Published in final edited form as:

Am J Physiol Regul Integr Comp Physiol. 2006 September ; 291(3): R533–R540.

Elevated body temperature during sleep in orexin knockout mice

Takatoshi Mochizuki¹, Elizabeth B. Klerman², Takeshi Sakurai³, and Thomas E. Scammell¹

¹ Department of Neurology, Beth Israel Deaconess Medical Center, Boston, MA 02115

² Division of Sleep Medicine, Brigham and Women's Hospital, Boston, MA 02115

³ Dept of Pharmacology, Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8575, Japan

Abstract

Core body temperature (Tb) is influenced by many physiological factors, including behavioral state, locomotor activity, and biological rhythms. To determine the relative roles of these factors, we examined Tb in orexin knockout (KO) mice, which have a narcolepsy-like phenotype with severe sleep-wake fragmentation. Because orexin is thought to promote heat production during wakefulness, we hypothesized that orexin KO mice would have lower Tb while awake. Surprisingly, the Tb of orexin KO mice was 0.4°C higher than wild-type (WT) littermates during the dark period. Orexin KO mice had normal diurnal variations in Tb, but the ultradian rhythms of Tb, locomotor activity, and wakefulness were markedly reduced. During sustained wakefulness, Tb was the same in both groups. During the first 15 min of spontaneous sleep, the Tb of WT mice decreased by 1.0°C, but Tb in orexin KO mice decreased only 0.4°C. Even during intense recovery sleep after 8 hr of sleep deprivation, the Tb of orexin KO mice remained 0.7°C higher than in WT mice. This blunted fall in Tb during sleep may be due to inadequate activation of heat loss mechanisms or sustained activity in heat-generating systems. These observations reveal an unexpected role for orexin in thermoregulation. In addition, because heat loss is an essential aspect of sleep, the blunted fall in Tb of orexin KO mice may provide an explanation for the fragmented sleep of narcolepsy.

Keywords

orexin; hypocretin; narcolepsy; body temperature; behavioral state; thermogenesis

INTRODUCTION

Core body temperature (Tb) is influenced by many physiological factors, including behavioral state, physical activity, and biological rhythms (for reviews, see Parmeggiani, 1987; McGinty and Szymusiak, 1990; Refinetti and Menaker, 1992). For example, wakefulness and motor activity are associated with heat generation and retention of heat through skin vasoconstriction, whereas sleep engages heat loss processes, such as reduced heat production and skin vasodilation (Alföldi et al., 1990; McGinty and Szymusiak, 1990).

Orexin A and B (hypocretin 1 and 2) are neuropeptides produced in the lateral hypothalamus that affect Tb, sleep-wake behavior, and motor activity (for reviews, see Sakurai, 2005; Scammell and Saper, 2005). Intracerebroventricular injection of orexin A increases Tb (Yoshimichi et al., 2001), perhaps in part by increasing wakefulness and locomotor activity

Corresponding author: T.E. Scammell, Department of Neurology, Beth Israel Deaconess Medical Center, 77 Avenue Louis Pasteur, Boston, MA 02115. E-mail: tscammel@bidmc.harvard.edu.

Senior Editor: Dr. Barry W. Connors

Section Editor: Dr. Richard Simerly

(LMA) (Hagan et al., 1999; España et al., 2002). In addition, orexin may affect Tb through direct autonomic effects. The orexin neurons heavily innervate sympathetic preganglionic neurons of the spinal cord (van den Pol, 1999; Llewellyn-Smith et al., 2003) and activate sympathetic outflow; injection of orexin in the lateral ventricle or arcuate nucleus increases arterial blood pressure, heart rate, oxygen consumption, renal sympathetic nerve activity, plasma catecholamines, and colonic temperature in rats (Samson et al., 1999; Shirasaka et al., 1999; Wang et al., 2003). Injection of orexin into the diagonal band also increases the temperature of interscapular brown adipose tissue in anesthetized rats (Monda et al., 2004). These findings suggest that orexin facilitates heat generation as a result of behavioral and/or sympathetic activation (Kanosue et al., 2001; Morrison, 2004). However, few of these studies have considered the effects of behavioral state on Tb control, even though orexin potently increases wakefulness.

