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Elevated Sleep Quality and Orexin Receptor mRNA in Obesity Resistant Rats

Vijayakumar Mavanji, PhD[1], **Jennifer A. Teske, PhD**[1,2], **Charles J. Billington, MD**[2,3,4], and **Catherine M. Kotz, PhD**[1,2,3]

[1]Department of Food Science and Nutrition, University of Minnesota Saint Paul, MN, USA

[2]Veterans Affairs Medical Center, Minneapolis, MN, USA

[3]Minnesota Obesity Center, University of Minnesota, Minneapolis, MN, USA

[4]Department of Medicine, University of Minnesota, Minneapolis, MN, USA

Abstract

Objective—To determine if resistance to weight gain is associated with alterations in sleep/wake states and orexin receptor gene expression.

Design—Three-month old obesity susceptible Sprague-Dawley (SD) and obesity resistant (OR) rats were fed standard rodent chow. Sleep/wake cycle was measured by radiotelemetry and orexin receptor profiles in sleep/wake regulatory areas of the brain were quantified by quantitative RT-PCR.

Subjects—Adult male obesity susceptible SD and selectively-bred OR rats.

Measurements—Body weight, food intake, energy efficiency, percent time spent in active wake, quiet wake, slow-wave sleep (SWS), rapid eye movement (REM) sleep, number and mean duration of sleep/wake episodes, number of stage transitions, SWS sleep delta power and orexin receptor mRNA levels were measured.

Results—Obesity resistant rats weighed significantly less and had lower energy efficiency than SD rats. Food intake was not different between SD and OR rats. Time spent in quiet wake was similar between groups, and therefore active wake and quiet wake were combined and are referred to as ‘wakefulness’. Obesity resistant rats spent significantly more time in wakefulness and less time in SWS compared to SD rats during the 24 h recording period. Relative to SD rats, OR rats had significantly fewer sleep/wake episodes and the duration of the episodes were prolonged, indicating less fragmented sleep. Further, OR rats had fewer transitions between sleep stages, which indicates that OR rats were behaviorally more stable and had more consolidated sleep than obesity susceptible SD rats. Obesity resistant rats exhibited lower delta power during SWS sleep,

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Please address correspondence to: Vijayakumar Mavanji, PhD or Jennifer A. Teske, PhD, Veterans Affairs Medical Center, Research Services (151), One Veterans Drive, Minneapolis, MN 55417, Ph: 612-467-2882 or 612-467-4956, Fax: 612-725-2084, mavan001@umn.edu or teskeja@umn.edu.

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indicating a lower sleep drive. Our results demonstrated greater orexin receptor gene expression in sleep regulatory brain areas in OR rats.

Conclusion—These results demonstrate that prolonged wakefulness, better sleep quality, lower sleep drive and greater orexin signaling may confer protection against obesity.

Keywords

Sleep quality; body weight; obesity resistance; wakefulness; sleep fragmentation; rat

Introduction

The rapid rise in obesity in the modern world has been paralleled by significant reduction in sleep quality.1–6 Recent epidemiological and animal studies indicate an association between obesity and disordered sleep.7–15 Modern trends show reductions in nocturnal sleep time and sleep quality while prevalence of excessive daytime sleepiness (EDS) has increased. Together these trends create a conducive environment for elevated risk of obesity apart from other obesigenic factors.8,17 Though bimodal sleep patterns have been documented in human obese subjects at night,1,5,8,12,13,16–21,23 EDS is common in obese individuals8, leading to the idea that obesity significantly and independently contributes to EDS and thus may reduce nocturnal sleep quality. Moreover, surgical weight loss has been associated with improved sleep quality, reduced stage-1 sleep, less sleep fragmentation and reduced daytime sleepiness.15,22 Furthermore, persons with the sleep disorder narcolepsy, characterized by increased daytime sleepiness, sleep fragmentation, low physical activity, and orexin deficiency, have elevated body mass index.30 Together these studies indicate that obesity is associated with poor sleep quality and EDS, which suggests that better sleep quality may confer protection against obesity.

Many animal studies also support the idea that disordered sleep may contribute to obesity. For example, following weight gain on a high fat diet, obese mice showed increased time spent in slow wave sleep (SWS)9, while time spent in wakefulness was decreased and the time spent in SWS was increased especially in the dark (active) period.9 In this model, greater body weight was positively correlated with more time spent in SWS, and negatively correlated with time spent in wakefulness in the dark period. Thus obese mice were unable to maintain wakefulness during the active period, a condition analogous to human EDS.9 Interestingly, weight loss was associated with reversal of these sleep abnormalities.7 A fragmented sleep/wake pattern is also observed in the genetically obese Zucker rat.11 Consistent with leptin deficient mice, which have significantly increased SWS time during the dark period10, leptin resistant mice also had increased SWS in the dark phase, increased sleep fragmentation, and altered sleep drive indicating poor sleep quality in these animals.34 Together, these studies suggest an association between obesity, excessive sleep during active periods and poor sleep quality.

Obesity is associated with decreased levels of orexin.24–26 Orexin regulates and consolidates sleep/wake patterns and plays important roles in food intake, physical activity and energy homeostasis.27,31–33 Narcoleptic patients, who lack orexin, have altered sleep patterns, highly fragmented sleep and elevated body mass index28, which highlights the

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importance of orexin in maintaining normal sleep/wake patterns and energy homeostasis. Similarly genetically obese db/db mice and obese Zucker rats with reduced orexin content in the lateral hypothalamus26,29,35 also exhibited disordered sleep/wake patterns.10,11 Thus alterations in orexin levels might be related to disordered sleep regulation observed in obese humans and animal models.

A decade ago Levin and colleagues showed that, when exposed to high fat diet, more than half of out-bred Sprague-Dawley (SD) rats developed diet-induced obesity, while the rest of the rats showed resistance to diet-induced obesity.47 Previously we showed greater spontaneous physical activity (SPA), orexin sensitivity and orexin receptor mRNA in the lateral hypothalamus of these obesity resistant (OR) rats.32,44,48,49 Relative to OR rats, SD rats had reduced orexin levels50, sleep fragmentation51, decreased physical activity and became obese with age.44, 52 Since obesity has been associated with poor sleep quality, we hypothesized that obesity resistance might be associated with better sleep quality, characterized by consolidated sleep/wake states. We further hypothesized that consolidated sleep/wake patterns in OR rats would be associated with elevated orexin receptor profiles in brain regions involved the regulation of vigilance states. Accordingly we measured 24h sleep/wake patterns and orexin receptor mRNA profiles in brain sites involved in sleep regulation, in OR and normally obesity susceptible SD rats at three months of age, an age when their weight gain profiles were significantly different.

Material and Methods

Animals

Male SD and selectively-bred male OR rats (Charles River, Kingston, NY; 225–250g and 175–200g, respectively, at the time of arrival) were individually housed in conventional hanging cages with a 12 h light/12 h dark photoperiod (lights on at 0600) in a temperature controlled room (21–22 °C). Chow (Teklad rodent diet 8604, Harlan, Madison, WI) and water was available ad libitum except where noted. In this diet protein, fat and carbohydrate provided 33%, 14% and 53% of calories respectively, with a metabolizable energy of 3.1kcal/g. Separate sets of OR and SD rats were used for sleep/wake recordings (n=8/group) and gene expression analysis (n=5–7/group). All experiments were approved by the local Institutional Animal Care and Use Committee at the Minneapolis VA Medical Center and the University of Minnesota.

Surgery

To record vigilance states, surface EEG and EMG leads connected to a radiotelemetry transmitter (F40-EET, Data Sciences International (DSI), St. Paul, MN), were implanted in each rat under ketamine and xylazine (50 and 7 mg/kg, respectively, I.P.) anesthesia. Landmarks for bilateral EEG leads (3.1mm posterior and 1.5mm lateral to bregma; incisor bar set at 3.3 mm below ear bars) were determined from the rat brain atlas. The transmitter was placed in a blunt dissected channel across the animals back. Animals were fed ad libitum and monitored for at least 7 d before the start of experiments.

