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Effect of a Hedonic Stimulus on the Sleep Architecture of Male Wistar Rats

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Abstract

Objective Nocturnal animals forage and eat during the night and sleep during the day. When food is available only for a short period during the day, animals develop a catabolic state and exhibit locomotor behavior before accessing food, termed *food anticipatory activity*. Consequently, there is a disruption in the sleep pattern. The present study aimed to explore how anticipatory arousal emerges under circadian exposure to a palatable meal (PM) and disrupts sleep architecture.

Materials and Methods Adult male Wistar rats were implanted with electrodes for continuous sleep recording and housed under a light/dark 12/12-hour cycle with free access to food and water. After basal recordings, the rats had access to a PM during the light period for eight days.

Results The anticipatory arousal started on the third day. On the eighth day, we found an increase in wake time and a decrease in the non-rapid eye movement sleep (NREMS) and rapid eye movement sleep (REMS) times 45 minutes before the PM compared with the basal recordings. The REMS transitions (events from NREMS to REMS) showed a significant reduction during the light period of the eighth day of PM. In contrast, the number of NREMS transitions (events from wakefulness to NREMS) remained unchanged.

Conclusion The results suggest that palatable food induces a motivational timing that leads the rat to wake by altering the sleep quota.

Keywords
- anticipatory locomotor behavior
- circadian entrainment
- palatable meal
- sleep

Introduction

In mammals, periods of rest/activity are organized to ensure that foraging and sleep behaviors occur at appropriate light and dark phases, depending on their behavioral patterns. The brain's suprachiasmatic nucleus (SCN) is the master clock that organizes the circadian oscillation of these alternate periods and is entrained by the light/dark cycle; it promotes arousal during the active phase and sleep during the rest phase.[1,2] In addition, circadian rhythms can also be modulated by behavioral patterns, physical activity, food intake, hedonic stimuli, sleep loss, and sleep disorders.[3] When food is restricted to a short period during the rest phase, rodents develop intense locomotor behavior before the scheduled feeding time, termed *food*

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anticipatory activity (FAA).[4] Other physiological measures, such as core body temperature and circulating corticosterone levels, increase during FAA in animals under restricted food schedules.[5] Likewise, the sleep pattern is disturbed around three to four hours before food availability: animals frequently awake, and rapid eye movement sleep (REMS) and non-rapid eye movement sleep (NREMS) are reduced.[4,6] After eight or more days of food restriction, the wake period during the rest phase increases, and REMS propensity and delta activity on electroencephalogram (EEG) during NREMS decrease.[7] Indeed, the food-restriction protocol to elicit FAA requires changes in the metabolism that lead subjects to a catabolic state,[8,9] which is crucial for food entrainment.[10] The metabolic challenge of food restriction induces a negative energy balance that forces subjects into a partially diurnal phenotype and leads to the disruption of the sleep pattern.[7,11,12] Consequently, during the rest period, foraging activity increases against sleep requirements to counter the energetic deficit.[13]

Anticipatory locomotor behavior similar to that observed in food-restricted rats can be elicited with a palatable-meal (PM) schedule in satiated rats.[9,14] However, subjects entrained by a PM schedule are not in a chronic catabolic state as those under food restriction.[9,14] Aside from those differences, subjects in a PM schedule also experience wake periods during their resting phase. Based on locomotor behavior recordings, a previous report[4] showed that the wake time among animals entrained by a PM schedule is considerably shorter than in animals under food restriction. However, the impact of entrainment by a PM on sleep-wake patterns has not been characterized.

In the present study, we have hypothesized that, despite the shortened wake period and absence of a catabolic state, exposure to a PM would alter sleep patterns. We have analyzed sleep recordings in rats exposed daily to a PM in the middle of the rest period to investigate whether a hedonic stimulus affects the wake-sleep architecture and determine when anticipatory arousal appears.

Material and Methods

Animals

Male Wistar rats (weighing between 300g and 400 g) were kept in individual cages in a temperature-controlled room (21° C ± 1°C) under a light/dark cycle (12/12 hours; lights on at 4 p. m., defined as zeitgeber time 0 [ZT0]) with access to food and water ad libitum. Experimental procedures were approved and conducted according to the ethical committee (CICUAL 2015–0018) in agreement with national (Norma Oficial Mexicana NOM-062-ZOO-1999) and international guidelines (Society for Neuroscience) for the production, care, and use of laboratory animals. Furthermore, all measures were taken to reduce unnecessary suffering of the animals.

