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Research report

Wake-sleep and cardiovascular regulatory changes in rats made obese by a high-fat diet

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HIGHLIGHTS

- Diet-induced obesity rats sleep more than lean controls during the daily activity period.
- Diet-induced obesity rats produces more sequential REM sleep than lean controls.
- Sleep homeostasis is maintained in diet-induced obesity rats.
- Arterial pressure is increased in diet-induced obesity rats throughout the different wake-sleep states.

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ABSTRACT

Obesity is known to be associated with alterations in wake-sleep (WS) architecture and cardiovascular parameters. This study was aimed at assessing the possible influence of diet-induced obesity (DIO) on sleep homeostasis and on the WS state-dependent levels of arterial pressure (AP) and heart rate in the rat. Two groups of age-matched Sprague-Dawley rats were fed either a high-fat hypercaloric diet, leading to DIO, or a normocaloric standard diet (lean controls) for 8 weeks. While under general anesthesia, animals were implanted with instrumentation for the recording of electroencephalogram, electromyogram, arterial pressure, and deep brain temperature. The experimental protocol consisted of 48 h of baseline, 12 h of gentle handling, enhancing wake and depressing sleep, and 36-h post-handling recovery. Compared to lean controls, DIO rats showed: i) the same amount of rapid-eye movement (REM) and non-REM (NREM) sleep in the rest period, although the latter was characterized by more fragmented episodes; ii) an increase in both REM sleep and NREM sleep in the activity period; iii) a comparable post-handling sleep homeostatic response, in terms of either the degree of Delta power increase during NREM sleep or the quantitative compensation of the REM sleep loss at the end of the 36-h recovery period; iv) significantly higher levels of AP, irrespectively of the different WS states and of the changes in their intensity throughout the experimental protocol. Overall, these changes may be the reflection of a modification in the activity of the hypothalamic areas where WS, autonomic, and metabolic regulations are known to interact.

1. Introduction

In both humans and animal models, the development of obesity is associated with alterations in wake-sleep (WS) architecture. Obese humans present poor quality of sleep at night, and these sleep disturbances, which can occur independently from sleep apneas, appear to be closely related to either the overweight condition or metabolic dysregulation [1,2].

It is well established that metabolic regulation and sleep influence each other. On the one hand, it has been shown that sleep

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restriction induces an increase in energy expenditure, followed by increased food intake and, consequently, weight gain [3–5]. On the other hand, it has been shown that obesity is associated with a decrease in time spent in wakefulness (Wake) and a parallel increase in the time spent asleep in humans [2], diet-induced obesity (DIO) mice [6,7], and DIO rats [8]. In rats, these changes have been shown to appear at an early stage of DIO development and to be more evident during the active phase of the rest-activity cycle, which corresponds to the Dark period of the Light-Dark (LD) cycle in this species [8]. WS pattern modifications have also been observed in genetically obese rodents that lack either leptin [9,10] or leptin receptors [11–13]. Overall, these results may be interpreted on the basis that homeostatic regulation of body metabolism and sleep regulation share common integrative mechanisms at a hypothalamic level [14].

Since the model of spontaneous sleep curtailment, mimicking modern human habits, cannot be applied to animals, we believe that the best way to investigate the relationship between obesity and sleep regulation is to further assess possible WS changes in animals that have been made obese by a high-fat diet. Since no data stemming from an assessment of the processes underlying sleep homeostasis in rats are available, we sought to enrich the results from our previous work [8] with a qualitative and quantitative analysis of the sleep rebound immediately following a 12-h sleep deprivation caused by gentle handling. This approach provided fruitful results in previous studies carried out in our lab, in which the analysis of the sleep homeostatic response was carried out at a behavioral and cellular level following deprivations caused by different environmental and pharmacological challenges [15–21].

In the present study, deep brain temperature (T_{brain}) was assessed as an index of the influence of diet-related changes in adaptive thermogenesis [22] on the regulation of WS states across the experimental protocol [18,14,8]. Furthermore, special attention was given to the assessment of cardiovascular parameters in DIO rats. As a matter of fact, obese humans present a higher arterial pressure (AP) compared to lean control subjects [23]; moreover, in obese subjects AP decreases less (non-dipping) than in lean controls on passing from the diurnal activity period to the nocturnal resting period [23]. Some experimental evidence suggests that circadian distribution and the quality of sleep states strongly affect the normal occurrence of AP fluctuations between activity and rest periods in both humans and animals [24,25,10]. Therefore, in the present work, the WS state-dependency of cardiovascular parameters was studied in DIO rats, with a specific focus on possible alterations observed during either the gentle handling period, in which Wake was enhanced, or the post-handling period, during which a sleep rebound occurred.

