Food Restriction Alters the Diurnal Distribution of Sleep in Rats

RACHIDA ROKY,* LEVENTE KAPÁS,† PING TAISHI,‡ JIDONG FANG‡ AND JAMES M. KRUEGER‡

*Department of Pharmacology, Faculty of Medicine and Pharmacy, Casablanca, Morocco; †Department of Biological Sciences, Fordham University, Bronx, NY 10458; and ‡Washington State University, College of Veterinary Medicine, Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, P.O. Box 646520, Pullman, WA 99164-6520

Received 30 December 1998; Accepted 15 June 1999

ROKY, R. L. KAPÁS, P. TAISHI, J. FANG AND J. M. KRUEGER. Food restriction alters the diurnal distribution of sleep in rats. PHYSIOL BEHAV 67(5) 697–703, 1999.—The purpose of the present study was to determine the effects of restricting food and water intake to the light period on sleep and brain temperature (Tbr). Sprague–Dawley male rats were anesthetized and provided with electrodes and thermistors for electroencephalographic (EEG) and Tbr recordings. Baseline recordings were performed after a 3-week recovery period. After baseline recordings, access to food and water was restricted (FWR) to the light period for 29 days. During FWR, the diurnal distribution of rapid-eye-movement sleep (REMS) and Tbr were reversed, while the distribution of non-REMS (NREMS) between the dark and light periods was attenuated. Daily food and water intake, body weight, and the diurnal distribution of EEG slow-wave activity within NREMS remained unchanged. In a separate study, sham-operated and pinealectomized rats were studied in a similar manner. The sleep responses of pinealectomized and sham-operated rats to FWR were similar. Further, FWR did not affect melatonin levels in the sham-operated rats, thereby suggesting that the pineal gland does not mediate the effects of FWR on sleep. © 1999 Elsevier Science Inc.

LITTLE information is available on the effects of altered feeding schedules on the diurnal distribution of vigilance and brain temperature (Tbr). The circadian rhythm of sleep is under the control of an endogenous clock located in the suprachiasmatic nucleus (SCN) (10,14,31). The SCN clock is entrained by various periodic environmental factors; of these, the light–dark cycle is the best characterized zeitgeber (28). Other signals, such as periodic food intake, also can entrain circadian rhythms of motor activity (1,11,12,17) and corticosterone secretion (2) in several species. One previous study reported increases in the time spent in rapid-eye-movement sleep (REMS) and Tbr following alteration of the feeding schedule in the rat (29). However, the time spent in sleep is only one parameter of sleep. Non-rapid-eye-movement sleep (NREMS) is also characterized by its intensity and sleep ultrastructure by the periodicity of NREMS–REMS episodes. A widely used indicator of NREMS intensity is the amplitude of electroencephalographic (EEG) slow waves during NREMS (6), which is also called slow-wave activity (SWA), and measured by the power density of the EEG in the 1/2–4-Hz frequency range. Sleep periodicity can be assessed by the intervals between REMS episodes. Similarly to the time spent in sleep, SWA and REMS intervals also show clear diurnal distributions. In rats, SWA during NREMS is the highest and REMS intervals are the longest during the behaviorally active phase. REMS intervals shorten and SWA gradually declines during the rest period (6).

We report herein that a food and water restriction (FWR) protocol alters the diurnal distribution of REMS, NREMS, and Tbr, but not EEG SWA during NREMS in rats. Further, the pineal gland plays an important role in the regulation of circadian rhythms. Pinealectomy, however, does not alter the effect of food and water restriction on sleep and Tbr.