Electroencephalogram and Electromyogram Electrode Implantation

A total of 8 rats were deeply anesthetized with ketamine and xylazine (at doses of 87 mg/kg and 13 mg/kg respectively), and standard sterile-surgical and stereotactic procedures were performed to implant electrodes. First, the skull was exposed, and four holes were drilled; then, stainless steel miniature screw electrodes were placed on top of the dura mater over the frontal and parietal bones. These electrodes were used to record the electroencephalogram (EEG) from two contralateral derivations. The electromyogram (EMG) activity was recorded using stainless-steel wires bilaterally coated with Teflon, which were placed on the trapezius muscles. The EEG and EMG electrodes were connected to leads. In turn, these were plugged into a lightweight pedestal (preamplifier, Pinnacle Technology, Inc., Lawrence, KS, United States) fixed into the skull with dental cement. After surgery, all animals were injected intramuscularly with ketorolac and enrofloxacin (at doses of 3.5 mg/kg and 0.2 mg/kg respectively) to reduce pain and inflammation, and to prevent infection. The surgeries were performed ten days before the sleep recordings began.

Sleep Recordings

A flexible recording cable connecting the preamplifier to an electrical swivel (8409 Rat Commutator, Pinnacle Technology, Inc.) was used for the sleep recording. After 48 hours of habituation, a 24-hour baseline (BL) recording was performed for each rat. The EEG and EMG signals were digitized and analyzed using the Sirenia Sleep Analysis (SSA) software (Pinnacle Technology, Inc.). The behavioral states of wakefulness, NREMS, and REMS were determined offline using the same software in ten-second epochs. Desynchronized EEG and phasic EMG activity identified the wakefulness. The NREMS corresponded to high amplitude waves (range: 100 µV to 200 µV), slow frequency (range: 0.5 Hz to 4 Hz), and low muscle tone. The REMS was identified by theta (range: 4 Hz to 8 Hz) activity and loss of muscle tone. The rats were recorded in their sleep for eight days.

Experimental Design

After the BL sleep recording, exposure to a PM started at ZT 6 for 8 days. Following a previous report,[14] the PM was a piece of chocolate (weighing 5 g, containing 10% of proteins, 51% of carbohydrates, and 34% of fat, with caloric value of 550 kcal/100 g), which was placed on the special plate inside the rat cage until it was completely consumed by the rat, which took between 10 to 15 minutes maximum. The anticipatory arousal (PM entrainment) was determined by a significant increase in the time spent in wakefulness 15 minutes before accessing the PM stimulus, compared with the BL recordings. Food and water were available ad libitum throughout the entire experiment.

Statistical Analysis

The statistical analysis was performed using the Rstudio integrated development environment (Posit PBC, Boston, MA, United States), version 1.1.383. Each day of sleep recording was summarized into a state-by-epoch array containing 8,640 assignments that were processed to demarcate episodes of wakefulness, NREMS, and REMS. The time spent in wakefulness displayed 15 minutes before ZT6 was compared regarding the day of the BL and every day of the PM protocol.

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The comparisons were made through one-way analysis of variance (one-way ANOVA) with repeated measures.

The sleep parameters analyzed were the time spent in wakefulness, NREMS, REMS, number of REMS and NREMS transitions, and mean duration of the REMS episodes. The REMS transitions correspond to NREMS-to-REMS transitions, whereas NREMS transitions correspond to wakefulness-to-NREMS transitions during the recording period. These data were analyzed using one-way ANOVA and two-tailed paired *t*-tests comparing the BL and PM protocol data. The Holm-Bonferroni correction was used for paired multiple comparisons.

Results

Rats without food restriction and exposed to the PM during the rest phase showed anticipatory arousal since the third day. According to the analysis of the sleep records, the wakefulness time 15 minutes before the PM increased progressively throughout the 8 days of sleep recording. In contrast, the NREMS and REMS time decreased significantly compared with the BL (►**Fig. 1**).

In addition, an analysis of the sleep recording on day 8 was performed every 15 minutes 1 hour before PM access. The results showed that, on the eighth day, PM-induced-anticipatory arousal appeared 45 minutes before access to the PM. The time spent in wakefulness on day 8 during the hour before PM access increased (BL: 4.23 ± 2.57 m versus day 8 PM: 37.35 ± 2.57 m; $p = 0.001$), whereas the duration of the NREMS (BL: 45.76 ± 2.23 m versus day 8 PM: 20.65 ± 5.27 m; $p = 0.001$) and REMS (BL: 10 ± 1.69 m versus day 8 PM: 1.98 ± 0.82 m; $p = 0.004$) were reduced (►**Fig. 2A**).