2. Methods

2.1. Animals

Adult male Sprague-Dawley rats (Charles River) were used (n = 52). Animals were housed under normal laboratory conditions (nLab): free access to food and water, ambient temperature (Ta) 24.0 ± 0.5 °C, 12-h:12-h LD cycle (L: 09:00–21:00; 100 lux at cage level). The experiments were approved by the National Health Authority and were carried out under the supervision of the Central Veterinary Service of the University of Bologna in accordance to the European Union Directive 2010/63/EU.

2.2. Experimental protocol

After their arrival at the laboratory, all animals were fed a standard normocaloric (NC) laboratory diet (D12450B: 3% fat, 10% calories from fat, Mucedola). Starting from the end of the sixth week of life, which was considered to be time = 0 of the experiment, the animals were randomly separated into two groups: the first group (n = 16) continued to be fed the standard NC diet, while the second group (n = 36) was fed a high-fat hypercaloric (HC) diet (D12492: 35% fat, 60% calories from fat, Mucedola). Both groups underwent electroencephalographic recordings (EEG) after 8 weeks of diet differentiation.

The population of HC candidates was more numerous than its NC equivalent since about 50% of Sprague-Dawley rats that are fed a HC diet appear to be obesity resistant (OR) [26]. In order to study the WS pattern, the animals assigned to each diet protocol had to undergo surgery by seven to ten days before the EEG recordings were carried out. The animals selected for the HC experimental group were among the heaviest of those whose weight was over the median value (465 g) of the population (obesity prone). Obesity prone (OP) and obesity resistant (OR) rats constitute two phenotypes, selected from the original outbred Sprague Dawley population by a selective inbreeding that followed the changes in weight induced by a high fat-high energy diet [27]. In these animals, the polygenic trait of weight gain appeared to be established at the 3rd filial generation. However, in spite of growing evidence [2,6–8], the relationship between changes in sleep regulation and diet-induced obesity has not yet been firmly linked to the OP phenotype. Since the DIO rats we selected came from an OP population that is not yet stable, as a control we decided to use outbred Sprague Dawley animals matched for age but not exposed to a HC diet (lean controls). This also had the advantage of consistently extending the results of our previous work in which the same experimental approach was followed [8].

For both groups, the EEG recordings were carried out in an undisturbed environment under nLAB for two consecutive days taken as baseline, and values were averaged for Light (BLL) and Dark (BLD) periods. During the third day of recording, a gentle handling (GeH) causing total sleep deprivation was carried out during the whole 12-h L (GeHL) phase of the LD cycle. Handling consisted in the kind manipulation of the animal by the experimenter when EEG synchronization occurred in order to prevent sleep consolidation. Handling was associated with the introduction in the cage of new objects which stimulated the curiosity of the animal favoring spontaneous awakening and exploratory activity. Such a procedure has long been used in sleep deprivation studies [28] since it minimizes stress compared to other deprivation procedures. Following GeH, animals were left undisturbed (post-handling) throughout the D period of the same day (PH0D) and during the whole of the following day (PH1L and PH1D); this was considered as a recovery period from the previous sleep deprivation.

2.3. Surgery

The surgery procedure has been described elsewhere [29]. Briefly, under deep general anesthesia (diazepam, Valium Roche, 5 mg/kg intramuscular; ketamine-HCl, Ketalar, Parke-Davis, 100 mg/kg intraperitoneal), animals were implanted with: i) electrodes for EEG and nuchal electromyographyc (EMG) recording; ii) a catheter placed into the femoral artery for the telemetric recording of arterial pressure (AP); iii) a thermistor mounted inside a stainless-steel needle (21 gauge) stereotaxically implanted above the left anterior hypothalamus (from Bregma: 2 mm posterior, 2 mm lateral and 6 mm ventral; cf. [30]) to record the deep brain temperature (T_{brain}). Immediately after surgery animals received 20 ml/kg of saline subcutaneously and a wide spectrum antibiotic intramuscularly. Animals were allowed to recover from surgery for at least one week, while adapting to the recording apparatus in individual Plexiglas cages kept in a thermoregulated and sound-attenuated box.