To whom requests for reprints should be addressed. E-mail: krueger@vetmed.wsu.edu
MATERIALS AND METHODS

Animal Housing and Surgery

Forty adult male, Sprague-Dawley rats (220–260 and 55–65 days old at the beginning of the experiment) were used. All rats were housed individually in sound-attenuated chambers with a controlled temperature (22 ± 1°C) and light cycle (lights on from 0900 to 2100 h, 15-W light bulbs, light intensity 45 lx). Rats were divided into five groups of eight animals each (Table 1). Rats in groups I, II, and III were anesthetized with ketamine and xylazine (87 and 13 mg/kg i.p., respectively) and surgically implanted with three stainless steel electrodes into the skull over the frontal and parietal cortex as previously described (20,21). Bipolar EEG recordings were obtained between two electrodes placed contralaterally. One recording electrode was placed 2 mm anterior to lambda and 4 mm lateral to the central suture, and the other one was placed 2 mm posterior to the bregma and 2 mm lateral to the central suture. The third electrode was used as a ground. A thermistor (Model 44008 Omega Technologies Co.) was placed over the parietal cortex to measure $T_{rb}$. The leads were insulated with dental cement and connected to a Teflon plug that was fixed to the skull by additional cement. Rats in groups II and III were also either sham pinealectomized (group II) or pinealectomized (group III) following the procedure of Bliss and Bates (5). Rats were transferred to individual plexiglas cages, and were connected via a recording tether to an electronic swivel. Ultrasonic motor detectors were used to record body movements. Rats in groups IV and V were used for measurement of body weight, hourly and total food intake, and water intake under control conditions (group IV) and during food and water restriction (group V).

Experimental Conditions

For the rats in groups I and V, the 65-day experiment consisted of three recording periods: 1) a control period (15 days) during which food and water were provided ad lib. The recording chambers were opened at 0900 and 2100 h daily. 2) An experimental period (29 days) during which food and water were removed at 2100 and given back at 0900 (FWR). 3) A recovery period (21 days) during which ad lib feeding was restored. In group I, EEG, $T_{rb}$ and motor activity were monitored. Recordings were performed for 24 h on Days 1, 8, and 15 of the control period, Days 1, 8, 15, 22, and 29 of the experimental period, and Days 7, 14, and 21 of the recovery period (recording days). Day 15 of the control period was used to obtain baseline values (baseline day). In group V, body weight, total food, and water intake were recorded daily; hourly food and water intake were measured on the days when the rats in group I were recorded. The animals in group IV were housed similarly to group I and V but they were allowed to eat and drink ad lib throughout the 65-day experiment. Body weight, water, and food intake in group IV were recorded as in group V. The animal cages were provided with fresh sawdust after the recording days at a random time of the day.

In groups II and III, rats were provided with food and water ad lib for an initial 22-day control period. During that period, the recording chambers were opened at 0900 and 2100 h daily. An experimental period of an additional 22 days followed; during the experimental period, food and water were removed at 0900 h, and given back at 2100 h. In groups II and III, recordings were taken during one 24-h period once a week throughout the 44-day period (Days 1, 8, 15, and 22 of the control and experimental periods).

In groups II and III on Day 45 after the end of the FWR period and polygraph recordings, blood samples were obtained from the lateral tail vein, either in the middle of the dark period or in the middle of the light period. Sampling during the dark period was performed under red light. Samples were immediately frozen in liquid nitrogen and stored at −70°C. Serum samples were extracted and melatonin measured using BUHLMANN Melatonin Radioimmunoassay test kits (ALPCO LTD, Windham, NH) according to the manufacturer’s instructions. Briefly, the C18 reversed-phase columns were initially primed with methanol and distilled water. After passing through 1 mL of sample, the columns were washed with 2 mL 1% methanol in water and 1 mL hexane. Then the extracts were eluted with 1 mL methanol, dried in a vacuum concentrator and reconstituted in 1 mL incubation buffer and stored at −20°C until assay. On the day of assay, 0.4 mL of each sample or standard (0.15, 0.5, 1.5, 5.0, 15.0, and 50 pg/tube) was combined with 0.1 mL of antiserum and 0.1 mL buffer. The $[^{125}]$I-melatonin tracer. After mixing, samples were incubated for 20 h at 4°C, then 0.1 mL of the second antibody was added to all tubes, mixed, and incubated for 15 min at 4°C. One millimeter cold distilled water was added and the precipitates were obtained by centrifugation at 2000 × g for 2 min at 4°C and then were counted in a gamma counter.

