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Research Report

Influence of long-term food restriction on sleep pattern in male rats

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Abstract

The present purpose was to determine the effects of different schedules of long-term food restriction (FR) applied to rats from weaning to the 8th week. Rats were distributed into FR and ad libitum groups at weaning and fed at 7 am, at 7 pm, and finally, restricted rats fed ad libitum. The restricted rats started with 6 g/day and the food was increased by 1 g per week until reaching 15 g/day by adulthood. The rats were implanted with electrodes to record electrocorticogram/eletromyogram signals. Their wake–sleep cycles were monitored over 3 consecutive days (72 h of recording). The FR group fed at 7 am showed an increase in awake time, and decrease in slow wave sleep (SWS) and paradoxical sleep (PS) during the three light periods compared with the control recordings whereas in the dark periods, these sleep parameters were the opposite. The restricted group fed in the evening showed no statistical significances at diurnal periods; however, a significant decrease was observed in the dark recordings for awake time, but the SWS and PS were increased in relation to controls. The analysis of the 24-h period demonstrated that both FR groups presented increase in SWS time. After being FR, the rats were fed ad libitum and their sleep was monitored for 3 additional days. During the first dark recording, the decrease in awake time and increase in SWS were still present; however, as ad libitum food continued, these sleep parameters returned to control values, reestablishing the normal sleep pattern. These results suggest that dietary restriction, regardless to the feeding schedule, caused increase in total sleep time, during the active period.

Theme: Neural basis of behavior
Topic: Biological rhythms and sleep

Keywords: Food restriction; Sleep; Diurnal variation; Paradoxical sleep; Rat

1. Introduction

Reduction of nutrient intake has been shown to extend the lifespan of diverse organisms [21], including rats [13]. For instance, since 1935, the effects of food restriction (FR) in rodents have been extensively investigated [14,16,19,24]. Indeed, rodents under dietary restriction since weaning show strikingly extended maximal survivorships [5,18]. According to McCay and coworkers [13], FR increases rats' maximum lifespan by about 80% if started at weaning. The application of FR has been extended to other species, in which similar effects have also been observed. Furthermore, there are indications of health benefits of FR in humans [9]. Of note, these nutritional strategies produce chronic undernutrition (not malnutrition) by limiting caloric intake while providing adequate amounts of other dietary essentials [23].

Sleep alteration is among the diverse behaviors observed in rats submitted to FR, but has been sparsely investigated. Studies of Roky et al. [20] reported that FR alters the diurnal distribution of sleep in rats and similarly [17] demonstrated that paradoxical sleep (PS) and slow wave sleep (SWS) also increased during the dark and decreased during the light period when food and water were available only during the light phase. Of note, in Roky's study, the access to both food and water were restricted.

In an era of energy homeostasis, research has increasingly been focused on obesity, which has become epidemic in Westernized societies. Highly palatable foods that stimulate intake aggravate the situation, especially in children and adolescents who may acquire poor eating habits that are kept throughout their entire lives leading them to obesity [7].

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Exploring the consequences of FR in the sleep architecture may provide insights into feeding-induced sleep modulation.

Since there has so far been no detailed study of different FR time-schedules on alterations of sleep pattern since weaning in rats, our goal was to perform a chronic electrocorticographic study to examine the time course, magnitude, and character of sleep architecture alterations occurring during 3 days following a long-term FR protocol, and to compare the effects on sleep of different feeding schedules on male rats.

2. Methods

2.1. Subjects

Male Wistar rats were bred and raised in the animal facility of the Department of Psychobiology, Universidade Federal de São Paulo. The animals were housed in a colony maintained at 22 °C in 12:12 h light–dark cycle (lights on at 0700 h) inside standard polypropylene cages. Rats used in this study were maintained and treated in accordance with the guidelines established by the Ethical and Practical Principles of the Use of Laboratory Animals [3]. The experimental protocol was approved by the Ethical Committee of UNIFESP (CEP N. 434/05). Since this experiment may result in a certain amount of stress-related behavior, the number of animals was kept at a minimum but sufficient to allow for statistical significances.