Orexin deficiency in mice strongly resembles human narcolepsy, with fragmented sleep-wake behavior and cataplexy (Chemelli et al., 1999; Mochizuki et al., 2004). Compared to wild type (WT) mice, orexin knockout (KO) mice also have less LMA (Kayaba et al., 2003; Mochizuki et al., 2004), slower heart rate, and lower blood pressure (Kayaba et al., 2003). Orexin KO mice thus provide an opportunity to study how sleep-wake behavior, LMA, and autonomic dysfunction affect the regulation of Tb. Because the orexin neurons are mainly active during wakefulness (Estabrooke et al., 2001; Lee et al., 2005; Mileykovskiy et al., 2005), we hypothesized that orexin deficiency would reduce Tb, especially during wakefulness. We examined Tb in orexin KO mice with an emphasis on how Tb varies in relation to behavioral state and LMA.

MATERIALS AND METHODS

Animals

Founder orexin KO mice were on a C57BL/6J-129/SvEV background, and their offspring were backcrossed with C57BL/6J mice for 6–8 generations (Mochizuki et al., 2004). These experiments used 7 male KO mice and 8 wild-type (WT) male littermates, all 11 weeks old and weighing 26–28 g. All experiments were approved by the Institutional Animal Care and Use Committees of Beth Israel Deaconess Medical Center and Harvard Medical School.

Surgery and EEG/EMG recordings

Mice were anaesthetized with ketamine-xylazine (100 and 10 mg/kg, i.p.) and implanted with electroencephalogram (EEG) and electromyogram (EMG) electrodes as described previously (Mochizuki et al., 2004). EEG signals were recorded using two ipsilateral stainless steel screws (1.5 mm to the right of the sagittal suture; 1 mm anterior to bregma and 1 mm anterior to lambda). EMG signals were acquired by a pair of multistranded stainless steel wires inserted into the neck extensor muscles. A telemetric temperature and LMA transmitter (TA-F20, Data Sciences International, St. Paul, MN) was placed in the peritoneal cavity. Transmitters were factory-calibrated to an accuracy of 0.1 °C. Nine days after surgery, mice were transferred to individual recording cages lined with woodchips. They had ad lib access to food and water and acclimated to the recording cables for another 5 days. All recordings were performed in a sound-attenuated chamber with a 12:12 hr light/dark (LD) cycle (30 lux; lights on at 7:00 A.M. and off at 7:00 P.M.) and a constant temperature of about 23°C.

The EEG/EMG signals were acquired using Grass Model 12 amplifiers (West Warwick, RI) and digitized at 128 Hz using a sleep scoring system (Sleep Sign, Kissei Comtec, Matsumoto, Japan). The signals were digitally filtered (EEG: 0.3–30 Hz, EMG: 2–100 Hz) and semi-automatically scored in 10 sec epochs as wake, NREM, or REM sleep. This preliminary scoring was visually inspected and corrected when appropriate. We operationally defined cataplexy to

begin with an abrupt transition from active wake to atonia with EEG theta (4–9 Hz) activity, and to end with an abrupt return from atonia and theta to active wake (Mochizuki et al., 2004). On rare occasions, orexin KO mice had brief atonia during sustained wake that lacked clear theta or delta (0.5–4 Hz) activity, and these indeterminate epochs were scored as a fifth state. These atypical states accounted for only 0.2% of the total recording time and were omitted from further analysis. WT mice never had cataplexy or this fifth state. We recently presented a detailed description of the sleep-wake behavior of these mice (Mochizuki et al., 2004).

Analysis of body temperature and locomotor activity at behavioral state transitions

The signal from the telemetry transmitter was received by an antenna (RPC-1, Data Sciences International) below the recording cage and digitally acquired (Dataquest, Data Sciences International). Body temperature (Tb) was sampled for 10 sec at 5 min intervals. Locomotor activity (LMA) was measured as movements around the cage and tallied in 5 min bins.

