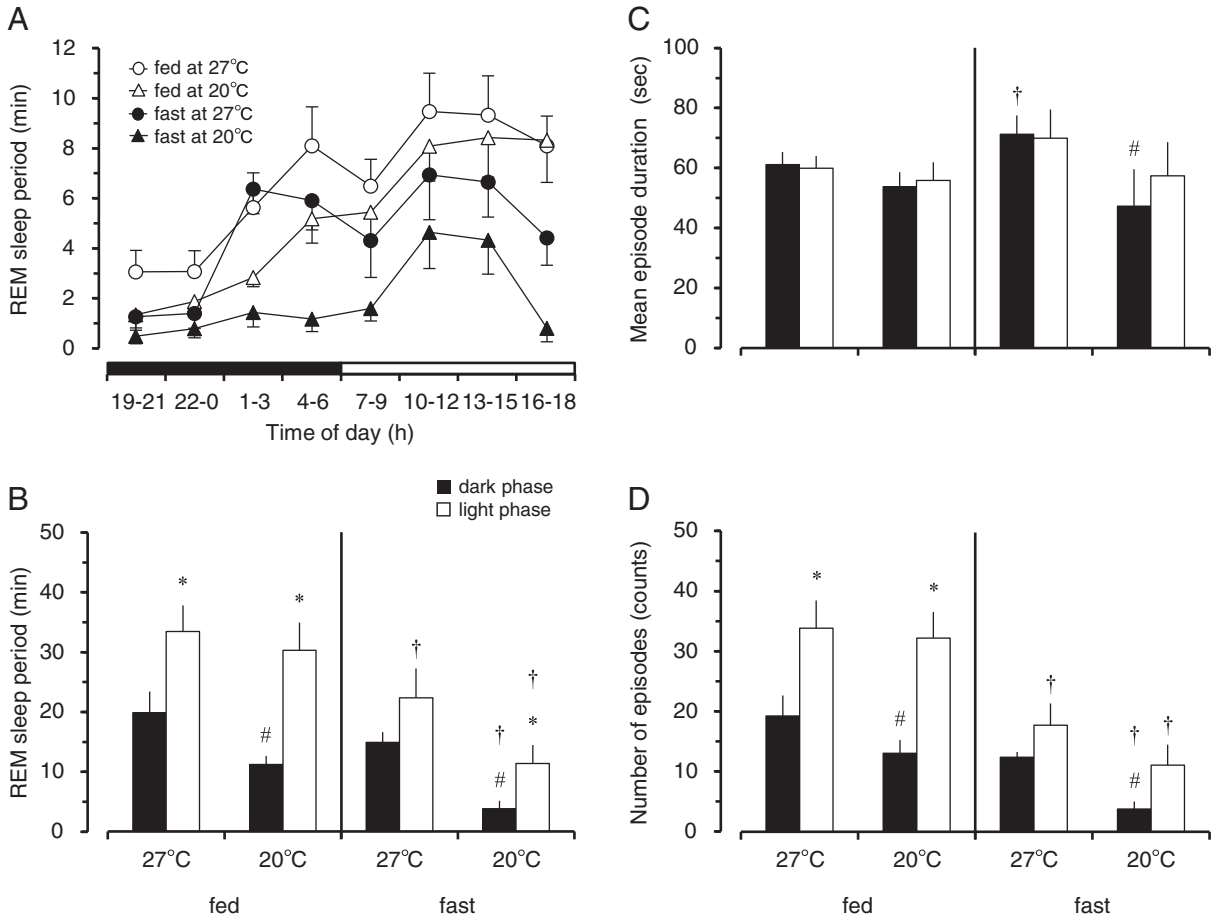

Fig. 4. Daily changes in the REM sleep period (A), and the sum (B), average episode duration (C), and number (D) of REM sleep periods during the light and dark phases. The analyses were conducted in the same manner as the total sleep time analyses. Values are means ± SEM ($n = 9$). *Significant differences between the dark and light phases in each trial ($p < 0.05$). #Significant differences between $T_a = 27°C$ and $T_a = 20°C$ under each feeding condition and in each phase ($p < 0.05$). †Significant differences between mice examined under fed and fasted conditions at each ambient temperature and during each phase ($p < 0.05$).

phases (fed condition: 98.5 ± 1.2% and 101.9 ± 1.7%; fast condition: 101.8 ± 5.8% and 105.3 ± 4.7%, at T_a of 27°C and 20°C, respectively). During both light phases, the power did not differ between feeding conditions [three-way ANOVA for repeated measures: phase: $F(1, 8) = 2.56$, $p = 0.148$; feeding condition: $F(1, 8) = 1.42$, $p = 0.268$; T_a: $F(1, 8) = 0.81$, $p = 0.395$; phase × feeding condition interaction: $F(1, 8) = 2.06$, $p = 0.190$; phase × T_a interaction: $F(1, 8) = 3.20$, $p = 0.111$; feeding conditions × T_a interaction: $F(1, 8) = 0.13$, $p = 0.732$; phase × feeding condition × T_a interaction: $F(1, 8) = 2.08$, $p = 0.187$].