2.2. Food restriction (FR)

From weaning (21 days), rats were fed 6 g of chow daily. This amount of food was increased 1 g/week until reaching 15 g in the 8th week. This amount represents 40–50% of ad libitum intake in the control group. The animals were kept apart in separated cages during feeding and remained there in the remainder of the time until the complete ingestion of all the food (approximately in 60 min). The protocol used in the calculation of chow was established in compliance to previous investigations described in the literature. The animals were distributed into two groups (n = 6/each):

(a) *Control*: animals allowed to eat and drink ad libitum from weaning throughout the experiment.
(b) *FR*: animals fed under the dietary restriction protocol described above.

In both groups, body weight was recorded weekly from weaning until the end of the experiment.

2.3. Protocol design

Exp. 1: The FR rats were fed at 7 am according to the experimental procedure described above.
Exp. 2: As the manipulation of dietary restriction makes it unclear whether the reward value is equivalent for the ad libitum and the restricted groups, and to ascertain whether the feeding shows a diurnal variation, we extended our proposal by including a restricted group fed at 7 pm. Thus, a clock-like mechanism defining the alteration of periods with high and low sleep propensity and being basically independent of sleep and waking could be predicted. Of note, on both experiments, the feeding procedure occurred at a random time around 7 am or 7 pm to avoid synchronization.
Exp. 3: In an attempt to revert the effects of FR on sleep pattern in this set of experiments, we provided food ad libitum to the restricted group and electrophysiological signals were recorded for three more consecutive days.

2.4. Surgical preparation

The rats were given ketamine hydrochloride and diazepam anesthesia (90 mg/kg and 4.5 mg/kg of body weight, i.p.). To record cortical electrocorticogram (ECoG) with a minimum of theta activity, one pair of screw electrodes was placed through the skull ipsilaterally in a lateral position: 1 mm posterior to bregma, 3 mm lateral to the central suture; and 1 mm anterior to lambda, 4 mm lateral to the central suture. To ensure the best recording of theta activity, two screw electrodes were placed ipsilaterally in a more medial posterior position: 3 mm anterior to bregma, 1 mm lateral to the central suture; and 4 mm anterior to lambda, 1 mm lateral to the central suture. The electromyogram (EMG) electrodes were implanted in the neck muscles. The electrodes were soldered to a socket containing 6 pins and covered with dental acrylic. After the surgery, the rats were individually placed in rounded transparent plastic cages and allowed a 14-day surgery recovery period comprising 10 days without the recording cable followed by a 4-day adaptation period with the polysomnography cable connected.

2.5. ECoG recording

During recording, the animals remained in their recording cages, inside a Faraday chamber in a soundproof room. The recording equipment was placed in an adjacent room.

The recordings were performed on a Nihon Koden Co. (Tokyo, Japan) model QP 223-A (acquisition of digital signal), using 3 channels for each animal: two for ECoG and one for head–muscle activity. The ECoG signals were amplified and filtered with the low pass at 0.1 s (1.6 Hz) and EMG activity was filtered with the low pass at 0.03 s (5.3 Hz). The sampling frequency was set at 200 Hz and DVD-R discs were used for polysomnographic recording storage. The data were collected every 12 h and started at 0700 h. The recording sessions were carried out over 3 days obtained during the diurnal (lights on at 0700 h) and dark periods (lights off at 1900 h) for 12 h each. The states of wakefulness (W), paradoxical sleep (PS), and SWS sleep (slow wave sleep) were identified and scored by a combination of ECoG, EMG, and behavioral criteria

[2,22], as detailed below, using Polysmith Neurotronics equipment (Gainesville, FL, USA).

The following sleep parameters were considered: total sleep time (percentage of total sleep time during the recording time), total awake time (W-percentage of all periods of wakefulness throughout the recording), SWS (SWS-percentage of all periods of with high delta content throughout the recording), and PS (percentage of all periods of PS throughout the recording). The number, average duration, and total amount of sleep and wake episodes were calculated for the entire recording session. Arousal was defined as events lasting at least 15 s with abrupt modification of baseline frequency of ECoG accompanied by high-amplitude EMG activity and followed by SWS sleep.