Recording and Scoring

Recording and determining the vigilance states were performed as described in (24). Briefly, signals for EEG, $T_{rb}$ and body motion were amplified, and digitized at a frequency of 128 Hz. The EEG was filtered below 0.1 and above 40 Hz. The

<table>
<thead>
<tr>
<th>Group Number</th>
<th>n</th>
<th>Surgical Treatment</th>
<th>Food/Water Restriction (length)</th>
<th>Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>8</td>
<td>EEG electrode/thermistor</td>
<td>Yes (29 days)</td>
<td>EEG, $T_{rb}$, FFT analyses of EEG</td>
</tr>
<tr>
<td>II</td>
<td>8</td>
<td>EEG electrode/thermistor</td>
<td>Yes (22 days)</td>
<td>EEG, $T_{rb}$, melatonin</td>
</tr>
<tr>
<td>III</td>
<td>8</td>
<td>EEG electrodes/thermistor</td>
<td>Yes (22 days)</td>
<td>EEG, $T_{rb}$, melatonin</td>
</tr>
<tr>
<td>IV</td>
<td>8</td>
<td>None</td>
<td>No</td>
<td>Food, water intake</td>
</tr>
<tr>
<td>V</td>
<td>8</td>
<td>None</td>
<td>Yes (29 days)</td>
<td>Food, water intake</td>
</tr>
</tbody>
</table>
FOOD RESTRICTION ALTERS SLEEP

699

body movements and T\textsubscript{br} were digitized at a frequency of 2 Hz. On-line Fast Fourier Transformation (FFT) was performed at 10-s intervals on 2-s segments of the EEG in 0.5-Hz bands in the 0.5–20-Hz frequency range. To reduce the undesirable effects related to spectral leakage due to the discontinuities at the beginning and the end of each 2-s EEG epoch (256 samples), the original data were weighted with a Hanning window. In this procedure, the original data were multiplied in the sample interval by a function that is 1 at its center and tapers to 0 at both endpoints. All signals were recorded and displayed by a computer. Recordings were visually scored off-line in 10-s epochs. Wakefulness (W) was characterized by low-amplitude EEG activity and frequent body motion; NREMS was distinguished by high-amplitude EEG slow waves, lack of body movement and gradually decreasing T\textsubscript{br}. REMS was distinguished by low amplitude fast EEG activity, theta rhythm, and lack of body movements interrupted by occasional twitches. The number of epochs containing artifacts was less than 1%; these epochs were excluded from spectral analyses. Percentages of time spent in each vigilance state were calculated for 1-h intervals. The EEG power density values (\( \mu \text{V}^2 \)) were calculated separately for W, NREMS, and REMS in four frequency bands: delta (0.5–4 Hz), theta (4.5–8 Hz), alpha (8.5–12 Hz) and beta (12.5–20 Hz). Epochs that included state transitions were excluded from the EEG spectral analyses.

Power density values were averaged over the 24-h control period to obtain a reference value for each rat in group I. For each hour on every recording day, power density in each frequency band was expressed as a percent of that value. REMS intervals were measured as the intervals between the end of one REMS episode that lasted at least 30 s and the beginning of the next one. The average lengths of REMS intervals were then calculated separately for the light and dark periods. For statistical analysis, analysis of variance (ANOVA) for repeated measures was performed on hourly values between the baseline and experimental recording days and between the baseline and recovery days. The acceptance level of significance was \( p < 0.05 \).

For statistical analysis of EEG power density, two-way ANOVA was performed because of several missing data points due to the lack of REMS or NREMS in some hours. When this happened in a control hour, the data corresponding to the same hour of the experimental recording were eliminated and vice versa.