Fig. 5 illustrates the correlations between T_c and TST, NREM sleep periods, and REM sleep periods during Day 3 (24 h) of each experiment. Each data point is the 3 h average. Strong negative and linear correlations ($r = -0.884$ to -0.987) were observed for each relationship between T_c and TST, NREM sleep periods, and REM sleep periods. However, this result was not observed for REM sleep during fasting at a T_a of 20°C ($p = 0.112$).

4. Discussion

In the present study, we assessed the relationship between T_c and sleep rhythms. Furthermore, we assessed the influence of T_a and/or fasting, both of which are factors affecting the two rhythms [2,4,14,26–29], on the relationship. The rhythms of T_c and sleep have long been thought to be linked to each other [26,30], but clear evidence has not been presented thus far. We analyzed the components of sleep and T_c in the dark and light phases, and then assessed the relationship between each component of sleep and T_c by using correlation analyses.

Aschoff suggested that circadian mechanisms have a strong influence on both sleep and T_c [30]. The present study also indicates that despite the stimuli of fasting and cold, the differences in T_c and sleep components between the dark and light phases were mostly preserved (Figs. 1–4), which suggests a strong circadian influence.

In the fed condition, T_c decreased during cold exposure at 20°C by 0.3°C and 0.3 °C in the dark and light phases, respectively (Fig. 1B). It has been reported that cold exposure decreases sleep in humans, rats, and mice [4,14,27,28,31,32]. In the present study, TST and the periods of NREM and REM sleep only decreased (Figs. 2–4B) during the dark phase in mice. Szentirmai et al. also showed that during a 3-day 17°C cold exposure in mice, REM and NREM sleep periods decreased in the dark phase, but the reduction in REM sleep was only observed on the first day [14]. Conversely, Cerri et al. reported that in rats, reductions in TST and in NREM and REM sleep periods in the cold (ranging from 10°C to −10°C) were observed during the light phase [28]. These results indicate that the effect of cold exposure on sleep periods during each phase may differ between mice and rats according to the degree of cold.

During fasting, T_c decreased under the fed conditions in both dark and light phases by 0.9°C and 1.0°C, respectively. In addition, cold conditions augmented the reduction by 2.2°C and 1.7°C from fed condition levels in the dark and light phases, respectively. However, the difference between the phases was consistent. Furthermore, Tokizawa et al. reported a similar reduction of T_c in fasted mice [20]. At a T_a of 27°C, TST and the NREM sleep period under the fasted condition did not differ from fed condition levels (Figs. 2B and 3B). The period of REM sleep decreased in the light phase, and the difference between the two phases was lost. The episode duration of NREM sleep increased in the dark