To examine the change in Tb and LMA at major behavioral state transitions, we analyzed sleep-wake behavior in 5 min bins. In an individual animal, when the amount of wakefulness in 5 min exceeded 60%, this bin was considered “wake”. During cataplexy, consciousness is preserved and some wake-promoting brain regions remain active (John et al., 2004). Therefore, cataplexy was included with wake when determining whether a 5 min bin met the above criterion. A “sleep” bin required more than 60% total sleep (NREM and REM) in 5 min. These 60% thresholds were chosen because the results parallel changes in Tb and in preliminary analyses, we found that more stringent thresholds (70% or 80% wakefulness or sleep/5 min) markedly reduced the numbers of acceptable bins, especially in orexin KO mice. Two consecutive “wake” or “sleep” bins were considered the beginning of a wake or sleep episode, and an episode ended if interrupted by a bin of sleep or wake, respectively. We aligned the Tb and LMA data to the onset of the sleep or wake episodes. In this report, an *epoch* lasts 10 sec, a *bin* lasts 5 min, and a sleep or wake *episode* lasts at least 10 min. A *bout* means one or more consecutive epochs of wake or sleep.

The fragmented sleep of orexin KO mice (Mochizuki et al., 2004) could alter Tb. Thus, to produce consolidated sleep, we used gentle handling to deprive WT and KO mice of sleep for 8 hr beginning at lights on (7 A.M.). In this experiment, analysis of Tb focused on the subsequent recovery sleep at 3:00 – 4:00 P.M.

Analysis of ultradian rhythmicity in body temperature, locomotor activity, and wakefulness

We used a cosine spectrum analysis (Circadia, Behavioral Cybernetics, Cambridge, MA) to measure the periods and amplitudes of the ultradian rhythms of Tb, LMA, and wakefulness. During the dark period, mice often have long, consolidated episodes of wakefulness, sometimes lasting several hours. In our preliminary analysis, these very long episodes of wakefulness interfered with the analysis of ultradian rhythmicity, so the present analysis focused on the 12 hr light period. Tb, LMA, and behavioral state data in 5 min bins were fit using a linear, least squares cosine routine. For controls, we randomized the same data series chronologically and analyzed them the same way.

Statistical analysis

All results are expressed as means $\pm$ SEM except Figure 2, which shows data from individual mice. Time-course changes in Tb and LMA were averaged across animals and compared between WT and KO mice using two-way, repeated measures ANOVAs with post hoc, two-tailed Student's *t*-tests.

RESULTS

Hourly variations in Tb, LMA, and wakefulness

Across 24 hours, the Tb of orexin KO mice was slightly higher than that of WT mice (Fig. 1) (genotype*time, $F = 1.844$, $p < 0.01$). The mean Tb of orexin KO mice was higher during the 12 hr dark period (37.6 vs. 37.3°C in WT and KO, respectively, $p < 0.05$) but not different during the 12 hr light period (36.0 and 36.1°C).

The hourly amounts of wakefulness did not differ between WT and orexin KO mice, but orexin KO mice had less LMA than WT mice (genotype*time, $F = 1.589$, $p < 0.05$). This reduction in movement was most apparent during the dark period, though orexin KO mice still had a normal daily pattern of LMA. Specifically, the amount of LMA during the dark period was 3 times higher than during the light period in both groups. These normal amounts of wakefulness and reduced LMA cannot account for the unexpectedly higher Tb of orexin KO mice during the dark period.

Short-term variations in Tb, LMA, and wakefulness

To better understand the control of Tb in orexin KO mice, we examined Tb, behavioral state, and LMA in 5 min bins. Viewed on this time scale, individual WT mice had long episodes of wakefulness at the beginning of the dark period that often lasted several hours (Fig. 2). These were followed by recurring episodes of wakefulness lasting 20–40 min in an ultradian pattern. The Tb of WT mice increased soon after each wake episode began, and decreased about 2°C during sleep. Changes in LMA closely paralleled the rhythms of wakefulness and Tb, with large bursts of LMA during the dark period.