RESULTS

Group I: Distribution of REMS and NREMS

In group I, the time spent in NREMS and REMS over the 24-h recording periods remained unchanged during the entire experiment. However, after 29 days of FWR during the dark period, there was a significant increase of NREMS (288 min during the 12-h period on Day 29 versus 230 min during the 12-h period on baseline day, \( p < 0.05 \)) and REMS (84 versus 43 min, \( p < 0.05 \)). During the light period, there was a significant decrease of NREMS (346 versus 410 min, \( p < 0.05 \)) and REMS (39 versus 82 min, \( p < 0.05 \)), compared to the baseline recording (Fig. 1). Changes in REMS (95% increase and 52% decrease) were proportionally greater than changes in NREMS (25% increase and 16% decrease), so that diurnal distribution of REMS was completely reversed, while that of NREMS was only attenuated. The decrease of REMS and NREMS in the light period began 1 h before the food was provided, suggesting that rats developed an anticipatory activity to feeding time. These statistically significant changes in sleep were observed as early as 1 week after FWR, and returned to control values 2 weeks after the restoration of ad lib feeding (data not shown).

REMS to REMS intervals were significantly decreased during the dark period (31 ± 3.8 min on baseline day versus 16 ± 1.2 min on Day 29) and significantly increased during the light period (18 ± 3.5 versus 31 ± 2.9 min).

Group I: Changes in EEG Power Density

Within REMS, the delta, theta, alpha and beta powers were significantly decreased during the dark and the light period after 29 days of FWR (Fig. 2). During NREMS, there was no significant change during the light period in the power density of any of the frequency bands. There was, however, a decrease in the delta and alpha activities that occurred at night, 1–2 h before the time of feeding. The diurnal distribution of SWA (delta activity) within NREMS was not significantly changed. Within W, power density of the four frequency bands showed a significant decrease, which was more pronounced during the light period. Changes observed in power density during FWR disappeared 7 days after the restoration of ad lib feeding (data not shown).

Group I: Changes in T\textsubscript{br} and W

In group I during the baseline recording, T\textsubscript{br} showed the well-known diurnal changes, i.e., it was higher during the night than during the day (Fig. 3). After FWR, there was no change in the first half of the dark period in T\textsubscript{br} compared to the baseline. In the second half of the dark period, however, there was a significant decrease of T\textsubscript{br} (~1°C). One hour before food was provided, T\textsubscript{br} started to increase reaching levels above the base-
line during the first hour of the light, and stayed elevated throughout the entire light period so that the diurnal distribution of T_{br} was reversed (Fig. 3). Changes in T_{br} appeared progressively after the beginning of the restriction period and became significant after 22 days (data not shown). The diurnal distribution of W mirrored that of sleep. In the dark, W was below the baseline and in the light period the amount of W increased. W was the highest at the light–dark transition. Motor activity showed a general decrease and a significant increase one hour before the food was provided (data not shown).

**Group II: Sleep in Sham-Operated Rats Subjected to FWR**

The changes in sleep induced by FWR in group II were similar to those described for group I. During the experiment, the amount of NREMS and REMS occurring over the 24-h recording periods remained unchanged. However, after 22 days of FWR there was a significant increase in NREMS in the dark period (338 versus 245 min; p < 0.05) and REMS (80 versus 38 min; p < 0.05) (Fig. 4A). During the light period significant decreases in NREMS (321 versus 382 min; p < 0.05) and REMS (53 versus 84 min; p < 0.05) compared to baseline recordings were observed. As in group I, the FWR-induced changes in REMS were proportionally greater than changes in NREMS. Further, as in group I, the decreases in REMS and NREMS during the light period began 1 h before food and water were provided, again suggesting that the rats developed an anticipatory activity to feeding time. FWR-induced changes in T_{br} were similar to those observed in group I (data not shown).