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EEG/EMG Recording

Rats were placed in a plastic solid-bottom cage with rodent bedding and habituated for 24 h in a sound-attenuated chamber (Med Associates, St. Albans, VT) and free-moving polysomnogram recording conditions followed by a 24 h recording period. Recording sessions began between 0900–1000. Body weight was measured before and after the recording period and food intake was measured during the recording period.

The EEG/EMG recording system includes the implanted transmitter with EEG and EMG leads, which transmit bioelectric signals (EEG and EMG) by radiotelemetry to the receiver (PhysioTel RPC-1). Electroencephalogram signals (1.0–30.0 Hz bandpass) and EMG signals (30–100.0 Hz bandpass) were amplified, filtered, recorded, digitized and stored electronically on a computer using a Data Exchange Matrix and Dataquest A.R.T 4.1 software (DSI). The EEG and EMG signals were displayed on the computer monitor during the recording sessions.53

Power Spectral Analysis

Digitized EEG signals were subjected to fast Fourier transformation (FFT) to calculate the power during the total period of time spent in wakefulness, SWS, and REM sleep in the 24 h recording session (10 s bins). Since SWS delta power is a marker for sleep drive34, we used the delta frequency band (0.5–4 Hz) during SWS sleep for power density calculations. Finally, the power of delta frequency during all the SWS sleep epochs were averaged and expressed as microVolt$^{2.}$ The resultant data were further divided into delta power during light and dark phases.

Determination of behavioral states

To score sleep/wake behavioral states, EEG and EMG signals were visualized with Neuroscore software (DSI, Saint Paul, MN) and sleep/wake behaviors were then scored manually on a personal computer. Specifically, consecutive 10 s epochs of EEG and EMG signals were graphically displayed on the computer monitor. Then the EEG/EMG data were classified into one of the following four behavioral states based on the EEG and EMG signals: (1) slow-wave sleep (SWS: spindling and high-voltage EEG with slow waves, low voltage EMG); (2) rapid eye movement sleep (REM sleep: low-voltage and fast EEG combined with EMG activity approximately 50% lower amplitude than that observed in SWS, with occasional short-duration, large-amplitude deflections due to muscle twitches); (3) quiet wake (QW: low-voltage fast EEG with EMG activity of a mean amplitude twice that observed in SWS); (4) active wake (AW: low-voltage fast EEG, sustained high-voltage EMG of approximately twice that observed in QW, with frequent movement deflections) as described previously.54 To be scored as a valid behavior state, the appropriate EEG and EMG activity patterns needed to persist for a minimum of 15 s. Percent time spent in each state was calculated from the scored data. The total number and mean duration of sleep/wake episodes for each behavioral state and total number of transitions between different stages were determined by Neuroscore based on the manual scoring results.

The polygraphic measures provided the following sleep/wake variables that were quantified for each recording session: (a) total sleep time (TST; SWS+REM); (b) percent time spent in

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QW, AW, total wake (W; QW+AW), SWS, and REM sleep (c) total number of sleep/wake episodes; (d) mean duration of these episodes; (e) number of state transitions between different sleep/wake states; and (f) mean delta power in SWS.

Gene expression studies

Rats were sacrificed at three months of age. Ventrolateral preoptic area (VLPO), substantia inmoninata magnocellular basal nucleus (SI-MBN), locus coeruleus (LC) and dorsal raphe (DR) were excised using a brain micropunch technique described previously.44 Brain sites were immediately frozen in liquid nitrogen following excision and were stored at −80 °C until analysis. To avoid potential effects of recent food intake on gene expression, food was removed from the cages between 0700–0800 h, and animals were sacrificed between 1100–1200 h. Relative orexin 1 receptor (OX1R), orexin 2 receptor (OX2R), and glyceraldehyde 3-phosphate dehydrogenase (GADPH) gene expression were measured by one-step real time RT-PCR and were expressed as a ratio of target to housekeeping gene, GADPH as described previously.44

Statistical analyses

Data were analyzed using Prism 5.0b (GraphPad Software, Inc., San Diego, CA) and are expressed as mean±SEM. An alpha level of .05 was used for all statistical tests. T-tests were used to determine the effect of group on body weight, food intake, energy efficiency defined as the ratio of caloric intake and body weight gain over seven days prior to EEG/EMG recordings, sleep/wake variables and gene expression. Sleep/wake parameters were also analyzed for the cumulative light phase, dark phase and the 24 h recording period. Finally sleep/wake data were binned into two-hour time bins across the 24 h recording period, analyzed for group differences and were reported as follows: 0800–1000 (10h), 1000–1200 (12h), 1200–1400 (14h), 1400–1600 (16h), 1600–1800 (18h), 1800–2000 (20h), 2000–2200 (22h), 2200–2400 (24h), 2400-0200 (2h), 0200–0400 (4h), 0400–0600 (6h), and 0600–0800 (8h). Pearson Product Moment Correlation was then used to determine the correlation between body weight and sleep/wake parameters for OR and SD rats.

Results

Body weight, food intake and energy efficiency

While 24 h food intake in three-month old OR and obesity susceptible SD rats was not significantly different (Table 1), OR rats weighed significantly less than the age-matched SD rats ($P<0.0001$). Moreover, body weight gain from two to three months of age was significantly greater in SD rats compared to OR rats ($P<0.001$). Analysis of energy efficiency over a week period revealed that OR rats have lower energy efficiency compared to SD rats (Table 1, $P<0.05$).

Cumulative time spent in sleep/wake states

In OR rats reduced body weight was associated with changes in percent time spent in W and SWS during 24 h recording. Obesity resistant rats had significantly lower TST compared with SD rats (OR vs. SD: 743±31 vs. 826±46 minutes, $P<0.05$). Lower TST in OR rats was due to less time spent in SWS (41.0±1.1% and 47.8±0.9%, respectively, $P<0.0005$) given

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that REM sleep was not significantly different between groups (9.7±0.65% and 9.2±0.69%, respectively, P=0.3, Fig. 1A). Because percent time spent in QW did not differ between OR and SD rats over 24 h (1.42±0.35% and 1.24±0.26%, respectively, P=0.3, Fig. 1A), we only analyzed W (AW+QW) and did not consider AW and QW separately. Obesity resistant rats spent significantly more time in W compared to SD rats during 24 h recording (49.8±0.85 % vs. 42.2±1.19%, P<0.0005). This increase was due to a significant increase in the percent time spent in AW in OR rats (48.57±1.3% and 40.85±0.92%, respectively, P<0.0005, Fig. 1A).

Time spent in QW, AW, W (OR vs. SD: 31.90±1.00% vs. 33.50±2.10%, respectively, P=0.26) and SWS sleep was not significantly different between OR and SD rats during the light phase (Fig. 1B). In contrast, OR rats spent significantly more time in REM sleep relative to SD rats during the light phase (P<0.05, Fig. 1B). During the dark phase, OR rats spent significantly more time in W (OR vs. SD: 67.40±1.0% vs. 57.20±1.70%, respectively, P<0.0005) and less time in SWS (P<0.0005, Fig. 1C) and REM sleep (P<0.05, Fig. 1C). Thus, total time spent in sleep was also significantly less (31± 1.8% vs. 41±1.3%, respectively, P<0.0005) and time spent in wakefulness was greater in OR rats compared to SD rats during the dark phase. The greater time OR rats spent in W was due to more time spent in AW (66.08±1.07% vs. 56.07±1.84%, respectively, P<0.0005), as QW was not different between groups (Fig. 1C).

As illustrated in Fig. 2, a detailed analysis of sleep/wake parameters in 2 h intervals across the 24 h recording period indicated that OR rats spent significantly less time in SWS during 20 h, 22 h, 0 h, 2 h and 4 h time intervals and significantly more time in wake during the 20 h, 22 h, 0 h and 2 h time intervals compared to SD rats (P<0.001 for all comparisons, Fig. 2A and 2B). Obesity resistant rats spent significantly less time in REM sleep during the 20 h (P<0.05), 22 h and 0 h (P<0.001) time intervals within the dark phase, however, they spent significantly more time in REM sleep during the 10 h, 12 h, 14h and 16h time interval within the light phase (P<0.001, Fig. 2C). Thus, OR rats spent less time in REM sleep during the dark phase and spent more time in REM sleep during the light phase, which led to lack of over-all difference in the percent time spent in REM sleep during the 24 h recording period between groups. The two hour analysis also indicates that differences between groups observed during the 24 h recording period were primarily due to differences in the vigilance states during the dark phase.

