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Late feeding in the active period decreases slow-wave activity

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ABSTRACT

Aims: Sleep and feeding behaviors closely interact to maintain energy homeostasis. While it is known that sleep disorders can lead to various metabolic issues such as insulin resistance, the mechanism for this effect is poorly understood. We thus investigated whether different feeding rhythms during the active period affect sleep-wake regulation.

Main methods: For 2 weeks, mice were randomly assigned to 1 of 3 feeding schedules as follows: free access to lab chow during the active period (ZT12-24, Ad-lib group), free access to lab chow during the first half of the active period (ZT12-18; Morning group), or free access to lab chow during the second half of the active period (ZT18-24, Evening group). Food intake, body weight, body temperature, locomotor activity, and sleep were evaluated. The hypothalamus and cerebral cortex were examined post-mortem.

Key findings: No alterations in food intake or body weight were observed among the 3 groups. The Evening group showed lower slow-wave activity (SWA) than the other 2 groups, in addition to higher expression of orexin mRNA in the hypothalamus and higher concentrations of dopamine and its metabolites in the cerebral cortex. AMPK phosphorylation was increased in the hypothalamus of mice in the Evening group; however, AMPK inhibition had no effect on SWA.

Significance: We concluded that late feeding reduces SWA in NREM sleep via a mechanism that involves orexin-mediated arousal in the hypothalamus and elevated monoamines in the cerebral cortex. These data have important implications for the relationship between sleep-wake disturbances and metabolic disorders.

1. Introduction

The circadian clock system plays a major role in the maintenance of physiological functions, including the sleep-wake cycle and energy metabolism [1]. The molecular mechanisms of the circadian clock system are well studied in rodents [2]. In particular, the perturbation of circadian oscillator components results in internal desynchronization in rodents, and is considered to be a risk factor for metabolic disorders such as obesity and diabetes. Several studies have observed the synchronization of peripheral clocks to different feeding schedules [3,4]. The restriction of feeding to the inactive period has been reported to shift circadian rhythm phases for clock gene expression in peripheral tissues [5]. These evidences indicate that feeding schedule can affect both metabolic state and circadian rhythm in rodents.

Sleep and feeding are two essential behaviors that closely interact to maintain energy homeostasis. It is thought that late feeding in the active phase promotes insulin resistance as a precursor to obesity and diabetes [6,7]. Indeed, recent studies have indicated that sleep disorders can lead to various metabolic disturbances [8–10]. In the hypothalamus, sleep and energy metabolism are regulated by common neuronal subsets including those expressing hypocretin/orexin, neuropeptide Y (NPY), agouti-related peptide (AgRP), and proopiomelanocortin (POMC).

In mammals, starvation increases wakefulness and decreases non-rapid eye movement (NREM) sleep [11–13]. Slow-wave activity (SWA) is a factor calculated during NREM sleep from electroencephalogram (EEG) delta power, and this factor has been used as a physiological marker of sleep depth and homeostatic sleep pressure in a variety of studies. SWA is independent of sleep timing and/or duration, which are factors that are tightly controlled by circadian rhythm. Some rodent studies have addressed the relationship between feeding during the inactive period and sleep disorders. In addition, acute sleep deprivation is generally thought to increase SWA in a compensatory manner in rodents; however, no study to date has investigated the effects of different feeding rhythms during the active period on sleep pattern and quality. We thus investigated whether different feeding rhythms during the active period affect sleep-wake regulation and SWA in mice. Our results indicate that late feeding in the active phase promotes arousal and reduces SWA during NREM sleep.

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2. Materials and methods

2.1. Animals and experimental design

Ten-week-old C57BL/6 male mice (Japan SLC, Shizuoka, Japan) were individually housed and maintained on a 12-hour light/dark cycle. Experimental animals of similar body weight were randomly assigned to 1 of 3 groups: free access to lab chow during the active period (Zeitgeber Time, ZT12-24, Ad-lib group), free access to lab chow during the first half of the active period (ZT12-18; Morning group), or free access to lab chow during the second half of the active period (ZT18-24, Evening group). Feeding conditions were maintained for 2 weeks (Fig. 1A). All experiments were approved by the Animal Study Committee of Tokushima University and conducted in accordance with the Guidelines for the Care and Use of Animals approved by the Council of the Physiological Society of Japan.

2.2. Sleep, body temperature, and locomotor activity recording and analysis

Mice received three surgical implants for the recording of physiological data. A telemetric device (TA10TA-F20; Data Sciences Int., USA) was implanted into the peritoneal cavity for the recording of body temperature and locomotor activity. Two stainless steel miniature screw electrodes were implanted into the skull for the recording of EEG data. Lastly, Teflon-coated stainless steel wires were bilaterally implanted into the muscles of the neck for the recording of electromyogram (EMG) data. Flexible cables were used to connect implanted EEG and EMG probes to a polygraph and computer-assisted data acquisition system. Off-line sleep scoring was conducted by visual assessment of EEG and EMG activities using the Spike2 analysis program (CED, Cambridge, UK). Vigilance states were evaluated using data binned in 6-s epochs and classified as wakefulness, rapid eye movement (REM) sleep, or NREM sleep. The EEG power spectrum representing the NREM sleep state was calculated by Fast Fourier Transform using the Spike2 analysis program. The EEG delta and theta frequency bands were set at 0.5–4.0 Hz and 4.0–7.8 Hz, respectively. In this study, the power density of the EEG delta band (the ratio of the delta and theta bands) during NREM sleep was used as a parameter of sleep pressure (SWA).