Sleep architecture was analyzed 24 hours and during the light and the dark periods on day 8 of PM synchronization (►**Fig. 2B**). The results showed that, compared with the beginning of the experiment, the total time spent in wakefulness during the eighth day increased (BL: 574.5 ± 45.49 m; day 8 PM: 707.9 ± 45.69 m; $p = 0.01$) due to the increase in wakefulness during the light period (BL: 126.4 ± 14.98 m; day 8 PM: 263.4 ± 20.25 m; $p < 0.001$). In consequence, we found a significant reduction in the total time spent (24 hours) in NREMS (BL: 748.5 ± 46.04 m; day 8 PM: 633.4 ± 31.78 m; $p = 0.02$) and REMS (BL: 116.9 ± 14.47 m; day 8 PM: 87.36 ± 14.63 m; $p = 0.041$). We also found a significant reduction in the time spent in NREMS (BL: 509.1 ± 20.81 m; day 8 PM: 391.1 ± 14.65 m; $p = 0.004$) and REMS (BL: 84.4 ± 9.14 m; day 8 PM: 51 ± 7.18 m; $p < 0.001$) during the light period. Interestingly, there were no significant changes in the wakefulness and sleep times during the dark period (►**Fig. 2B**).

The REMS transitions showed a significant reduction during the light period of the eighth day of PM ($p = 0.04$; ►**Table 1**). In contrast, the number of NREMS sleep transitions remained unchanged (►**Table 1**; ►**Fig. 3**). Besides, there were no statistically significant differences in the duration of REMS periods between the BL and PM conditions (BL: 2.87 ± 0.32 m; day 8 PM: 2.39 ± 0.30 m; $p = 0.965$).

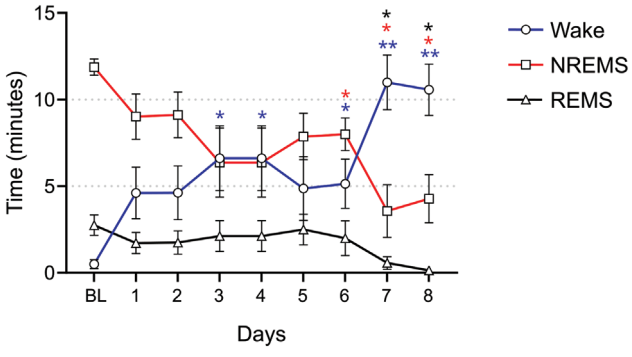

Fig. 1 Anticipatory arousal induced by a palatable meal (PM). The wake time during the 15 minutes before the access to the chocolate increases progressively along the days of synchronization to the PM stimulus. A robust reduction in non-rapid eye movement sleep (NREMS) and rapid eye movement sleep (REMS) is found on days 7 and 8 of the stimulus. Notes: Data are expressed as means ± standard error of the mean (SEM). $^{*}p < 0.05$; $^{**}p < 0.001$. Differences between PM and baseline (BL) in terms of wake time, NREMS, and REMS.

Discussion

In agreement with our hypothesis, we found that a daily hedonic meal to rats with free access to water and food, that is, not in a catabolic state, is enough to induce an adaptation in the sleep/wake cycle. According to sleep recordings in food-restricted rats,[7] an adaptation of their sleep pattern is first observed after at least seven days, parallel to an apparent increase in locomotor behavior before food presentation. Similarly, continuous recording of EEG activity during food entrainment in mice revealed that seven days are necessary to detect clear sleep changes. In sharp contrast, in the present study, we found that only three days of circadian exposure to a piece of chocolate are necessary for animals to show evident anticipatory arousal. A previous study on rats[15] entrained with chocolate reported that they also need three days to show significant anticipatory activity. Furthermore, satiated rats that received a piece of chocolate during the day, in a model of circadian desynchronization by jet lag, required just four days to reach the new acrophase, in contrast to seven days for rats that did not receive chocolate.[16] Thus, anticipatory arousal develops gradually from day one, as observed in a previous report[15] and in the present study. Likewise, we found that only three days of PM exposure can induce a cortical desynchronized EEG and phasic EMG activities. In our subjects, anticipatory arousal activity reached a maximum of 45 minutes after 8 days of exposure to the hedonic stimulus.