In contrast, orexin KO mice had fragmented wakefulness, and much less variation in Tb. Ultradian rhythms were much less apparent, but Tb still dropped during the light period. LMA was less than in WT littermates, especially during the dark period. All orexin KO mice exhibited these dampened rhythms in wakefulness, Tb, and LMA.

We examined these ultradian rhythms using a cosine fitting analysis (Fig. 3). During the 12 hr light period, WT mice had clear rhythms of Tb, LMA, and wakefulness with periods of about 100 min (compared to the randomized data, condition*time, Tb: $F = 3.586$, $p < 0.0001$, LMA: $F = 2.277$, $p < 0.0001$, wake: $F = 2.772$, $p < 0.0001$). These rhythms were much less apparent in orexin KO mice; the amplitudes of their rhythms at the 90–110 min periods were reduced by 64% (Tb), 55% (LMA), and 40% (wake) compared to WT mice. The amplitudes of these rhythms in orexin KO mice did not differ statistically from the randomized data.

Changes in Tb at the onset of spontaneous sleep

Because the Tb of orexin KO mice appeared to vary less with changes in behavioral state, we analyzed Tb around the onset of spontaneous sleep episodes (Fig. 4). During the wake episodes in the dark period, both groups had similar average Tb (around 37.7°C) even though orexin KO mice had less LMA and slightly less wakefulness. Once WT mice started sleeping, their Tb dropped about 1.0°C in the initial 15 min and remained low through the rest of the sleep episode. In contrast, the Tb of orexin KO mice dropped much less (genotype*time, $F = 6.636$, $p < 0.0001$), falling only 0.4°C in the first 15 min of sleep ($p < 0.01$). This blunted fall in Tb is probably not caused by persistent muscle activity because both KO and WT mice had almost no LMA after the onset of sleep. Although the durations of sleep-wake bouts were not considered in this analysis, gross differences in behavioral state did not contribute either because both groups had similar amounts of sleep and similar types of sleep (REM sleep accounted for 12% and 13% of the total sleep in WT and KO mice, respectively). The pattern was similar during the light period: the Tb of WT mice dropped 0.8°C after 15 min of sleep,

but the Tb of orexin KO mice decreased only 0.4°C (genotype*time, $F = 5.182$, $p < 0.0001$). Both groups had similar amounts of sleep and similar ratio of REM sleep to the total sleep in the light period (WT 16%; KO 13%). These results demonstrate that orexin KO mice have higher Tb during sleep, and this cannot be easily explained by differences in the amount or type of sleep.

Changes in Tb at the onset of spontaneous wakefulness

We also examined the Tb of these mice as they transitioned from sleep to wakefulness (Fig. 5). During the dark period, the Tb of WT mice increased 1.2°C over the first 15 min of wakefulness. The average Tb of orexin KO mice during sleep was 0.9°C higher than that of WT mice (from -15 to 0 min, genotype*time, $F = 11.061$, $p < 0.0001$), and it rose 0.6°C during the first 15 min of wakefulness ($p < 0.05$ compared to the gain in WT mice). After 20 min of wakefulness, both groups of mice plateaued at the same temperature, even though orexin KO mice had less LMA (genotype*time, $F = 2.347$, $p < 0.01$) and slightly less wakefulness (genotype*time, $F = 1.947$, $p < 0.05$). During the light period, the Tb of WT mice rose 1.1°C after 15 min of wakefulness. Orexin KO had a slightly higher initial Tb, and their temperature rose 0.5°C after 15 min of wakefulness. Later in the wake episode, the Tb of orexin KO mice did not achieve the same steady level as in WT mice (genotype*time, $F = 7.787$, $p < 0.0001$), possibly because the wake bouts of orexin KO mice during the light period are so short (Mochizuki et al., 2004). Orexin KO mice also had less LMA after wake onset (genotype*time, $F = 2.868$, $p < 0.01$). These results illustrate that when they produce sustained wakefulness, orexin KO mice maintain a normal temperature.