2.6. Statistical analysis

Data with homogeneity of variance were analyzed using two-way ANOVA for repeated measures with recording (days 1 to 3) and group (control and restricted) as main factor. Multiple post hoc comparisons were performed using Duncan test. Student's *t* test was used to analyze the data from the third experiment. The level of significance was set at $P < 0.05$. Data are presented in the figures and text as means ± SEM.

3. Results

3.1. Observation of "spontaneous" behavior

In the restricted rats fed at 7 am, we noted a very active and attentive pattern of behavior and even hyperreactivity to any stimuli. While the control group did not display any abnormal behavior throughout the study and were normally drowsy in their cages occasionally interceded by drinking and food, the FR animals were in a great majority of the observations awake in an attentive posture and moving around the cages. The observations took place in the first half of the light period.

3.1.1. Body weight

The initial body weight of the control rats was 87 g at 21 days old whereas the FR groups were 106 g (7 am) and 123 g (7 pm) ($P > 0.05$) as depicted in Fig. 1. As expected, the control animals gain weight gradually (differed from baseline at all time-points) and presented a significant increase throughout the experiment, and at the end of the experiment presented a mean of 335 g which is statistically higher than the FR groups ($P < 0.05$, see inset of Fig. 1). Body weight under FR conditions also showed increase; however, the restricted group fed at 7 am showed an increase from the third week compared to baseline (and remained statistically different until the end of the study). A slower rate of increase in weight was observed in the group fed at 7 pm that only had a significant increase in the 7th and 8th week.

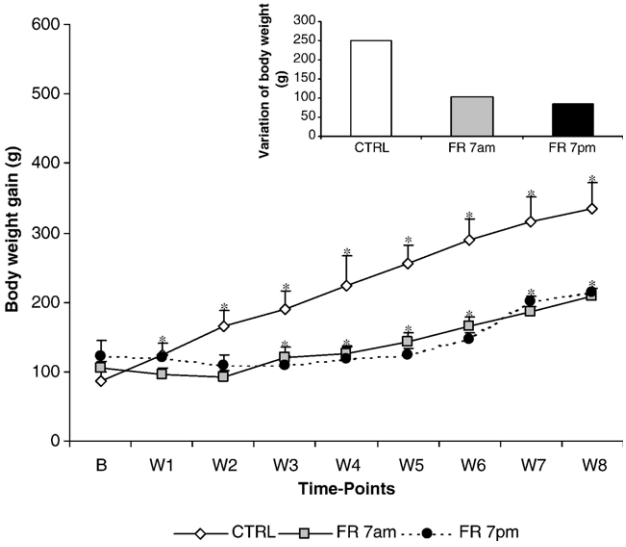

Fig. 1. Mean body weight (mean ± SD) over 8 weeks for males submitted to food restriction (fed at 7 am or at 7 pm) or maintained as control (fed ad libitum) from weaning (21 days, $n = 6$ per group). *Relative to baseline ($P < 0.05$).

3.2. Sleep parameters

3.2.1. Experiment 1 (feeding time at 7 am)

3.2.1.1. Awake. The FR rats showed significant increase in the total awake time during the light hours [$F_{(1,10)} = 26.84$; $P < 0.0005$] on days 1 to 3 of recording ($P < 0.0001$, $P < 0.001$, and $P < 0.01$, respectively) as depicted in Fig. 2A. During the dark period, a statistical difference [$F_{(1,10)} = 61.95$; $P < 0.00001$] was observed on all days of recording in comparison to control group. As illustrated in Fig. 2B, the FR group showed statistical decrease in awake time compared to control animals (Ps < 0.0001).

3.2.1.2. SWS. FR rats showed diurnal variation in sleep percentage time with significantly less SWS [$F_{(1,10)} = 16.71$; $P < 0.002$]. Reduced SWS was observed on all days compared to the control group ($P < 0.001$, $P < 0.01$, and $P < 0.02$, respectively). During the dark period, the percentage of SWS [$F_{(1,10)} = 22.81$; $P < 0.001$] was increased in FR rats compared to controls (Ps < 0.0001).