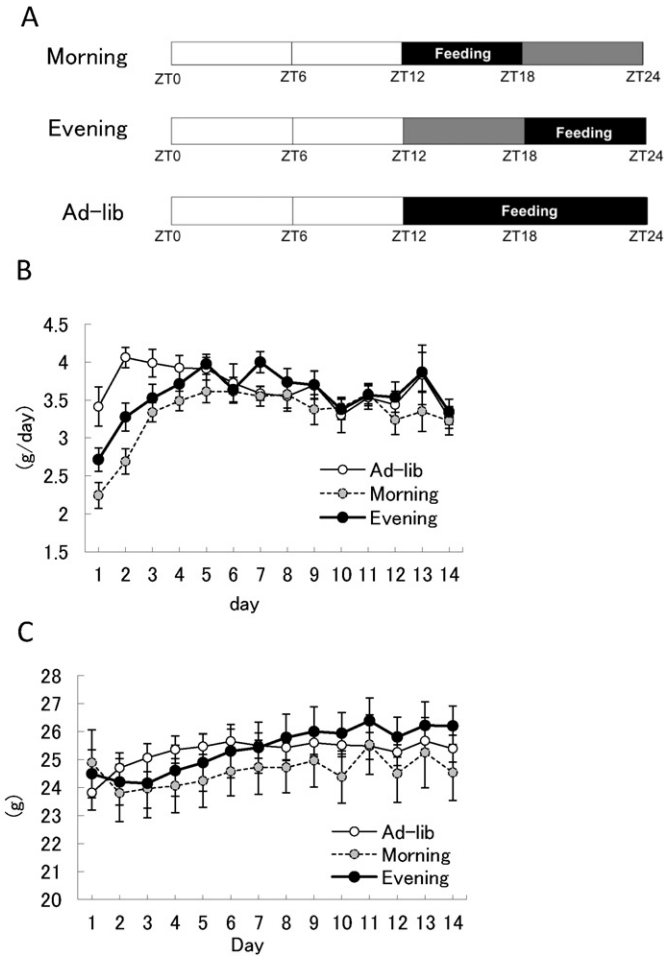

Fig. 1. A schematic chart of the feeding schedule paradigm (A). Food intake (B) and body weight (C) were measured during the 2 weeks following the onset of each feeding schedule. Data are presented as the mean ± SE, n = 6–8.

2.3. Procedure for sleep deprivation

Mice were sleep deprived for 6 h (ZT0-6) by persistent waking with the touch of a small soft brush on the back. The sleep deprivation period was terminated at the beginning of the second half of the light phase; subsequently, EEG and EMG were recorded for an 18-h period of uninterrupted sleep recovery.

2.4. Pharmacological treatments and injection procedures

Intracerebroventricular (icv) cannulae were implanted at the time of probe implantation surgery. A 25-gauge guide cannula was stereotactically implanted into the lateral ventricle using the following coordinates from the Franklin and Paxinos atlas [14]: 0.3 mm posterior to bregma, 1.0 mm lateral to the midline, and 2.5 mm below the surface of the skull. The cannula was fixed to the skull using the screw electrodes placed for EEG recording. A 31-gauge dummy cannula was inserted into the guide cannula to maintain cannula patency. After surgery, mice were allowed to recover for one week to ensure the return of body weight and food intake to baseline prior to the onset of experiments. Mice were injected with the AMPK inhibitor compound C (CC, Sigma Chemical Co., St. Louis, MO, USA) or vehicle at ZT-1. CC (5 μg dissolved in 1.0 ml of 25% dimethyl sulfoxide in artificial cerebrospinal fluid) or vehicle was injected slowly over 1 min using a Hamilton microsyringe. The injection cannula (31-gauge) was then left in place for 30 s prior to removal to minimize reflux.

2.5. Western blotting

Homogenized hypothalamus samples from each group were subjected to SDS-PAGE and transferred to a PVDF membrane. After blocking with 5% milk dissolved in Tris-buffered saline containing 0.05% Triton X-100 (TBS-T), membranes were incubated with anti-phospho-Thr172 AMPKα antibody or anti-AMPKα antibody (Cell Signaling Technology, Beverly, MA) (1:1000) overnight at 4 °C On the following day, membranes were washed with TBS-T and incubated with an appropriate horseradish peroxidase-conjugated secondary antibody (1:2000). After washing once more with TBS-T, blots were developed using an Immobilon chemiluminescent HRP substrate (Merck Millipore, Darmstadt, Germany). Blots were scanned into a computer and analyzed using Image J software (National Institutes of Health, Bethesda, MD).

2.6. Brain monoamine quantification

After EEG recording, experimental mice were decapitated and the whole brain was removed and dissected into 2 regions (the cerebral cortex and hypothalamus). All tissue was extirpated using tweezers, snap frozen in liquid nitrogen, and stored at −80 °C until use. Monoamine levels (norepinephrine, dopamine, 3,4-dihydroxyphenylacetic acid [DOPAC] and serotonin [5-HT]) were quantified by high-performance liquid chromatography according to previously published methods with slight modifications [15].