Changes in Tb during recovery sleep

Orexin KO mice have fragmented sleep (Chemelli et al., 1999; Mochizuki et al., 2004) that may disrupt the normal fall in Tb during sleep episodes. To produce more consolidated sleep, we deprived WT and orexin KO mice of sleep for 8 hours and then analyzed the subsequent recovery sleep (Fig. 6). Both WT and orexin KO mice started sleeping about 5 min after the end of sleep deprivation. The Tb of WT mice dropped 1.6°C in the first 15 min of recovery sleep, then further decreased to a steady state of around 34.7°C in the next 30 min of recovery sleep (2.6°C lower than at sleep onset). Orexin KO mice showed a similar initial drop of Tb (1.3°C over the first 15 min of the recovery sleep; statistically no different from the drop in WT mice) but then plateaued 0.7°C higher than WT mice (genotype*time, $F = 4.26$, $p < 0.0001$). Neither LMA nor the amounts of wake in the recovery period differed between groups.

This difference in Tb during recovery sleep is probably not influenced by brief awakenings. Orexin KO mice had more awakenings than WT mice in the first 20 min of the recovery period (Fig. 6, bottom), but both groups had similar falls in Tb during this interval. In the next 25–60 min period, orexin KO mice had persistently higher Tb even though the number and duration of awakenings was the same as seen in WT mice. Thus, simple fragmentation of sleep is unlikely to explain the smaller drop in Tb of orexin KO mice.

DISCUSSION

We found that the Tb of orexin KO mice falls much less than normal during sleep. Although prior studies implied that orexin KO mice should have lower Tb during wakefulness, we found that Tb is normal during wakefulness despite substantially less LMA. These surprising observations highlight an essential role for orexin in the control of Tb.

Technical considerations

A few technical aspects of our experiments warrant comment. First, several minutes are required for Tb to change in mice, so we analyzed the relation between Tb, LMA, and sleep-

wake behavior by collapsing data into 5 min bins. Although WT mice often have brief bouts of sleep and wakefulness that are not fully represented by this time scale (Mochizuki et al., 2004), the 5 min bins of sleep-wake behavior closely paralleled the changes in Tb over time. Second, we defined a bin as wake or sleep when an animal spent more than 60% of the 5 min awake or asleep because analysis with more stringent criteria (70% or 80% of the 5 min) substantially reduced the number of acceptable bins. Nevertheless, this criterion nicely permits demonstration of ultradian and state-dependent changes in Tb.

Orexin KO mice have elevated Tb during sleep

During sleep, Tb is regulated at a lower level (Alföldi et al., 1990; McGinty and Szymusiak, 1990). In part, this results from decreased heat production from reduced muscle activity, less non-shivering thermogenesis, and lower basal metabolism. Tb also falls because of an increase in heat loss from tail, ear, and skin vasodilation. In humans, vasodilation of hands and feet correlates well with the onset of sleep (Kräuchi et al., 1999). The mechanisms that produce these responses are not well understood, but sleep-promoting neurons in the preoptic area also activate heat loss mechanisms (McGinty and Szymusiak, 1990; Gong et al., 2000; Kanosue et al., 2001; Yoshida et al., 2005). Thus, a fall in Tb is an essential aspect of sleep.

Prior studies suggested that orexin deficiency should result in lower sympathetic activity, and so it is surprising that orexin KO mice have higher Tb during sleep. However, people with narcolepsy also have slightly higher than normal Tb during sleep (Mosko et al., 1983; Pollak and Wagner, 1994), and this abnormal response can be explained by several mechanisms.

First, with the onset of spontaneous sleep, Tb falls more slowly in orexin KO mice, suggesting that heat loss mechanisms might not be fully engaged. Perhaps, orexin KO mice may readily fall asleep (Mochizuki et al., 2004), even without much activity in sleep-promoting preoptic neurons. Because these preoptic neurons also activate heat loss mechanisms, Tb would fall less during sleep.

In support of this idea, the Tb of orexin KO mice falls at a normal, rapid rate during the initial sleep episode after sleep deprivation. The activity of ventrolateral preoptic area neurons is particularly high during recovery sleep after sleep deprivation (Szymusiak et al., 1998), and the initial, rapid fall in Tb in orexin KO mice may reflect strong activation of these and other sleep-generating preoptic neurons that reduce Tb. However, this hypothesis is not fully satisfying because, despite presumably similar sleep drive, the Tb of orexin KO mice then remains at a higher level. This persistently higher Tb in orexin KO mice is not a consequence of fragmented sleep-wake behavior, because both groups of mice have the same number of awakenings during this period.