Sleep Structure

To understand sleep structure, mean number and duration of the individual episodes of each sleep/wake state was compared between OR and SD rats. Obesity resistant rats had significantly fewer wake episodes (combined episodes of AW and QW) than SD rats during the 24 h recording period (P<0.005, Fig. 3A). Fewer wake episodes were due to significantly less AW episodes (OR vs. SD rats: 229±18 vs. 278±20, P<0.05) as the number of QW episodes was similar between groups (65±13 vs. 64±16, P=0.46). However, the duration of these wake episodes was significantly longer in OR rats than that in SD rats (P<0.005, Fig. 3D) due to a significant prolongation of the AW episodes (191±11 s vs. 150±10 s, P<0.01), as there was no difference in the duration of QW episodes between groups (18±2 s vs.

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15±11 s, P=0.1). Similarly, OR rats had significantly fewer REM sleep (P<0.05) and SWS (P<0.05) episodes compared to SD rats during 24 h period (Fig. 3A). As observed for W, duration of the REM sleep and SWS episodes were significantly longer in OR rats (P<0.005 and P<0.01, respectively, Fig. 3D). However, due to fewer REM episodes, the total percent time spent in REM did not differ during this recording period. Similarly, fewer episodes of SWS resulted in less time spent in SWS for OR rats, despite a prolonged duration for each SWS episode. The prolonged durations of W, SWS and REM sleep episodes show that OR rats were behaviorally more stable than SD rats.

Further, we analyzed the mean number and the duration of individual episodes of each sleep/wake state during the light and dark phase between groups (Fig. 3). During the light phase, no differences were observed for number of episodes of sleep/wake states (OR vs. SD rats; QW: 39±9 vs. 44±11, respectively, P=0.39; AW: 134±6 vs. 150±10, respectively, P<0.1; W: P=0.06 and SWS: P=0.14; REM: P=0.2, Fig. 3B) or for the duration of W and SWS episodes (QW: 19±2 s vs. 17±2 s, respectively, P=0.17; AW: 120±12 s vs. 102±10 s, respectively, P=0.14; W: P=0.25; SWS: P=0.08, Fig. 3E). In contrast, the mean duration of the REM sleep episodes were significantly longer in OR rats during the light phase (P<0.05, Fig. 3E). Thus, although there was no difference in the number of episodes of REM sleep between groups, OR rats spent more time in REM sleep during the light phase due to the prolonged duration of these episodes.

In contrast to the light phase, episodes of W, REM sleep and SWS were significantly fewer in OR rats compared with SD rats during the dark phase (P<0.005, P<0.05 and p<0.05, respectively, Fig. 3C). Fewer W episodes in OR rats was due to less AW episodes (OR vs. SD rats: 86±4 vs. 110±12, P<0.05), as QW episodes did not differ between groups (25±6 vs. 31±5, P=0.17). The average duration of W and SWS episodes was significantly greater in OR rats (P<0.05 and P<0.005 respectively, Fig. 3F). The prolonged duration of W episodes resulted from an increase in the duration of AW episodes in OR rats as no change was observed in the QW episode duration between groups (AW: 300±24 s vs. 228±26 s, P<0.05 and QW: 19±2 s vs. 16±1 s, P=0.1). Despite the increased duration of REM sleep in OR rats, differences were not statistically significant during the dark period (P = 0.06).

Thus, OR rats spent significantly more time spent in W and less time in SWS during the dark phase, due to the prolonged duration of AW episodes and the fewer SWS episodes compared to SD rats. Although there was no significant difference in the mean duration of REM sleep episodes between groups, OR rats spent less time in REM sleep during the dark phase due to fewer REM sleep episodes.

Transitions between sleep/wake stages

Stage shifts or transitions between different stages during the 24 h recording period were analyzed as a measure of sleep quality. The results demonstrated that OR rats had a less fragmented and more consolidated sleep/wake pattern, as indicated by fewer stage transitions compared to SD rats. During 24 h recording, OR rats had significantly fewer transitions from QW to AW, QW to SWS, AW to SWS, REM sleep to SWS, and from SWS to REM compared to SD rats (P<0.05 for all comparisons, Table 2).

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We further analyzed the sleep/wake data for transitions during the cumulative light and dark phase. The data showed that OR rats exhibited better sleep quality indicated by consolidated sleep with fewer transitions between different stages during both light and dark phases. Specifically, OR rats had fewer transitions from QW to AW, AW to SWS, REM sleep to QW, REM sleep to SWS, SWS to AW and from SWS to REM sleep compared to SD rats ($P<0.05$ for all comparisons, Table 2) during light phase. During the dark phase OR rats had fewer transitions from QW to AW, AW to SWS, REM sleep to AW, REM sleep to SWS and from SWS to REM sleep compared to SD rats ($P<0.05$ for all comparisons, Table 2). This indicates that OR rats entered into wake states fewer times and were able to maintain wakefulness for a prolonged period as indicated by fewer transitions from wake states into sleep.

SWS delta power

To obtain a measure of sleep drive, we compared mean power density in the EEG delta band (0.5–4 Hz) during SWS sleep between OR and SD rats. EEG slow-wave activity during SWS was lower in OR rats compared to OR rats during the 24 h recording period ($P<0.05$, Fig. 4). The pattern of sleep drive was further assessed by analysis of the SWS delta power separately for light and dark phase. In contrast to SD rats, OR rats had lower SWS delta power in the light phase when sleep pressure is normally low, although this was not statistically significant ($P=0.07$). However, the delta power was significantly lower in the dark phase among OR rats, which is normally associated with more wakefulness and increased pressure for sleep ($P<0.05$). This indicates that OR rats exhibit lower sleep drive, which may promote reduced body weight gain. Overall, OR rats exhibited behavioral stabilization, highly consolidated sleep as indicated by fewer episodes, prolonged duration and fewer transitions between sleep/wake states as well as lower sleep drive. Consolidated wake episodes were also prominent in OR rats as depicted in the hypnogram (Fig. 5).

Correlation of sleep/wake parameters with body weight

Regression analysis comparing body weight and sleep/wake parameters among OR and obesity susceptible SD rats indicated that body weight was positively correlated with total percent time spent in sleep and SWS (Fig. 6A and 6B). There was a negative correlation between body weight and percent time spent in W and AW (Fig. 6D and 6E). Body weight was also positively correlated with total number of stage transitions and SWS delta power (Fig. 6G and 6H). However, there was no correlation between body weight and percent time spent in QW or REM sleep (Fig. 6C and 6F).

Gene expression

Gene expression studies showed that OX2R mRNA in the VLPO and DR were significantly greater in OR rats compared to obesity susceptible SD rats ($P=0.02$ and $P=0.004$ respectively, Fig. 7). Further, OX1R and OX2R mRNA was greater in the LC of OR rats relative to SD rats ($P=0.01$ and $P=0.03$, respectively, Fig. 7). In contrast, OX2R mRNA in the SI-MBN and OX1R mRNA in the VLPO, DR and SI-MBN were not significantly different between groups (Fig. 7). Thus, orexin receptor mRNA expression was greater in sleep regulatory sites in OR rats.