Sleep is regulated by a combination of homeostatic and circadian mechanisms. The homeostatic process refers to the need for sleep that increases throughout wakefulness.[17,18] The circadian process involves adaptive mechanisms to temporarily coordinate the body's metabolic, physiological, and behavioral functions. In addition, the circadian mechanisms enable the body to synchronize and anticipate daily environmental events.[19] For example, previous sleep studies[6,7] reported prominent food anticipatory arousal that appears after a week of food restriction, characterized by

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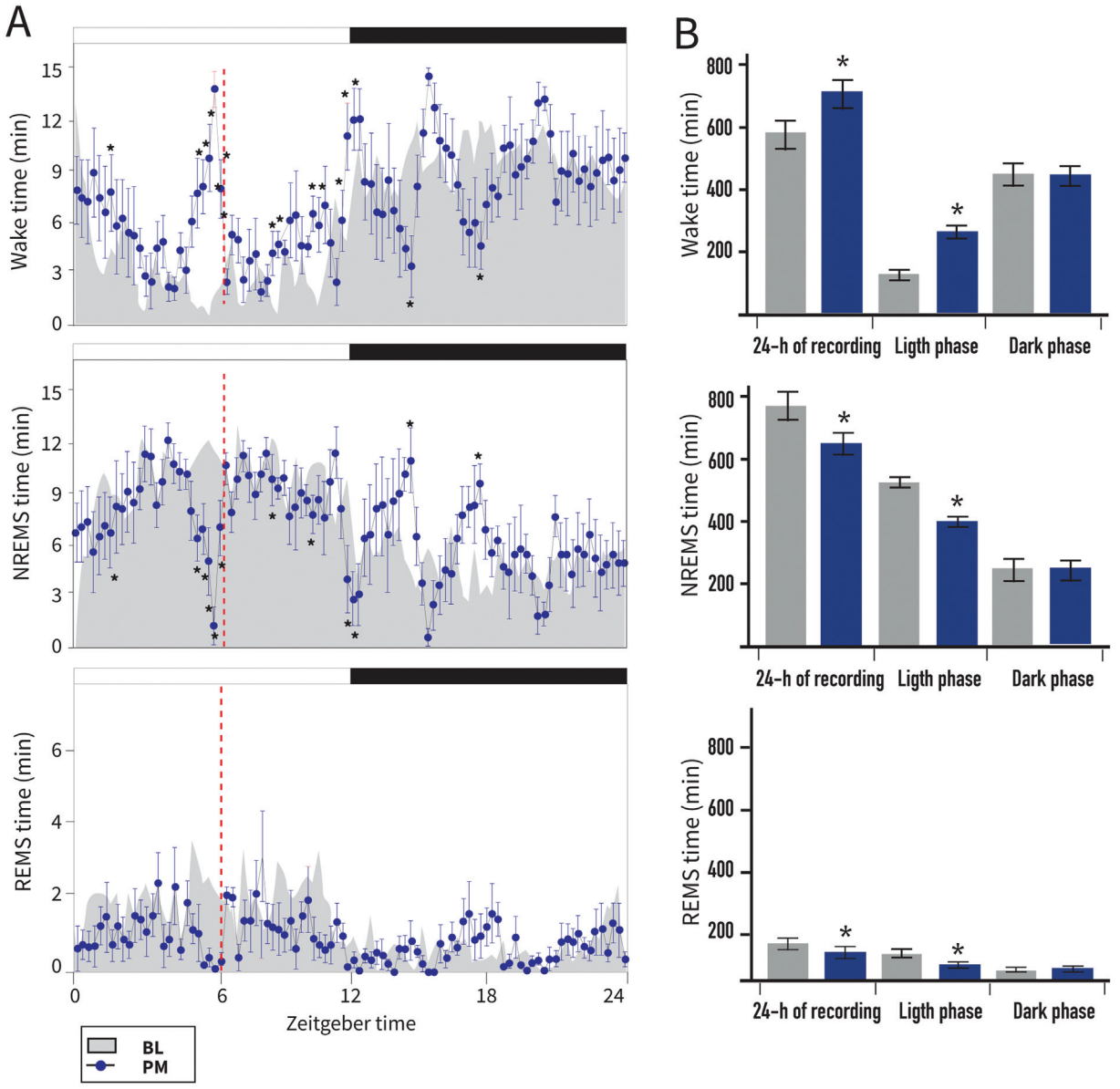