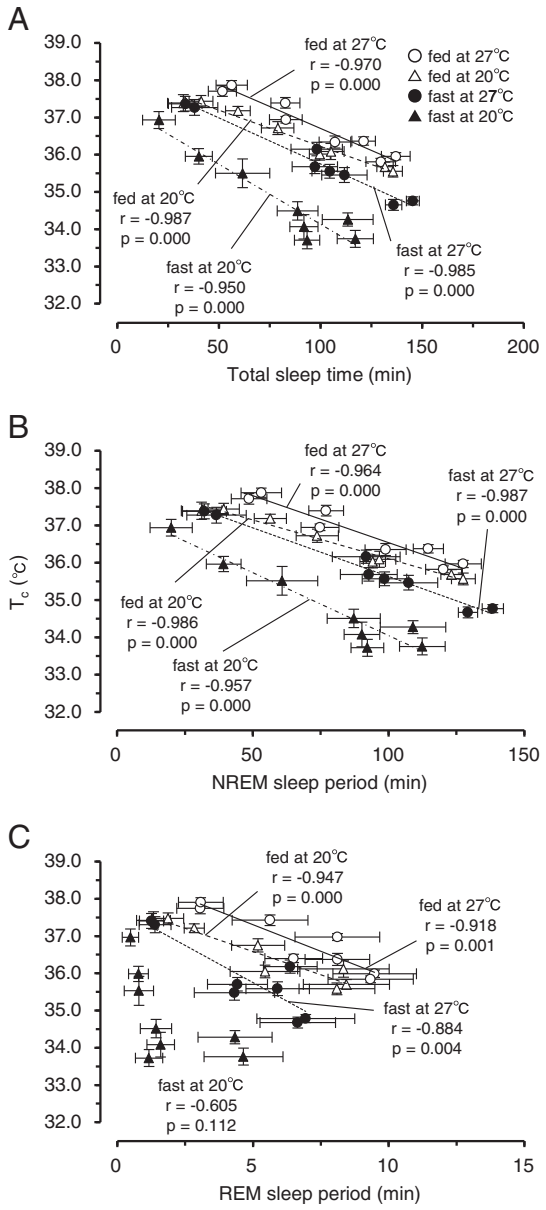

Fig. 5. The relationship between T_c and total sleep time (A), NREM sleep periods (B), and REM sleep periods (C) during 24 h of Day 3. Each data point denotes the 3-h average. Correlation coefficients and *p*-values for each relationship are indicated. The regression lines are superimposed, and it is assumed that the sleep periods are independent factors of T_c. Values are means ± SEM ($n = 9$).

phase, and the difference between the phases was lost. On the contrary, the number of NREM sleep episodes decreased during the dark phase. These results indicate that fasting affects sleep during both phases, which differs from cold exposure. Considering a similar reduction in T_c under cold and fasting conditions, the reduction in T_c would not be the sole factor determining sleep periods. Szentirmai et al. suggested a possible involvement of the preproghrelin gene, which is associated with metabolism, in the mechanism [14].

As opposed to the results found in the present study, several studies have shown that fasting decreases TST and the NREM sleep period [22–24,33]. Even among studies using mice, the effects of fasting on the REM sleep period differ (e.g., decreased [22] and unchanged [24, 33]). Shimizu et al. [34] compared the effects of sleep in the light phase and the dark phase according to when fasting was initiated. They reported that NREM sleep decreased in the first half of the dark phase and latter half of the light phase only when food deprivation began at the onset of the dark phase [34]. This suggests that depending on the time of onset, the influence of fasting on sleep may change. Body weight is another possible factor underlying the difference in sleep observed during fasting. We used ICR mice, which have body weights that are greater than the strains used in other studies [22,24] (e.g., C57BL6J). In addition, because we used each mouse for more than 2 months, the influence of fat mass could not be excluded. Body size has a large influence on T_c and sleep [23,35,36]. However, in the present study, the order of the trials was randomly chosen, and there were no interactions between body weight and T_c or sleep components during fasting. Therefore, we assume that body weight did not affect the present findings.

In the fasted condition, cold exposure augmented the reduction of T_c in both dark and light phases. During the dark phase, cold conditions decreased the NREM sleep episode duration (Fig. 3C) and period, as well as the episode duration and number of REM sleep episodes (Fig. 4B–D). In addition, cold conditions decreased the TST, the period, and the episode duration of NREM sleep during the light phase (Figs. 2B, 3B, and C). The ratio of the NREM sleep period to the TST in the dark phase increased under cold conditions. These results suggest that cold affects sleep in both phases during fasting, but the manner of the effect differs.

It has been suggested that EEG delta power reflects sleep depth and intensity [34,37–40]. Although we found changes in the NREM sleep period in cold and/or fasting conditions, there were no differences in EEG delta power among the trials. The reason remains unclear.

Strong correlations were observed between T_c and TST, NREM sleep periods, and REM sleep periods, except for REM sleep in the combined fasting and cold conditions (Fig. 5). This indicates that the mechanism that links the circadian T_c rhythm and sleep periods is preserved, even when mice are exposed to either fasting or cold conditions.

Daily torpor is defined as a decrease in physiological activity, which is usually accompanied by a reduction in T_c during energy deficit and/or cold conditions. Tokizawa et al. reported that in mice, the reduction in T_c during daily torpor occurs approximately at the onset of light [20], which is similar to that observed in the present study. Assuming that T_c is a dependent factor of sleep periods, we assessed the regression analyses for T_c and the sleep periods by using the least-squares method. The regression lines for TST and the period of the NREM shift downward in the combined fasting and cold conditions showed no significant relationship in REM sleep. Although we have no evidence that the sleep periods determine T_c, the relationships may be important for maintaining a lower T_c (i.e., torpor state). In addition, the reduction in sleep during fasting and/or cold may prevent a further reduction in T_c.