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Discussion

The purpose of this study was to determine if sleep/wake patterns and orexin receptor gene expression in sleep regulatory brain sites differed between obesity-susceptible SD and OR rats, which display divergent body weight gain profiles.47 These data are novel and demonstrate first that in contrast to REM sleep, OR rats spend more time in wakefulness and less time in SWS during the 24 h period due predominantly to differences in sleep/wake patterns present during the dark phase. Second, OR rats had fewer episodes of sleep/wake states, a prolonged duration of these episodes, fewer transitions between states and lower SWS sleep delta power, which indicates that OR rats were behaviorally more stable, exhibited better sleep quality and had a lower sleep drive compared to SD rats. Third, there was a positive relationship between sleep duration and body weight, which is consistent with earlier animal studies.9–11,34 Finally, OX1R mRNA was greater in VLPO and DR and both OX1R and OX2R mRNA levels were higher in the LC in OR rats. As OR rats exhibited more wakefulness, consolidated sleep/wake patterns and less fragmented behavioral states, this suggests that improved sleep quality and greater orexin receptor mRNA in areas related to the regulation of sleep/wake states may confer protection against obesity.

To date, few studies have addressed directly the relationship between obesity resistance and sleep. In humans, sleep quality improved after surgical weight loss.15,22 In diet-induced obese mice, weight gain was associated with increased sleep time and weight loss was associated with enhanced wakefulness.7 Moreover, obese leptin-deficient ob/ob mice, leptin resistant db/db mice and obese Zucker rats slept more during the dark (active) period, indicating poor quality sleep analogous to human EDS.10–12,15,34 The positive relationship between sleep duration and body weight in our study is consistent with these experimental findings and for the first time demonstrates that this rodent model of obesity resistance is associated with consolidated sleep and greater time spent in wakefulness.

We compared time spent in sleep/wake states to determine whether group differences were present. OR rats spent more time in W and less time in SWS during the 24 h recording period. However, during the light phase, time spent in W and SWS was similar between OR and SD rats, which suggests that group differences were present during the dark phase. Interestingly, OR rats also had fewer AW episodes and a prolonged duration of these episodes, which contributed to OR rats being more awake during the dark phase. Therefore, OR rats entered into wake states less frequently and were able to maintain the wake state for a longer period of time during the normal active period. Obesity resistant rats weighed significantly less and were less energy efficient than obesity prone SD rats. Hence, lean OR rats were behaviorally more stable and spent more time awake during normal active period than obese SD rats, which is consistent with studies demonstrating greater EDS in obese humans.1,8,16,21

While time spent in REM sleep was not different between OR and SD rats during 24 h recording, OR rats spent less time in REM in the dark phase and more time in REM in the light phase. Less time spent in REM sleep during dark was due to fewer REM episodes as mean duration of REM sleep episodes did not differ between groups. Human studies also show shorter nocturnal REM sleep among obese persons compared to lean controls.14

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Similarly, obese ob/ob mice spent less time in REM sleep during the light phase compared to wild-type animals10 and narcoleptic orexin knock out mice exhibit increased REM sleep during the dark phase.65 These data indicate that increased REM sleep duration during the normal rest period may be important for normal body weight maintenance since REM sleep during the normal rest phase was decreased in obese humans and several rodent models of obesity.

Obesity resistant rats had more REM sleep during the light phase than during the dark phase, but SD rats did not show this normal diurnal pattern in REM sleep. The lack of circadian variation in REM sleep in the SD rats was not related to short recovery from surgery, as rodents were given a post-surgical recovery period of at least 7 days before recording sleep/wake. Sprague-Dawley rats had higher REM sleep during the early dark phase (Fig. 2C) and they spent more time in REM sleep during the dark phase than during the light phase. This abnormal diurnal sleep pattern may be due to excessive body weight as in the present study SD rats weighed significantly more than the OR rats which displayed a normal and clear circadian variation in REM sleep. Consistent with this idea, an abnormal diurnal sleep pattern has been observed in leptin resistant genetically obese mice.10

We quantified the number and duration of sleep/wake episodes and the number of transitions between sleep/wake states, to determine whether sleep quality differed between OR and SD rats. Persons with poor sleep quality are unable to remain asleep at night, awake during the day, and show frequent sleep/wake transitions. In its most severe form, this sleep pattern is a hallmark of narcolepsy. Further, these patients may not necessarily sleep more than non-narcoleptics but instead display a fragmented sleep pattern. Thus, we hypothesized that OR rats would have “better” sleep quality characterized by fewer transitions between sleep/wake states, prolonged sleep periods, and prolonged wake periods relative to SD rats. Consistent with our hypothesis, OR rats exhibited fewer sleep/wake episodes, the duration of these episodes were prolonged, and there were fewer transitions between sleep/wake states, which indicates that OR rats had a more stable sleep pattern. Despite the increased duration of SWS episodes in OR rats, they had fewer episodes overall, and thus spent less time in SWS during the dark phase and during 24 h recording. Additionally, OR rats had fewer transitions between different stages compared to SD rats. This indicates that OR rats had less fragmented sleep, which is consistent with other obese rodent models that show more fragmented sleep, including obese leptin deficient mice, leptin resistant mice and obese Zucker rats.10,11,34 A highly fragmented sleep pattern has been associated with a higher body mass index in humans and an increased risk for obesity.18,57 Likewise, surgical weight loss has been associated with less fragmented sleep in humans.15 This infers that reduced body weight gain in OR rats may be in part due to less fragmented sleep, a measure of sleep quality.

SWS delta power is a measure of sleep drive.10 Our results showed that OR rats displayed lower power in the delta band during SWS indicating a lower sleep drive. We also showed that OR rats exhibited less fragmented sleep in the 24 h period and the duration of individual sleep episodes were longer in these rats. Thus, though the absolute amount of sleep was lower in OR rats, the high sleep quality and consolidated sleep/wake pattern observed in these rats might have contributed to the lower sleep drive. Earlier studies suggest that SWS

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delta power functions to prolong SWS episodes and that increased sleep episode duration decreases sleep drive and hence SWS delta power.36,55,56 The SD rats with poorly consolidated sleep (short SWS episodes) and a higher sleep drive indicated by higher SWS delta power, spent more time asleep. This suggests that increased total sleep time may be a compensatory mechanism for poor quality sleep, which may have resulted in chronic sleep debt. Our data further suggest that body weight status influences sleep quality. We found a positive association between body weight and SWS delta power and total transitions between sleep/wake states. Consistent with this idea, others showed that genetically obese mice had fragmented sleep and higher sleep drive indicated by higher SWS delta power compared to wildtypes.34 Thus it seems reasonable to believe that quality of sleep, in addition to amounts of prior wakefulness determine sleep drive and SWS delta power. OR rats with prolonged SWS episodes, have a low sleep drive indicated by a low SWS delta power due to the consolidated sleep/wake patterns and prolonged sleep episodes.

The observed difference in SWS delta power between groups was not due to a general change in power spectrum but instead was specific to EEG slow wave activity during SWS as there was no difference in other frequency bands and at other sleep/wake states. However, future experiments are needed to determine if reducing the number of SWS episodes (experimental sleep fragmentation) affects SWS delta power in OR rats.

Though altered sleep/wake phases are associated with elevated body weight, the mechanisms underlying the differences in sleep/wake parameters between OR and SD rats are unknown. Orexin deficiency is associated with the sleep disorder narcolepsy, characterized by decreased physical activity, sleep fragmentation,28,30,33,37,65 impaired energy balance and obesity, which suggests that orexin plays an important role in regulating and maintaining sleep-wake states as well as energy homeostasis.33,70 Behavioral and electrophysiological studies further implicate orexin in sleep/wake behavior stabilization. 37,65 Orexin administration increased wakefulness39,43 and orexin antagonism by various methods increased sleep and sleep fragmentation.39–42 Similarly, orexin levels increase after sleep deprivation.45,46 Orexin neurons activate cholinergic and aminergic neurons that are important for arousal mechanisms.33 Orexin inhibits the VLPO33 and oral pontine reticular nucleus (PnO), areas which are involved in the generation of SWS and REM sleep respectively.38 Our previous studies demonstrated increased orexin receptor mRNA expression and behavioral response to orexin in OR rats. Thus, we measured OX1R and OX2R mRNA in sleep regulatory brain sites to investigate mechanisms underlying sleep/ wake differences between OR and SD rats.

