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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.

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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).

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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