A second explanation is that orexin deficiency may directly impair mechanisms that reduce Tb. Stimulation of the caudal lateral hypothalamus triggers tail vasodilation in rats (Zhang et al., 1997), and the lateral hypothalamic orexin neurons may contribute to this response. Furthermore, the orexin neurons directly innervate many brain regions implicated in thermoregulation including the preoptic area, dorsomedial hypothalamus, ventromedial hypothalamus, posterior hypothalamus, periaqueductal gray matter, medullary raphe, and intermediolateral cell column of the spinal cord (Peyron et al., 1998; Marcus et al., 2001; Llewellyn-Smith et al., 2003; Berthoud et al., 2005). These neurons work together to regulate Tb depending on physiological needs, and orexin may be a key signal to control or synchronize the activity of these neurons. Though orexin neurons are mainly active during wakefulness (Estabrooke et al., 2001; Lee et al., 2005; Mileykovskiy et al., 2005), it is possible that orexin has lingering effects during sleep that reduce heat production and facilitate heat loss.

A third possibility is that the reduced fall in Tb could be caused by sustained thermogenic activity during sleep. Orexin deficiency may reduce sympathetic tone because orexin KO mice have lower blood pressure (Kayaba et al., 2003) and people with narcolepsy have higher than normal distal skin temperatures during wakefulness and sleep (R. Fronczek, personal communication). If orexin deficiency reduces sympathetic activity and LMA during wakefulness, then heat-generating mechanisms may compensate to produce normal Tb during wake. For example, increases in non-shivering thermogenesis or thyroid hormone could increase heat production—normalizing Tb during wake, but producing an inappropriately high Tb during sleep. Further studies are needed to determine whether neural or endocrine signals inappropriately drive thermogenesis in orexin KO mice.

Orexin and ultradian rhythms

The circadian rhythms of sleep-wake behavior, Tb, and LMA do not require orexin (Gerashchenko et al., 2003; Mochizuki et al., 2004), but the ultradian rhythms of Tb and wakefulness are clearly attenuated in orexin KO mice. Few researchers have examined ultradian rhythms in mice, but their Tb varies rapidly with changes in behavioral state, probably because of their small thermal mass. WT mice exhibit large amplitude and parallel rhythms of Tb and wakefulness with a period of about 100 minutes. In contrast, orexin KO mice appear to have much smaller ultradian rhythms. Most likely, this reduction in rhythmicity is due to three masking effects: orexin KO mice have smaller falls in Tb during sleep; LMA is reduced so Tb may not rise as rapidly during wakefulness; and the duration of wake and sleep bouts is much shorter so there is less opportunity for sustained increases or decreases in Tb (Mochizuki et al., 2004).

Conclusions

A moderate fall in Tb may be necessary for good quality sleep (Gilbert et al., 2004). Tb falls less than normal during sleep in individuals with poor quality sleep from aging, insomnia, or disorders of autonomic dysfunction such as multisystem atrophy (Zepelin and McDonald, 1987; Lushington et al., 2000; Pierangeli et al., 2001). No model of orexin neurobiology has adequately explained why people and animals with narcolepsy have fragmented sleep. If reduced heat production and activation of heat loss mechanisms are essential for sleep, then a smaller fall in Tb may contribute to the fragmented sleep of narcolepsy.

Acknowledgements

We thank C.B. Saper, K. Yoshida, and M.P. Anderson for their thoughtful comments on this manuscript. We thank A. Crocker and S. McCormack for their technical assistance. This study was supported by NIH grants MH62589, HL60292, and HD045459.