Our gene expression study shows that OX2R mRNA in the VLPO area was greater in OR rats. Similarly, we observed greater OX2R mRNA in the DR in OR rats. Moreover, the level of both OX1R and OX2R mRNA in the LC was greater in OR rats. It has been suggested that OX2R may regulate SWS and enhance behavioral stabilization while OX1R may modulate REM sleep.53,58 Application of orexin-A into the VLPO inhibited sleep and enhanced wakefulness, by inhibiting the GABAergic sleep active neurons.59 Thus, enhanced orexin signaling resulting from greater OX2R expression in the VLPO may be responsible for greater wakefulness observed in the OR rats. The raphe neurons suppress both REM and SWS sleep.33 In OR rats, elevated orexin signaling due to greater OX2R

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mRNA may activate the raphe neurons, inhibiting sleep during inappropriate times and stabilizing behavior. The LC maintains alertness and suppresses both SWS and REM sleep. 33 Enhanced activation of these neurons in OR rats due to greater OX1R and OX2R gene expression may contribute to their ability to maintain prolonged wakefulness and inhibit SWS and REM sleep during normal active periods. To the extent that gene expression may reflect capacity for signaling, greater orexin receptor mediated signaling in OR rats may enhance wakefulness and decrease sleep.

OR rats exhibited more consolidated sleep, demonstrating improved sleep quality. Earlier studies show that orexin plays an important role in behavioral state stabilization apart from maintaining wakefulness.37 Moreover, the sleep disorder narcolepsy is characterized by excessive daytime sleep and also by profound sleep fragmentation, indicating that lack of orexin results in sleep fragmentation and less consolidated sleep.30 Similarly, aging is associated with poor sleep consolidation, reduced sleep episode duration, unwanted awakening in the night and excessive napping during the daytime.50 Aging is also associated with decreased preproorexin and orexin A and B peptide levels, indicating again that orexin is important for behavioral consolidation.50 Orexin receptor activation in some brain areas release excitatory neurotransmitters, whereas these receptors stimulate the release of inhibitory neurotransmitters in other brain regions.37 Loss of these inhibitory and excitatory influences in narcolepsy and aging, leaves the sleep/wake regulating system less stable. In turn this may lead to poor sleep/wake consolidation including fragmented sleep and shortened wake episodes. 37 Together with the current data, this suggests that OR rats with higher orexin receptor sensitivity have a tighter regulation of the excitatory and inhibitory input into the sleep/wake regulatory areas, and hence are able to stabilize their sleep/wake states for a longer period of time. This could lead to increased duration of sleep episodes and hence sleep consolidation.

There are other potential mechanisms which may contribute to differences in sleep/wake patterns observed between OR and SD rats. For example, TNF-alpha is involved in the physiological regulation of sleep8,60 and obesity has been associated with low degree inflammation characterized by increased expression of TNF-alpha.8 Diet-induced obesity has been shown to be associated with increased TNF-alpha mRNA levels and increased sleep9,60,61, while neutralization of TNF- alpha in obese apnoeic men reduces sleepiness.8 Moreover, diet-induced obese rats had increased TNF-alpha mRNA, whereas rats that remained lean when fed with the same diet showed no significant changes in TNF-alpha mRNA in white adipose tissue.62 This suggests that TNF-alpha may be involved in the improved sleep/wake structure observed in the selectively-bred OR rat used in this study.

Another possible mechanism underlying greater wakefulness and more consolidated sleep in OR rats may be the greater level of physical activity observed in OR rats.44,49 Orexin stimulates SPA in rats44,63 and we shown that OR rats exhibit greater SPA and are more sensitive to the SPA enhancing effects of orexin-A than obese rats.44 Conversely, orexin-deficient mice are less active, resulting in less energy expenditure, and weigh more despite lower food intake relative to orexin-sufficient mice.64 Together, this suggests that orexin-mediated SPA is important for the maintenance of energy balance. Increased physical exercise suppresses the expression of sleep-inducing TNF-alpha66, decreases sleep,

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consolidates sleep/wake states and enhances wakefulness.68,69 Physical exercise also increases wakefulness and behavioral stabilization, as orexin knock out mice have increased sleep duration, decreased wheel running and increased sleep fragmentation.67 Thus, increased physical activity mediated by enhanced orexin sensitivity might play a role in the increased wakefulness and behavioral consolidation observed here in OR rats.

In summary, we provide evidence that sleep/wake patterns and orexin receptor mRNA profiles in sleep regulatory brain areas differ between obesity susceptible SD and OR rats. Obesity resistant rats spend greater time awake primarily during the dark phase, have fewer number of and greater duration of sleep/wake episodes, less frequent transitions between different sleep/wake states, and a lower sleep drive. These data indicate that during the normal active period, OR rats spent more time awake and had better sleep quality than obesity susceptible SD rats. This study lends additional support to our hypothesis that increased orexin signaling in sleep/wake regulatory sites enhances sleep quality and positively influences obesity resistance.

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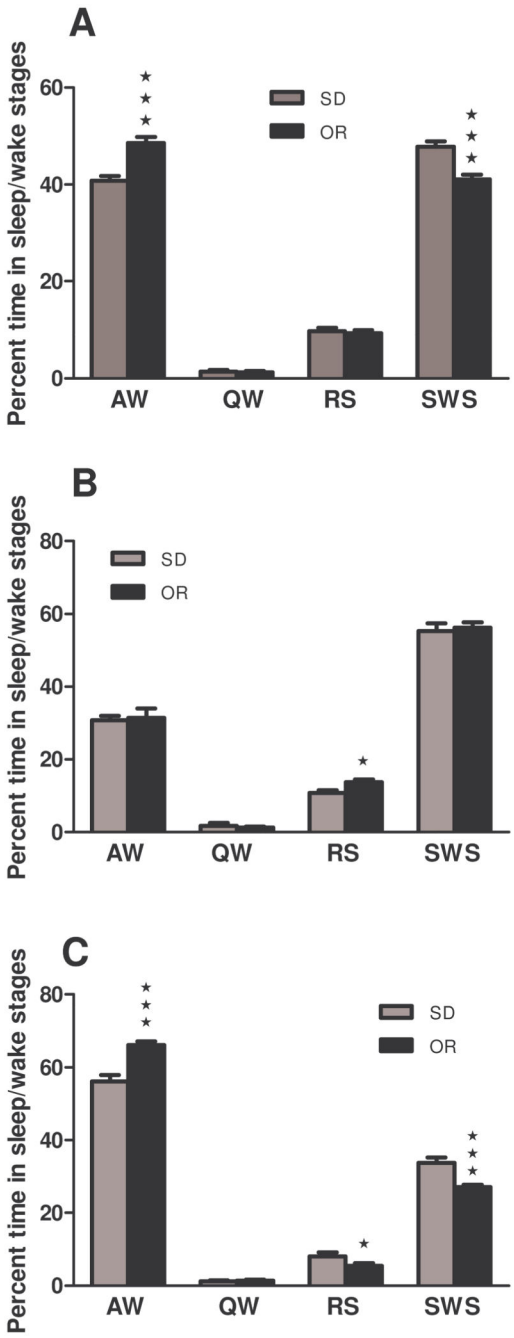

Figure 1.

OR rats spent more time in active wake (AW) and less time in slow wave sleep (SWS). Percent time spent in AW, quiet wake (QW), REM sleep (RS) and SWS in the 24 h recording period (A), 12h light phase (B) and 12h dark phase (C) in obesity resistant (OR) and Sprague-Dawley (SD) rats. Results are expressed as the percentage of total recording time, and are represented as means ± S.E.M. N=8/group. *P<0.05 and ***P<0.0005 as compared to SD rats for each sleep/wake state.