Fig. 2 Distribution of time spent awake, in NREMS, and in REMS during the 24-hour recordings of rats that displayed anticipatory arousal at day 8 of PM exposure. (**A**) Every blue point represents the value obtained every 15 minutes during the sleep/wake recording on day 8. The gray areas represent the mean BL values. The vertical red line in zeitgeber time (ZT) 6 indicates the moment of access to the PM. The horizontal white and black bar represents the light and dark periods respectively. (**B**) Total time awake, in NREMS, and in REMS during the 24-hour recordings and during the light and dark periods in rats that displayed anticipatory arousal on day 8 (blue bars) compared with BL values (gray bars). Notes: Values are expressed as mean ± SEM. $^{*}p < 0.05$.

increased wakefulness, suppression of REMS time, and dampening of NREMS EEG delta activity.

In contrast, the present study showed a significant decrease in NREMS and REMS during the light period, and no change during the dark period. These results suggest that the homeostatic component of sleep is affected by exposition to a daily PM scheduled in the rest period. In contrast, previous reports showed that rodents under food restriction during the day display significant changes in the distribution of sleep patterns, whereas the homeostasis sleep process is kept in rats[6] and mice.[12] Although the homeostatic component of sleep was maintained in these studies, the neural control of the entraining process by food restriction and a palatable treat is different. As aforementioned, circadian food restriction induces a catabolic state and entrains hypothalamic structures, as the paraventricular, dorsomedial, and ventromedial nuclei are involved in controlling metabolism.[9]

Nevertheless, this effect in the hypothalamus was not observed in animals entrained by a PM.[9] Thus, entrainment by chocolate, but not by food, caused a strong induction of FOS protein during anticipatory activity in corticolimbic structures such as the central amygdala, the nucleus accumbens, and the prefrontal cortex, in agreement with the high motivational state of the subjects due to the hedonic stimulus.[9] Beyond FOS

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Table 1 Mean values of NREMS-to-REMS and wakefulness-to-NREMS transitions during the 24-hour sleep recordings of rats during baseline and on day 8 of the palatable meal schedule

Total number of transitions			
	24-hour	Light phase	Dark phase
NREMS-to-REMS (events)			
Baseline	49.14 ± 10.91	34.42 ± 7.08	14.71 ± 4.72
Palatable meal schedule – day 8	47.21 ± 10.32	26.64 ± 5.46*	20.57 ± 5.14
Wakefulness-to-NREMS (events)			
Baseline	58.71 ± 17.26	31.85 ± 12.15	26.85 ± 5.95
Palatable meal schedule – day 8	67.57 ± 18.51	33.92 ± 10.98	33.64 ± 8.75

Abbreviations: NREMS, non-rapid eye movement sleep; REMS, rapid eye movement sleep.
Notes: Values are expressed as mean ± standard error of the mean (SEM); *$p < 0.05$.