3.2.1.3. PS. During the light periods, percentage of PS decreased in FR group compared to ad libitum fed rats [$F_{(1,10)} = 45.38$; $P < 0.0001$]. Decreased PS duration was due to reduced periods of PS [$F_{(1,10)} = 37.05$; $P < 0.0001$] rather than to longer duration of each episode ($P > 0.05$). Recordings during the dark period showed that RF presented higher PS percentage on all days than control rats [$F_{(1,10)} = 67.38$; $P < 0.00001$]. This increase in PS was due to a greater number of episodes [$F_{(1,10)} = 16.38$; $P < 0.002$] and to longer duration [$F_{(1,10)} = 29.01$; $P < 0.0003$].

The 24-h analysis of the sleep parameters revealed that there was a significant decrease in total time awake [$F_{(1,10)} =$

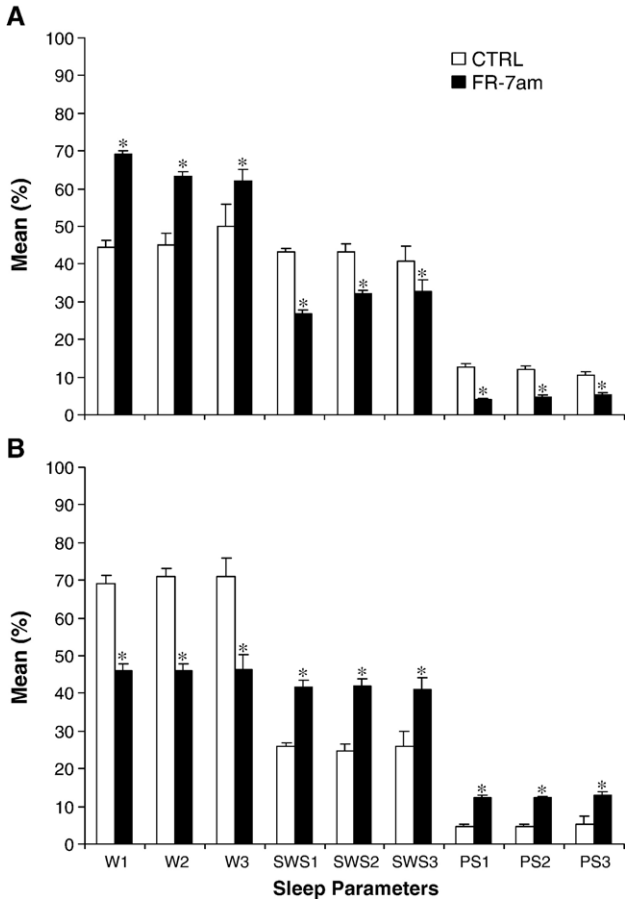

Fig. 2. Sleep parameters for 3 consecutive days during light (A) and dark (B) session recordings for food restricted (FR, $n = 6$) fed at 7 am and ad libitum control groups (CTRL, $n = 6$) during light (A) and dark (B). W indicates the percentage of total awake time during the recording time; SWS: slow wave sleep; PS: paradoxical sleep. Values are expressed as means ± SEM. Statistical data throughout reflect two-way ANOVA followed by Duncan test. *Significant differences between the two groups. See text for P values.

7.87; $P < 0.01$] and an increase in SWS [$F_{(1,10)} = 5.21$; $P < 0.004$] compared to ad libitum fed rats on the third day of recording ($P < 0.03$ and $P < 0.01$, respectively) as illustrated in Fig. 4A.

3.2.2. Experiment 2 (feeding time at 7 pm)

3.2.2.1. Awake. Fig. 3 shows the effect of FR on awake, SWS, and PS. After 8 weeks of FR, there was no significant alteration in these parameters compared to control group for all days of light recordings. However, in dark recordings, awake time decreased [$F_{(1,10)} = 18.30$; $P < 0.001$] in the FR group compared to control (Ps < 0.001).