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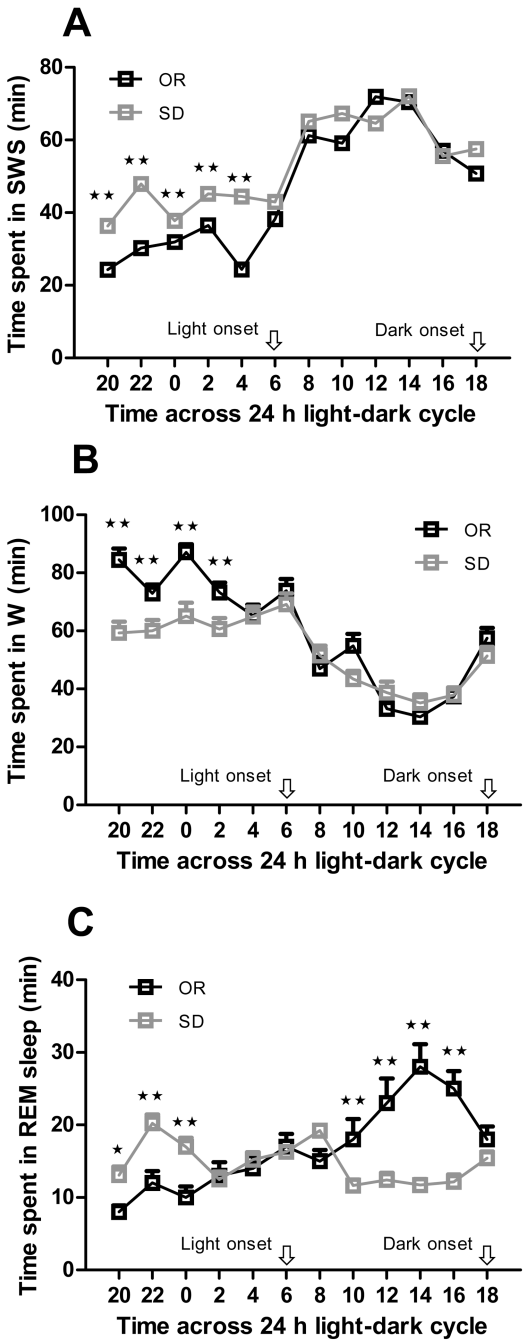

Figure 2.

Amount of (minutes ± SEM) of W (A), REM sleep (B), and SWS (C) in 2h intervals across the 12:12-h light-dark cycle. There are a number of time points in which OR rats and SD rats differed in the light and dark phases. Time of day is depicted on the X axis. Hour 6 in the X axis marks the light onset and hour 18 marks dark onset. Phenotype difference at respective time intervals (*$P < 0.05$, **$P < 0.001$).

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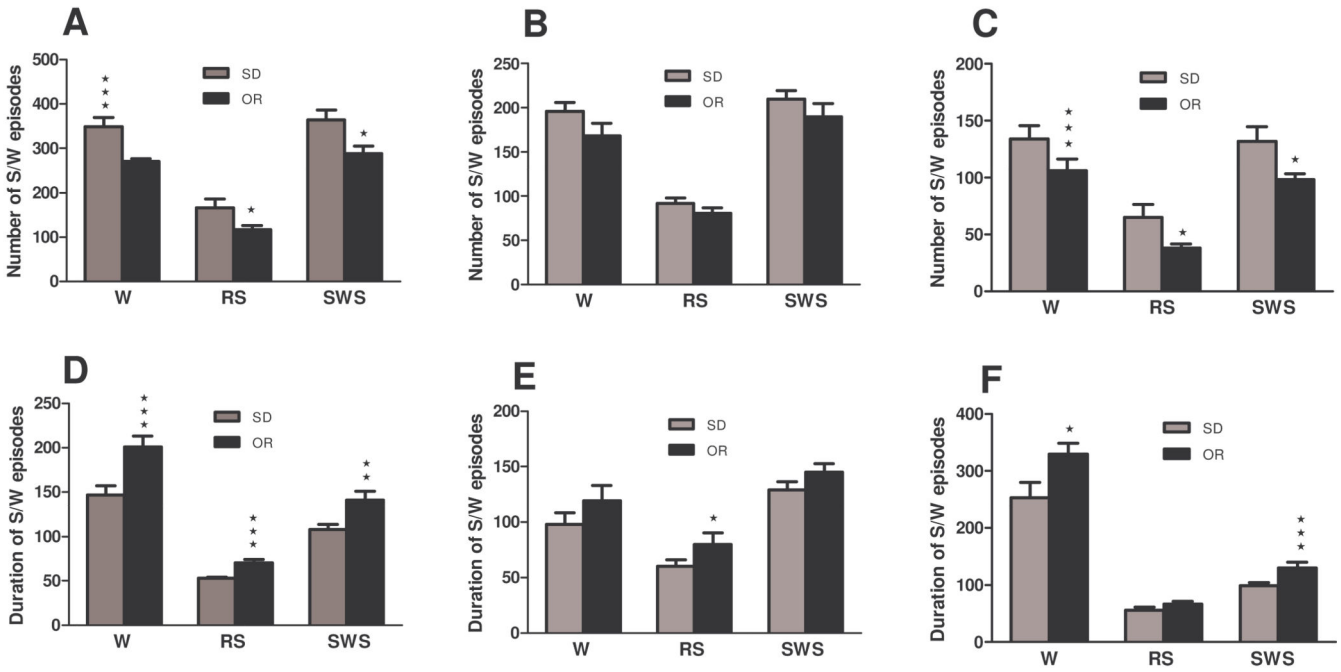

Figure 3.

Average number of wakefulness (W), REM sleep and SWS episodes (upper panel), and average duration (in sec) of W, REM sleep and SWS episodes (lower panel) in obesity resistant (OR) and Sprague-Dawley (SD) rats. A, B and C represent the number of episodes during the 24 h recording period, light phase and dark phase, respectively. D, E and F represent the average duration of episodes during the 24 h recording period, light phase and dark phase, respectively. Results are expressed as means ± S.E.M. n = 8/group. *P < 0.05, **P<0.01 and ***P <0.005 as compared to Sprague-Dawley rats for each sleep/wake state.

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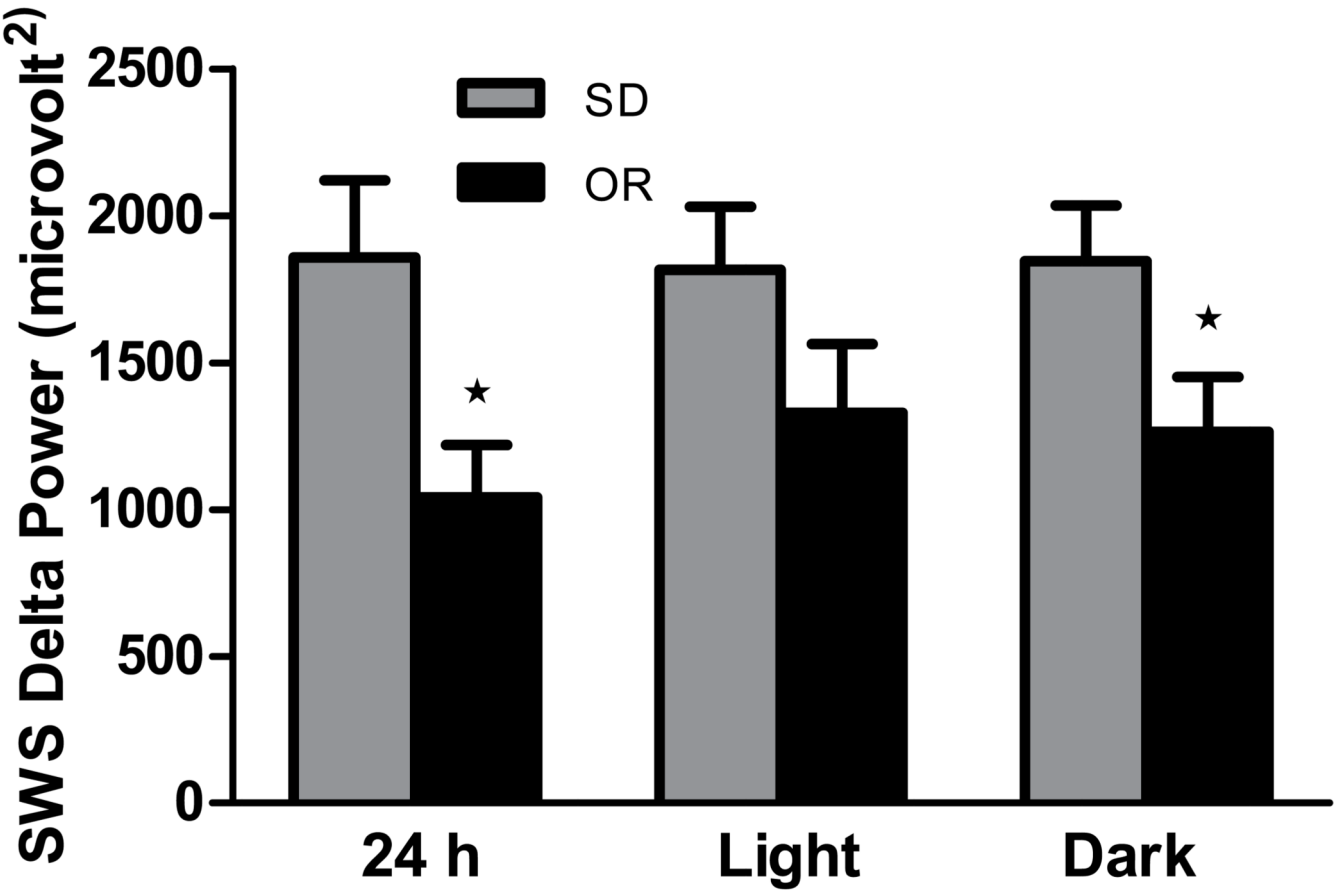