Cold and/or fasting are stimuli that change sleep and T_c rhythms in mice. Sleep rhythms are influenced by cold in the dark phase (i.e., decrease in sleep periods), and fasting affects sleep in both phases. In the present study, the close relationship between sleep periods and T_c was maintained, except for REM sleep periods where fasting and cold conditions were combined. To our knowledge, this is the first study to demonstrate the relationship between sleep and T_c rhythms, and the influence of cold and/or fasting on this relationship.

References

[1] Partinen M, Hublin C. Epidemiology of sleep disorders. In: Kryger MH, Roth TG, Dement WC, editors. Principles and Practice of Sleep Medicine: Fifth Edition. Philadelphia: Elsevier; 2010. p. 694–715.
[2] K. Krauchi, T. Deboer, The interrelationship between sleep regulation and thermoregulation, Front. Biosci. 15 (2010) 604–625.
[3] K. Okamoto-Mizuno, K. Mizuno, Effects of thermal environment on sleep and circadian rhythm, J. Physiol. Anthropol. 31 (2012) 14.
[4] P. Alföldi, G. Rubicsek, G. Cserni, F. Obál Jr., Brain and core temperatures and peripheral vasomotion during sleep and wakefulness at various ambient temperatures in the rat, Pflugers Arch. 417 (1990) 336–341.
[5] E.J.W. Van Someren, Mechanisms and functions of coupling between sleep and temperature rhythms, Prog. Brain Res. 153 (2006) 309–324.
[6] D.J. Dijk, R.E. Kronauer, Commentary: models of sleep regulation: successes and continuing challenges, J. Biol. Rhythm. 14 (1999) 569–573.
[7] Kräuchi K, De Boer T. Body temperature, sleep, and hibernation. In: Kryger MH, Roth TG, Dement WC, editors. Principles and Practice of Sleep Medicine: Fifth Edition. Philadelphia: Elsevier; 2010. p. 323–34.