**Group III: Sleep in Pinealectomized Rats Subjected to FWR**

The sleep patterns of the pinealectomized rats under baseline conditions were similar to those of intact animals (group I) or sham-operated animals (group II) (Figs. 1 and 4A and 4B). Further, FWR-induced changes in NREMS and REMS in pinealectomized rats were not significantly different from those changes observed in sham operated rats (compare Fig. 4A to B). Compared to their own baseline values, the pinealectomized rats had a significant increase in NREMS after 22 days of RFW (331 versus 230 min; p < 0.05) and REMS (86 versus 43 min; p < 0.05) during the dark period. During the light period, NREMS (43 versus 54%) and REMS (7.3 versus 12.1%; p < 0.001) were significantly decreased. Similarly, RFW-induced changes in T_{br} in pinealectomized rats were similar to those described for group I rats (data not shown).

**Groups II and III: Melatonin Levels**

RFW treatment did not significantly change melatonin blood levels in sham-operated rats (group II) (Fig. 5). In pine-
FOOD RESTRICTION ALTERS SLEEP

Groups IV and V: Changes in Body Weight and Hourly Food and Water Intake

The body weight gain and the 24-h food and water intake during the 29 days of the experiment were not different between the control (group IV) and the experimental (group V) rats (Fig. 6) except during the first week of FWR when experimental rats ate less than controls (data not shown). During a single 24-h day, food and water intake were the highest at the beginning of the feeding period under FWR condition (Fig. 6).

DISCUSSION

The present study showed that the diurnal distributions of $T_{nn}$, REMS and NREMS, but not EEG SWA during NREMS, were altered by restricting food and water to the light period. The observed effects on sleep distribution are comparable to those reported by Mouret et al. (29) under similar conditions. In their study, NREMS and REMS were also increased during the night and decreased during the light period when food and water were available only during the light phase. In their study, the total time spent in NREMS during a 24-h day did not change but the time spent in REMS increased from a control level of $\sim 6.8$ to $\sim 9\%$ during the fourth week of food re-
striction. In our experiment, the amount of REMS during 24 h did not change significantly under the FWR protocol. Another study is also consistent with our findings; the infusion of insulin, a lipogenic hormone, during the normally lipolytic light period followed by infusion of epinephrine, a lipolytic hormone, during the normal lipogenic dark period, induced an inversion of the diurnal distribution of sleep and food intake patterns (9).

The total amount of food intake and the body weight were not affected by 1 month of FWR, indicating that the effects of this regime on sleep and T_	ext{m} are not due to malnutrition. In our experiment, the availability of both food and water were restricted. It is likely that the food restriction plays the more significant role in the observed effects because in other studies, food restriction was more important than water restriction to the change in circadian variation of plasma corticosterone (13). Water restriction alone does not entrain circadian locomotor rhythm in rats (25), although it can entrain anticipatory circadian activity rhythms (26).

It is known that the metabolic rate is high after eating and low during fasting periods (4). Moreover, body temperature strongly correlates with metabolic rate (3). The present results are consistent with the hypothesis that sleep and metabolic rate are negatively correlated. It is unlikely that the decrease of T_	ext{m} during FWR was due to increased sleep because the changes in sleep occurred within 7 days of FWR, whereas changes in T_	ext{m} took 22 days to develop. It has been suggested that decreased T_	ext{m} during NREMS, compared to W, is necessary for the appearance of REMS in normal (35) and pontine cats (19). The present finding that REMS was maximum when T_	ext{m} was at the lowest level is consistent with that notion.