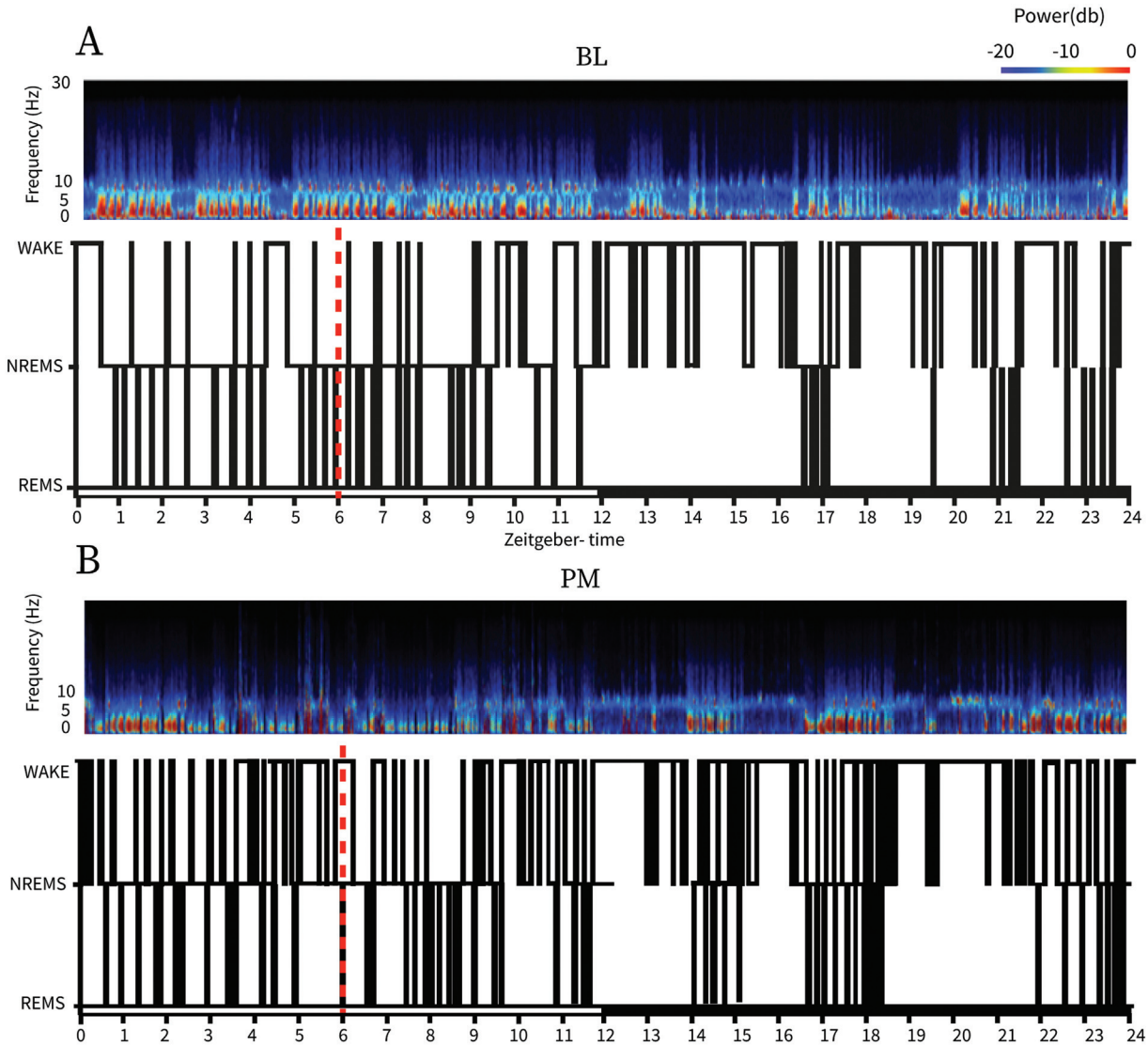

Fig. 3 Representative spectrogram and hypnogram during BL and after the PM schedule. Recording of one rat during BL (**A**); display of anticipatory arousal on day 8 (**B**). Over each hypnogram, the multitaper sleep EEG spectrogram reveals patterns of oscillatory dynamics that correspond to the sleep pattern represented on the hypnogram. The spectrograms show spectral power as a function of time (x-axis) and frequency (y-axis). The vertical red line in ZT 6 indicates the moment of access to the PM. The horizontal white and black bar represents the light and dark periods respectively.

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induction, the clockwork machinery is also affected differently by food and PM stimulus. Both food restriction and PM schedules induce a shift in the rhythm of the PER1 protein in corticolimbic structures, but with more robust and higher oscillation in the chocolate group.[14] Based on this evidence, we propose that, in our subjects, the palatable stimulus also induced a shift in the clockwork machinery similar to that reported in the corticolimbic system,[14] in agreement with the hedonic properties of the stimulus.

In considering the hedonic nature of the palatable stimulus, dopamine may be involved. This neurotransmitter, which is synthesized by ventral tegmental area neurons, among other areas, plays a central role in motivated behaviors and stimulates wakefulness.[20] Dopamine also controls sleep through a complex interaction with other neuromodulators, such as adenosine.[21] However, to our knowledge, the interaction of dopamine with other sleep/wake neuromodulators has not been explored in the entrainment model by a PM. Further studies need to address this issue.

In conclusion, the results of the present study indicate that the palatable treatment induces the activation of a timing system that leads the subject to be awake during their resting period, altering the sleep quota. Therefore, the hedonic properties of a stimulus such as chocolate are sufficient to cause a change in sleep architecture.

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Conflict of Interests
The authors have no conflict of interest to declare.

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