Figure 4.

SWS delta power in OR and SD rats. The values were calculated for 12h light period, 12h dark period and for the entire 24h recording period. EEG average power density (microVolt^2) for delta (0.5–4 Hz) frequency during SWS is shown. *P<0.05

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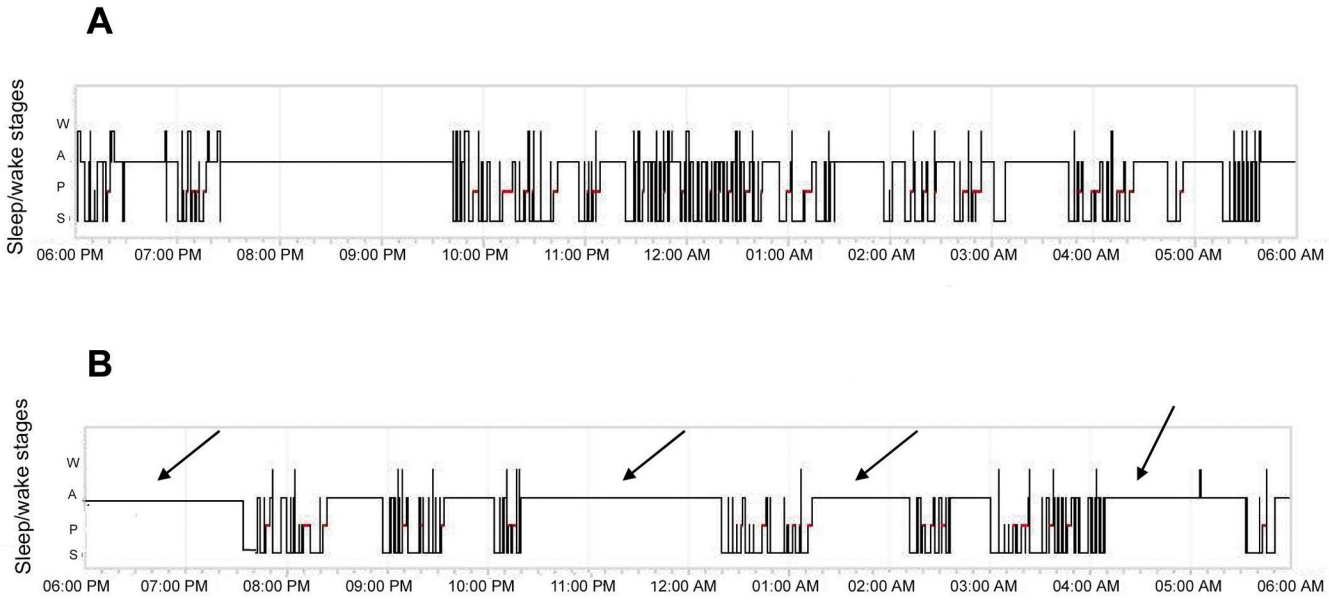

Figure 5.

Representative hypnograms of SD (A) and OR (B) rats during the dark phase obtained by concatenating 10 s epoch EEG/EMG stage scores. The height of the horizontal line above baseline indicates the vigilance state of the rat at the time (hr) from the beginning of the dark phase. A and W represent periods of wakefulness; S represents SWS and P represents REM sleep. Arrowheads highlight the prolonged wake bouts in the OR rats.

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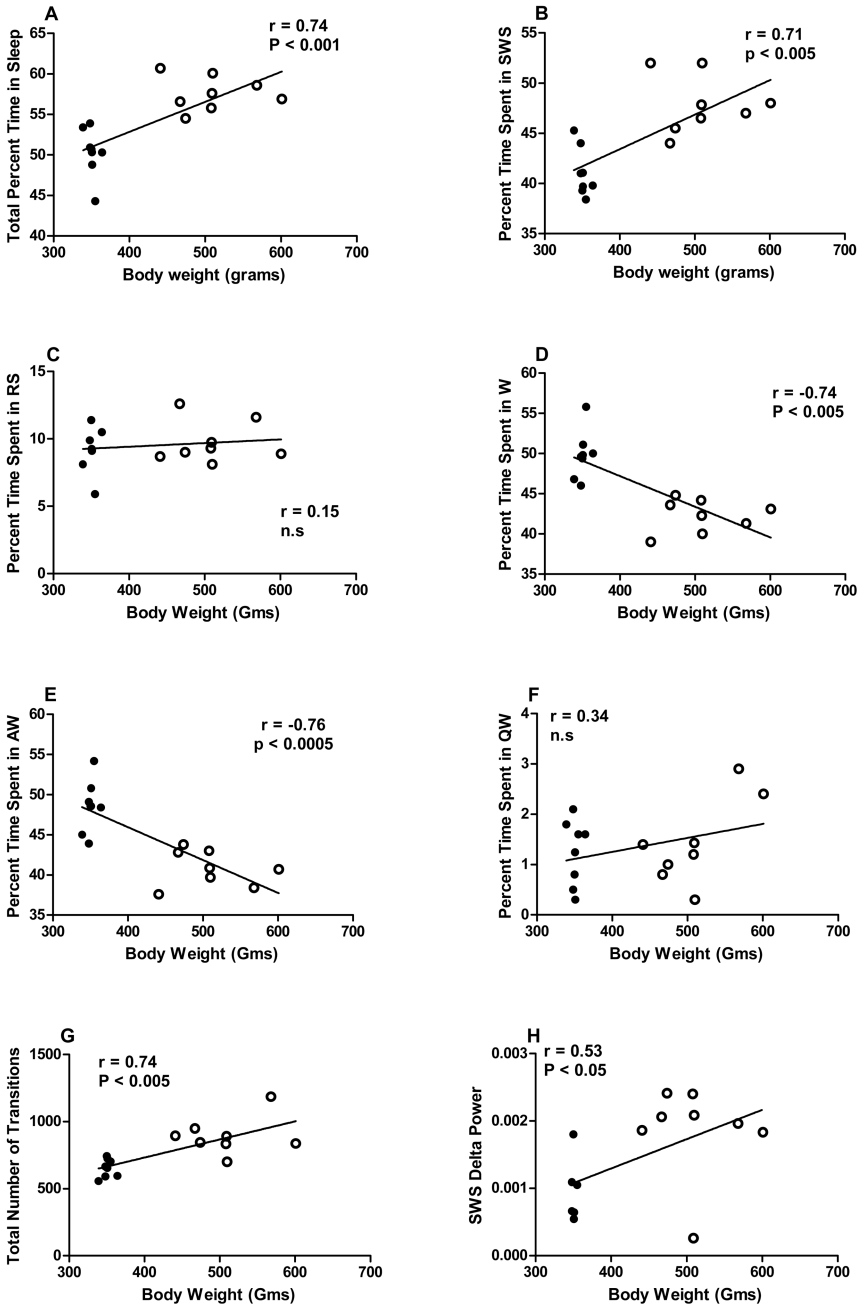

Figure 6.