Under baseline ad lib feeding conditions, pinealectomy did not significantly alter the distribution of sleep stages across the day. A similar result was obtained by Mouret et al. (30) 14 days after pinealectomy although 30 days after pinealectomy, there was an effect on the circadian rhythm of REMS. In our study, pinealectomy did not prevent the effects of FWR on sleep distribution. Further, in sham-operated animals, FWR did not affect blood melatonin levels. Another study reported that melatonin levels do not change during a 24-h period when the food was restricted to 2 h in the beginning of the light period (7). Those findings, along with current findings, suggest that the pineal does not mediate the effects of FWR on the sleep cycle.

Several studies support the idea that periodic feeding represents a zeitgeber that could entrain an oscillator different from the SCN (1,11,12,17). Lesion of the SCN does not prevent anticipatory rhythms (8,26), and periodic feeding cannot entrain the neural activity of the SCN (15,37). It is possible, however, that periodic feeding has an effect on the structures modulated by the SCN. The lateral hypothalamus and the ventromedial hypothalimus (VMH) are candidates because they receive innervation from the SCN (38,39), and their activity is modulated by food intake (22,32,36). Furthermore, alteration of neural output from the VMH attenuates the expression of motor activity and melatonin circadian rhythm (27). In addition, lesion of the VMH eliminates the anticipatory rhythm developed by periodic feeding (16) and prevents the subsequent appearance of the phase shift at the time of peak plasma cortisol concentration and body temperature seen in rats after exposure to periodic feeding (23). Involvement of structures outside the hypothalamus in the effect of periodic feeding on circadian rhythms is also possible because the brain stem or other structures outside the brain may also contain an oscillator (18).

The present study showed that even though the distribution of NREMS was altered during FWR, EEG SWA during NREMS was not. Compared to the baseline, SWA was decreased only for 2 h before the time of feeding under FWR condition; this is likely related to the food-anticipatory activity. In normal rats, SWA progressively declines during the light period and increases during the dark period (6). Further, supranormal EEG slow waves occur during NREMS rebound after sleep deprivation (34). In both cases it is posited that the high amplitudes are indicative of a more intense NREMS, and this is a manifestation of the relatively little sleep that occurred in the period preceding the NREMS episodes. The current results suggest that this explanation is incomplete because EEG SWAs did not shift, whereas NREMS did under the FWR protocol. Previously, our laboratory provided other evidence showing a separation of time spent in NREMS and EEG SWA during NREMS. Thus, the effects of interleukin-1 on these parameters are independent, and differentially depend on the time of day interleukin-1 is given; for example, high doses of interleukin-1 given intracerebroventricularly to rats inhibit NREMS yet enhance EEG SWA (low doses enhance both) (33). Immunoleoson of nerve growth factor receptive neurons causes increases in NREMS and decreases in EEG SWA during the dark hours (21). The time course of the effects of nitric oxide donor substances on NREMS and EEG SWA are different (20). Furthermore, during REMS (this study), the power density of the EEG was decreased during the light and during the dark period, even though the amount of REMS was significantly increased during the dark period. During W, the theta, alpha, and beta activities were selectively decreased during the light period. The amount of W was increased during the light period and decreased during the dark period, indicating that the power density did not change in parallel with the duration of vigilance state. These results suggest that the power density of the EEG is, in part, regulated independently from vigilance state duration. Finally, the fact that the changes in the power density of the EEG disappeared when the food and water were restored indicates that these changes were not due to aging or habituation.

Regardless of such speculation, we conclude that in rats food and water restricted to the light period reversed the diurnal rhythm of REMS and T_	ext{m}, attenuated that of NREMS and decreased the power density of the EEG during REMS and W, particularly during the light period. These effects of the FWR are independent of the pineal gland.

ACKNOWLEDGEMENTS

The technical assistance of Mr. Ying Wang is gratefully acknowledged. This research was supported in part by the National Institutes of Health (NS-25378, NS-27250, NS-31453, and NS-30514).

REFERENCES

3. Berger, R. J.; Phillips, N. H.: Comparative physiology of sleep, thermoregulation and metabolism from the perspective of energy...
FOOD RESTRICTION ALTERS SLEEP

703