3.2.2.2. SWS. FR rats presented higher SWS time [$F_{(1,10)} = 17.45$; $P < 0.001$] in the three dark recordings than that of control ad libitum animals (Ps < 0.001) as depicted in Fig. 3B.

3.2.2.3. PS. During the dark periods, percentage of PS increased [$F_{(1,10)} = 14.45$; $P < 0.003$] in FR group in relation to controls ($P < 0.001$, $P < 0.0001$, and $P < 0.001$, respectively).

Fig. 4B illustrates the results on the 24-h sleep parameters. As shown, this FR group fed at 7 pm presented a significant decrease in awake time [$F_{(1,10)} = 12.04$; $P < 0.006$] and an increase in SWS [$F_{(1,10)} = 11.56$; $P < 0.01$] compared to ad libitum fed rats on days 2 and 3 (Ps < 0.01).

3.2.3. Experiment 3 (FR + ad libitum)

3.2.3.1. Awake. The data analyzed by Student's t test revealed that during light recording, animals with free access to food showed no significant alterations compared to FR and FR + AD groups. Regarding the dark period, FR animals showed significantly reduced awake time than the control rats on the 3 days of recording. On day 1, the FR + AD group also differed from the ad libitum rats ($P < 0.01$) as shown in Fig. 5B.

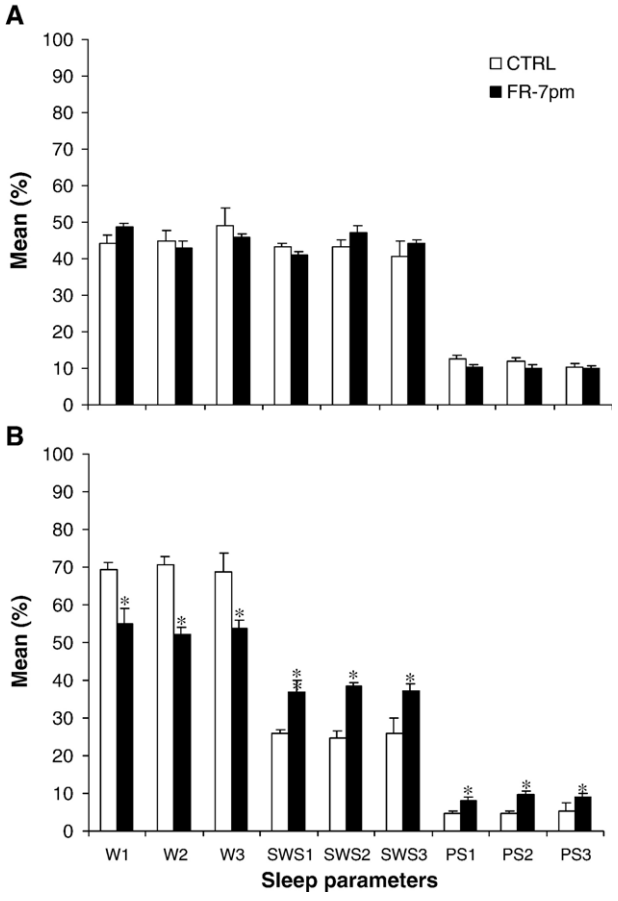

Fig. 3. Sleep parameters for 3 consecutive days during light (A) and dark (B) session recordings for food restricted (FR, $n = 6$) fed at 7 pm and ad libitum control groups (CTRL, $n = 6$) during light (A) and dark (B). W: awake; SWS: slow wave sleep; PS: paradoxical sleep. Values are expressed as means ± SEM. Statistical data throughout reflect two-way ANOVA followed by Duncan test. *Significant differences between the two groups.