Correlation between body weight and percent time spent in different sleep/wake states in obesity resistant (OR) rats (filled circles) and Sprague-Dawley (SD) rats (open circles). Body weight was positively correlated with total percent time spent in sleep (A), slow wave sleep (SWS, B), number of sleep/wake transitions (G), and SWS delta power (H). A negative correlation was observed between body weight and total percent time spent in wake (W, D) and percent time spent in active wake (AW, E). r = correlation coefficient; n.s. = not significant.

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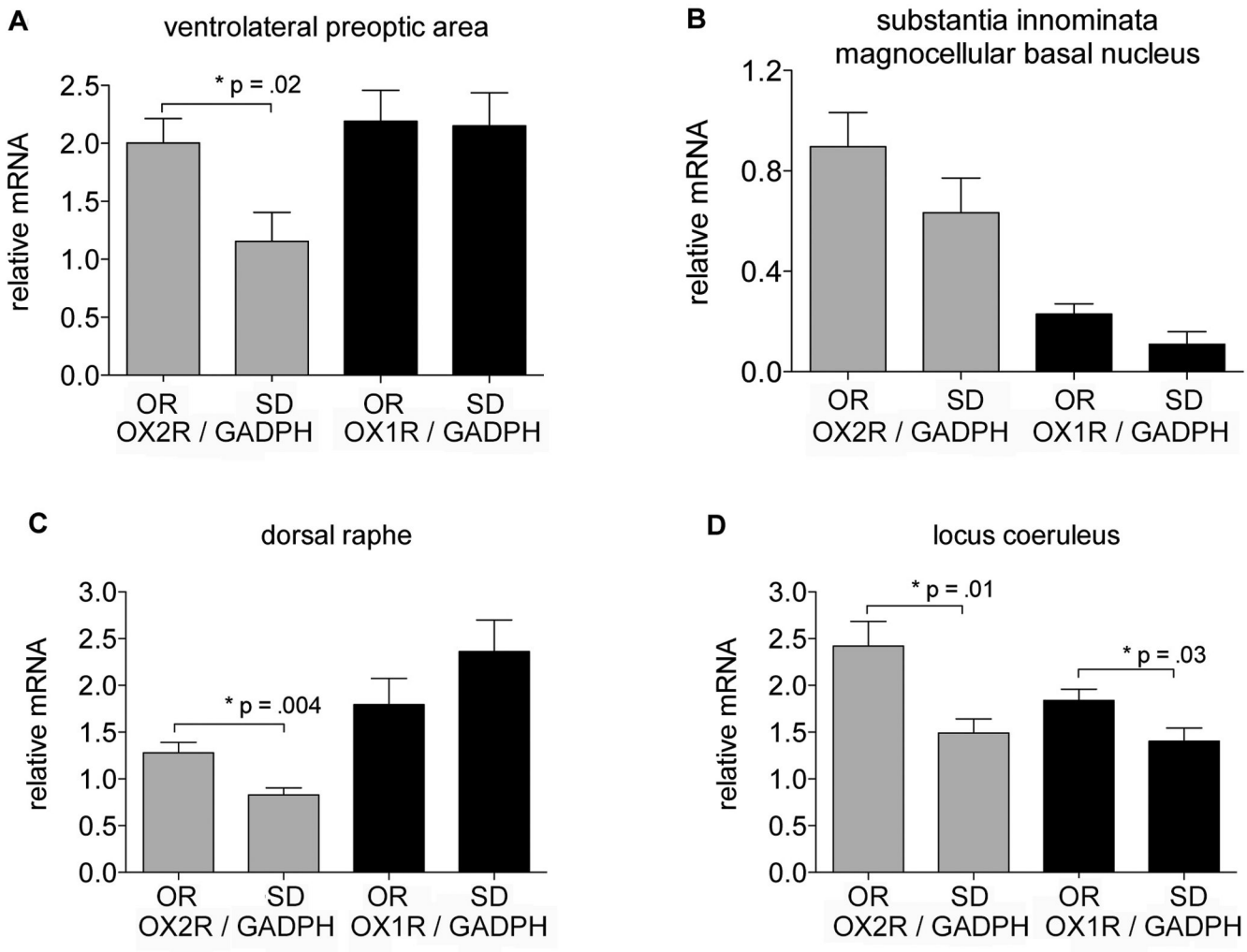

Figure 7.

Relative orexin one (OX1R) and two receptor (OX2R) mRNA corrected for glyceraldehyde-3-phosphate dehydrogenase (GADPH) in the (A) ventrolateral preoptic area, (B) substantia innominata medial basal nucleus, (C) dorsal raphe and (D) locus coeruleus in three month old obesity resistant (OR) and Sprague-Dawley (SD) rats. Data represent mean ± SEM. N = 5–7/group.

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Table 1

Body weight, food intake, and energy efficiency in 3 month old Sprague-Dawley and Obesity Resistant rats

	Sprague-Dawley	Obesity Resistant
Body weight (g)	509.80 ± 23.30	350.80 ± 3.10***
Body weight change (g)	275.00 ± 24.80	167.80 ± 6.10**
Food intake (g/bw$^{.75}$)	0.23 ± 0.01	0.24 ± 0.01
Energy efficiency	0.155 ± 0.007	0.125 ± 0.012*

Data are means ± SE. SD, Obesity prone Sprague-Dawley rats. OR, Obesity resistant rats. N= 8 rats per group.

*P<0.05,

P<0.001

***P<0.0001.

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Table 2

Number of transitions between different stages in Sprague-Dawley (SD) and Obesity Resistant (OR) rats.

	24 h SD vs. OR	**Dark phase SD vs. OR**	**Light phase SD vs. OR**
Quiet wake to active wake	23.1±5.5 vs 9.2±1.9*	9.5±3.7 vs 2.5±0.7*	12.8±3.3 vs 6.2±1.2*
Quiet wake to slow wave sleep	63.8±7.9 vs 40.2±6.9*	15.1±4.9 vs 19.2±5.3	39.1±4.5 vs 28.5±4.2
Active wake to quiet wake	45.4±6.7 vs 42.7±5.9	17.6±3.7 vs 19.1±4.4	26.5±4.1 vs 40.0±11.0
Active wake to slow wave sleep	231.2±27 vs158.5±11.2*	89.6±9.4 vs 63.8±2.9*	123±15.1 vs 89.5±8.9*
REM to quiet wake	6.5±1.9 vs 4.4±1.9	2.7±0.9 vs 2.1±1.2	3.7±1.2 vs 1.2±0.4*
REM to active wake	75.2±13.8 vs 59.7±5.1	36.1±5.5 vs 21.4±2.9*	35.4±7.1 vs 35.4±3.5
REM to slow wave sleep	84.5±15.1 vs 53.1±5.6*	26.1±7.3 vs 12.8±0.6*	54.0±9.2 vs 33.7±5.9*
Slow wave sleep to quiet wake	16.85±7.4 vs 5.8±1.50	4.7±2.6 vs 1.2±0.4	11.8±5.8 vs 4.4±1.2
Slow wave sleep to active wake	180.5±14.2 vs 163.7±22.7	62.2±6.1 vs 58.8±3.6	104.7±8.4 vs 83.4±7.8*
Slow wave sleep to REM	164.4±19.5 vs 116.4±10.3*	59.8±13.6 vs 35.1±2.5*	91.8±9.1 vs 65.2±9.2*

The number of transitions between sleep/wake states in obesity resistant (OR) and obesity prone Sprague-Dawley (SD) rats. The data (means ± SEM; n = 8/group) were calculated for the cumulative 24 h recording period, 12 h dark phase and 12 h light phase.

*P < 0.05 as compared to SD rats.