[8] S.S. Gilbert, C.J. van den Heuvel, S.A. Ferguson, D. Dawson, Thermoregulation as a sleep signalling system, Sleep Med. Rev. 8 (2004) 81–93.
[9] S.S. Campbell, R.J. Broughton, Rapid decline in body temperature before sleep: fluffing the physiological pillow? Chronobiol. Int. 11 (1994) 126–131.
[10] P.J. Murphy, S.S. Campbell, Nighttime drop in body temperature: a physiological trigger for sleep onset? Sleep 20 (1997) 505–511.
[11] C.J. Van den Heuvel, J.T. Noone, K. Lushington, D. Dawson, Changes in sleepiness and body temperature precede nocturnal sleep onset: evidence from a polysomnographic study in young men, J. Sleep Res. 7 (1998) 159–166.
[12] K. Kräuchi, C. Cajochen, E. Werth, A. Wirz-Justice, Functional link between distal vasodilation and sleep-onset latency? Am. J. Physiol. Regul. Integr. Comp. Physiol. 278 (2000) R741–R748.
[13] D.J. Dijk, C.A. Czeisler, Contribution of the circadian pacemaker and the sleep homeostat to sleep propensity, sleep structure, electroencephalographic slow waves, and sleep spindle activity in humans, J. Neurosci. 15 (1995) 3526–3538.
[14] E. Szentirmai, L. Kapás, Y. Sun, R.G. Smith, J.M. Krueger, The preproghrelin gene is required for the normal integration of thermoregulation and sleep in mice, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 14069–14074.
[15] D. McGinty, R. Szymusiak, D. Thomson, Preoptic/anterior hypothalamic warming increases EEG delta frequency activity within non-rapid eye movement sleep, Brain Res. 667 (1994) 273–277.
[16] V. Candas, J.P. Libert, A. Muzet, Heating and cooling stimulations during SWS and REM sleep in man, J. Therm. Biol. 7 (1982) 155–158.
[17] S. Sakurada, O. Shido, N. Sugimoto, Y. Hiratsuka, T. Yoda, K. Kanosue, Autonomic and behavioural thermoregulation in starved rats, J. Physiol. 526 (2000) 417–424.
[18] T. Yoda, L.I. Crawshaw, K. Yoshida, L. Su, T. Hosono, O. Shido, et al., Effects of food deprivation on daily changes in body temperature and behavioral thermoregulation in rats, Am. J. Physiol. Regul. Integr. Comp. Physiol. 278 (2000) R134–R139.
[19] K. Nagashima, S. Nakai, K. Matsue, M. Konishi, M. Tanaka, K. Kanosue, Effects of fasting on thermoregulatory processes and the daily oscillations in rats, Am J Physiol Integr Comp Physiol. 284 (2003) R1486–R1493.
[20] K. Tokizawa, Y. Uchida, K. Nagashima, Thermoregulation in the cold changes depending on the time of day and feeding condition: physiological and anatomical analyses of involved circadian mechanisms, Neuroscience 164 (2009) 1377–1386.
[21] Y. Uchida, K. Tokizawa, K. Nagashima, Characteristics of activated neurons in the suprachiasmatic nucleus when mice become hypothermic during fasting and cold exposure, Neurosci. Lett. 579 (2014) 177–182.
[22] A. Yamanaka, C.T. Beuckmann, J.T. Willie, J. Hara, N. Tsujino, M. Mieda, et al., Hypothalamic orexin neurons regulate arousal according to energy balance in mice, Neuron 38 (2003) 701–713.
[23] J. Danguir, S. Nicolaidis, Dependence of sleep on nutrients' availability, Physiol. Behav. 22 (1979) 735–740.
[24] M. Esposito, J. Pellinen, L. Kapás, É. Szentirmai, Impaired wake-promoting mechanisms in ghrelin receptor-deficient mice, Eur. J. Neurosci. 35 (2012) 233–243.
[25] K. Tokizawa, T. Yoda, Y. Uchida, K. Kanosue, K. Nagashima, Estimation of the core temperature control during ambient temperature changes and the influence of circadian rhythm and metabolic conditions in mice, J. Therm. Biol. 51 (2015) 47–54.
[26] E.J.W. Van Someren, More than a marker: interaction between the circadian regulation of temperature and sleep, age-related changes, and treatment possibilities, Chronobiol. Int. 17 (2000) 313–354.
[27] B. Roussel, P. Turrillot, K. Kitahama, Effect of ambient temperature on the sleep–waking cycle in two strains of mice, Brain Res. 294 (1984) 67–73.
[28] M. Cerri, A. Ocampo-Garces, R. Amici, F. Baracchi, P. Capitani, C.A. Jones, et al., Cold exposure and sleep in the rat: effects on sleep architecture and the electroencephalogram, Sleep 28 (2005) 694–705.
[29] R.J. Berger, N.H. Phillips, Energy conservation and sleep, Behav. Brain Res. 69 (1995) 65–73.
[30] J. Aschoff, Circadian control of body temperature, J. Therm. Biol. 8 (1983) 143–147.
[31] W.R. Schmidek, K. Hoshino, M. Schmidek, C. Timo-Iaria, Influence of environmental temperature on the sleep–wakefulness cycle in the rat, Physiol. Behav. 8 (1972) 363–371.
[32] A.G. Buguet, B.H. Roussel, W.J. Watson, M.W. Radomski, Cold-induced diminution of paradoxical sleep in man, Electroencephalogr. Clin. Neurophysiol. 46 (1979) 29–32.
[33] E. Szentirmai, L. Kapás, Y. Sun, R.G. Smith, J.M. Krueger, Restricted feeding-induced sleep, activity, and body temperature changes in normal and preproghrelin-deficient mice, Am. J. Physiol. Regul. Integr. Comp. Physiol. 298 (2010) R467–R477.
[34] N. Shimizu, S. Chikahisa, K. Kitaoka, S. Nishino, H. Séi, Refeeding after a 24-hour fasting deepens NREM sleep in a time-dependent manner, Physiol. Behav. 104 (2011) 480–487.
[35] R. Amici, M. Cerri, A. Ocampo-Garcés, F. Baracchi, D. Dentico, C.A. Jones, et al., Cold exposure and sleep in the rat: REM sleep homeostasis and body size, Sleep 31 (2008) 708–715.
[36] R. Refinetti, The circadian rhythm of body temperature, Front. Biosci. 15 (2010) 564–594.
[37] Krueger JM, Rector DM, Roy S, Van Dongen HP, Belenky G, Panksepp J. Sleep as a fundamental property of neuronal assemblies. Nat. Rev. Neurosci. 2008;9:910–9.
[38] C. Cirelli, The genetic and molecular regulation of sleep: from fruit flies to humans, Nat. Rev. Neurosci. 10 (2009) 549–560.
[39] P. Achermann, A.A. Borbély, Sleep Homeostasis and Models of Sleep Regulation. Principles and Practice of Sleep Medicine, Fifth Edition Elsevier, Philadelphia, 2010. 431–444.
[40] R.W. Greene, M.G. Frank, Slow wave activity during sleep: functional and therapeutic implications, Neuroscientist 16 (2010) 618–633.