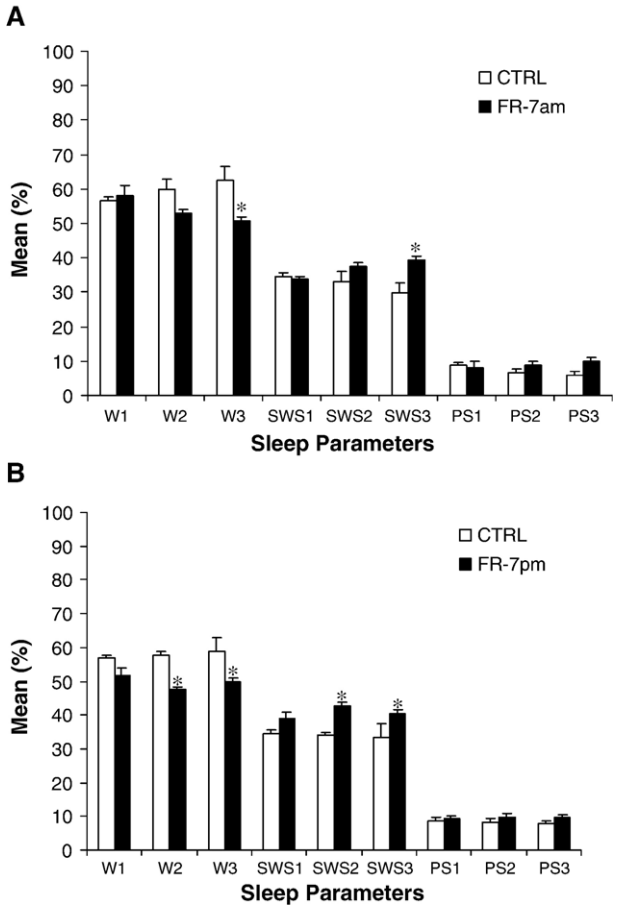

Fig. 4. Sleep parameters for 3 consecutive days during a 24-h period for food restricted after being fed at 7 am (A, $n = 6$) and fed at 7 pm (A, $n = 6$). W: awake; SWS: slow wave sleep; PS: paradoxical sleep. Values are expressed as means ± SEM. Statistical data throughout reflect two-way ANOVA followed by Duncan test. *Differs from control group.

3.2.3.2. SWS. There was no significant alteration in this parameter at light recordings. At dark recordings, SWS was increased in the FR group compared to control. This enhancement also occurred in the FR + AD rats ($P < 0.01$). On days 2 and 3, a statistical reduction in SWS was observed in this group compared to FR animals ($P < 0.01$).

3.2.3.3. PS. The percentage of PS in the light period was not modified during the recording days; however, the three recordings at dark period revealed that FR animals showed an increase in PS compared to controls. Additionally, FR + AD animals showed lower PS time than that of FR rats on days 2 to 3 (Ps < 0.01).

4. Discussion

To evaluate FR on sleep regulation, different feeding paradigms were investigated. This study shows that long-term FR in rats produces significant alterations in sleep patterns. Restricted-diet animals fed exclusively at 7 am showed significant altered diurnal distribution of the wake–sleep cycle. By feeding the animals at 7 pm, no statistical differences were observed in light recordings whereas a significant increase in sleep was observed in recordings of the dark phase. Finally, food was offered ad libitum to FR rats and sleep recording revealed that the dark recording continued to show marked alterations in dark periods. In the first day that the FR rats received food ad libitum, they still showed increase in SWS, but on the second and third days, these statistical differences disappeared and what remained were the values observed in control group.

Alterations in sleep distribution in FR rats have been reported [8,17,20]. In Roky's study, after baseline recordings, food and water were restricted to the light period for 29 days that resulted in a significant decrease of SWS and PS during the light period. Following similar conditions, Mouret et al. [17] restricted food and water to the light period and also reported decreased sleep during the light period.

As a decrease of PS and SWS in the light period was observed 1 h before food was provided [20], we elected to perform two different time-schedules to dissect the anticipatory activity to feeding time by focusing on the light/dark cycle. Indeed, illumination is a very strong factor that influences physiology, morphology, and behavior of rats. Although we carried out only FR with free access to water, our observations are comparable to previous studies, suggesting that FR is what leads to the observed alterations. We estimated that an exclusively FR condition provides more conclusive evidence of the effect of dietary restriction on sleep mechanisms than the associated effect of food and water restriction. Furthermore, the scenario of water withdrawal does not find correlation in survival strategy and is not applicable to homeostasis values.

In regards to the evening feeding protocol, our data add the evidence that regardless of the feeding time, the effect occurs most markedly in the dark period, when the animals are most active and highly sensitive to FR conditions. Considering that the animals were fed immediately before their active period, it could be predicted that the relative inability of FR to disrupt sleep might indicate the functioning of a homeostatic mechanism which is capable of coping with naturally occurring stress.

Although much can be extracted from fasting states, we elected to focus the investigation on FR and not by total food deprivation (FD). The main reason for this was that the sole critical factor responsible for FR's antiaging action is reduced calories, not dietary nutrients [25] or fasting conditions. In fact, reduced lipid or proteins and their components as usually believed seem to play a minor role in the life-extension action [11]. In this sense, by using this protocol, no animals died, indicating that the manipulation did not induce terminal suffering or undernourished animals. Of note, the diet that was adopted did not lead to malnutrition since the total amount of food provided to

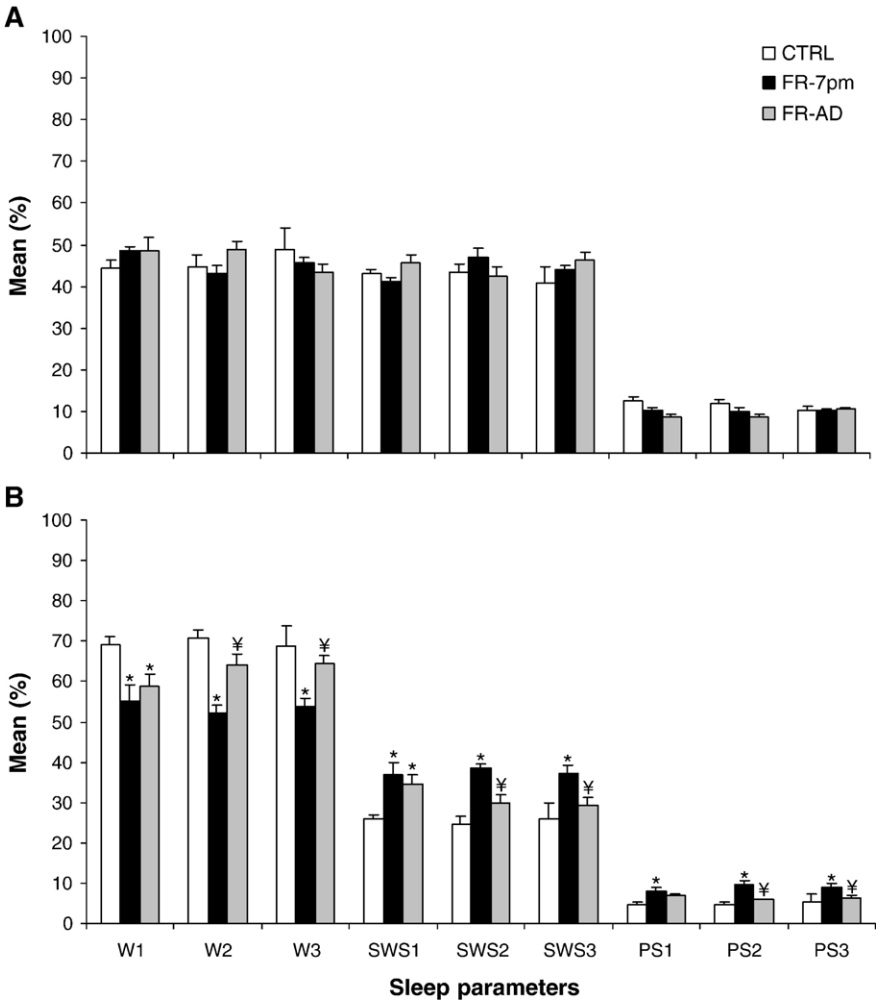

Fig. 5. Sleep parameters for 3 consecutive days during light (A) and dark (B) session recordings for food restricted after being fed ad libitum (FR-AD, $n = 6$) during light (A) and dark (B). W: awake; SWS: slow wave sleep; PS: paradoxical sleep. Values are expressed as means ± SEM. Statistical data throughout reflect Student's t test. *Differs from control group; ¥differs from FR group.

the restricted animals led to an increase in their body weight.

After 6–11 days of FD in male rats, Jacobs and McGinty [10] reported that rats that did not eat spontaneously when offered food ad libitum died within the next 24 h. The light session recordings indicated that SWS declined gradually during the first days of food deprivation until suppression whereas PS increased on the first day of deprivation and then stayed close to control levels until 2 days before it disappeared completely. The apparent discrepancy with the present observations may be due to methodological differences such as restriction vs. total FD, time from the beginning of the experiment (weaning vs. adult), duration of FR, and recording as well. Still, the contradiction of acute and chronic effect of FR warrants a clearer comprehension.

Borbély [6] determined the vigilance states of rats subjected to 80 h of FD and 64 h following restitution of food. During FD, though the diurnal rhythm of total sleep and waking was not altered by FD, the light–dark distribution of the sleep states was changed. PS was markedly reduced in the dark and increased in the light phase. In contrast to the remarkable invariance of three major sleep parameters, it was the shortening of sleep episodes that constituted the most prominent change during the FD period. Although the authors argue that the shortening of PS episodes was due to ECoG cable connection which interfered with sleep, our data demonstrated that alterations of the PS episodes may be a direct consequence of FR since our animals were firstly adapted to the cable prior to the recordings and no change in the cable connection occurred once the recording started. The dark recordings revealed that the increase in PS was due to longer duration of PS episodes suggesting that under FR, adult animals regulate their need for sleep and energy by a succession of more frequent and prolonged episodes (data not shown).

Of note, the sharp differences observed between the wake–sleep recordings resulted from the FR rats fed in the morning in relation to evening time. This differential effect of FR on sleep during the onset of light and dark phases may

be partly due to differences in metabolism. Le Magne [12] reported that the rat normally overeats at night with respect to its energy needs and undereats during the day. The dark period represents a lipogenetic phase in which fat is synthetized, whereas the light phase constitutes a lipolytic phase in which fat is broken down and converted to energy [6]. Despite the fact that both latencies to the onset of sleep and to PS were significantly increased in the FR rats compared to the control groups (data not shown), the total sleep time was increased in the restricted animals at dark recordings. Although additional studies are warranted, this increase in sleep time in the group fed at 7 pm may occur due to an anticipatory activity. This behavior could shift the sleep rhythm raising the possibility that the nocturnal increase of sleep in this group could be due to a phase advance in sleep.

Restriction of food is a common condition for animals living in their natural habitat and is often mimicked by different experiment paradigms (e.g., learning procedures). Although very well documented positive effects on longevity have been described, the consequences of FR on the sleep–wake cycle is still patchy. In part due to great discrepancies of the methodological approaches, like starting FR at different ages, alternating food and water diets, and duration of the restriction as well. Our results demonstrated that despite the significant alterations in sleep patterns in FR groups, the ad libitum refeeding rats presented a complete reversion in the sleep parameters observed previously in the dark phase.

In the past few years, there has been a great amount of data demonstrating that sleep disturbance reduces lifespan. Likewise, sleep is accepted as having restorative function on several physiological processes [15] whereas disordered sleep and sleep loss are thought to alter hormone levels and behavioral responses [1,4] as well as exacerbate several diseases. Interestingly, this increase in sleep occurs during a period the rat is most active, suggesting the relevance of a resting period during our active day.

Additional factors may be involved since FR may alter metabolism and affect brain monoamines (such as dopaminergic and noradrenergic systems) and consequently sleep through mechanisms currently under investigation. Such have a modulating effect on neurophysiological excitatory states that involve sleep/wake and eating behavior.

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