References

- Alföldi P, Rubicsek G, Cserni G, Obál F Jr. Brain and core temperatures and peripheral vasomotion during sleep and wakefulness at various ambient temperatures in the rat. *Pflügers Arch* 1990;417:336–341.
- Berthoud HR, Patterson LM, Sutton GM, Morrison C, Zheng H. Orexin inputs to caudal raphe neurons involved in thermal, cardiovascular, and gastrointestinal regulation. *Histochem Cell Biol* 2005;123:147–156. [PubMed: 15742197]
- Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammell T, Lee C, Richardson JA, Williams SC, Xiong Y, Kisanuki Y, Fitch TE, Nakazato M, Hammer RE, Saper CB, Yanagisawa M. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell* 1999;98:437–451. [PubMed: 10481909]
- España RA, Plahn S, Berridge CW. Circadian-dependent and circadian-independent behavioral actions of hypocretin/orexin. *Brain Res* 2002;943:224–236. [PubMed: 12101045]

- Estabrooke IV, McCarthy MT, Ko E, Chou TC, Chemelli RM, Yanagisawa M, Saper CB, Scammell TE. Fos expression in orexin neurons varies with behavioral state. *J Neurosci* 2001;21:1656–1662. [PubMed: 11222656]
- Gerashchenko D, Blanco-Centurion C, Greco MA, Shiromani PJ. Effects of lateral hypothalamic lesion with the neurotoxin hypocretin-2-saporin on sleep in Long-Evans rats. *Neuroscience* 2003;116:223–235. [PubMed: 12535955]
- Gilbert SS, van den Heuvel CJ, Ferguson SA, Dawson D. Thermoregulation as a sleep signalling system. *Sleep Med Rev* 2004;8:81–93. [PubMed: 15033148]
- Gong H, Szymusiak R, King J, Steininger T, McGinty D. Sleep-related c-Fos protein expression in the preoptic hypothalamus: effects of ambient warming. *Am J Physiol Regul Integr Comp Physiol* 2000;279:R2079–R2088. [PubMed: 11080072]
- Hagan JJ, Leslie RA, Patel S, Evans ML, Wattam TA, Holmes S, Benham CD, Taylor SG, Routledge C, Hemmati P, Munton RP, Ashmeade TE, Shah AS, Hatcher JP, Hatcher PD, Jones DN, Smith MI, Piper DC, Hunter AJ, Porter RA, Upton N. Orexin A activates locus coeruleus cell firing and increases arousal in the rat. *Proc Natl Acad Sci USA* 1999;96:10911–10916. [PubMed: 10485925]
- John J, Wu MF, Boehmer LN, Siegel JM. Cataplexy-active neurons in the hypothalamus: implications for the role of histamine in sleep and waking behavior. *Neuron* 2004;42:619–634. [PubMed: 15157423]
- Kanosue, K.; Yoshida, K.; Maruyama, M.; Nagashima, K. The central organization of the thermoregulatory system. In: Kosaka, M.; Sugahara, T.; Schmidt, KL.; Simon, E., editors. *Thermotherapy for neoplasia, inflammation, and pain*. Tokyo: Springer-Verlag; 2001. p. 2–11.
- Kayaba Y, Nakamura A, Kasuya Y, Ohuchi T, Yanagisawa M, Komuro I, Fukuda Y, Kuwaki T. Attenuated defense response and low basal blood pressure in orexin knockout mice. *Am J Physiol Regul Integr Comp Physiol* 2003;285:R581–R593. [PubMed: 12750151]
- Kräuchi K, Cajochen C, Werth E, Wirz-Justice A. Warm feet promote the rapid onset of sleep. *Nature* 1999;401:36–37. [PubMed: 10485703]
- Lee MG, Hassani OK, Jones BE. Discharge of identified orexin/hypocretin neurons across the sleep-waking cycle. *J Neurosci* 2005;25:6716–6720. [PubMed: 16014733]
- Llewellyn-Smith IJ, Martin CL, Marcus JN, Yanagisawa M, Minson JB, Scammell TE. Orexin-immunoreactive inputs to rat sympathetic preganglionic neurons. *Neurosci Lett* 2003;351:115–119. [PubMed: 14583395]
- Lushington K, Dawson D, Lack L. Core body temperature is elevated during constant wakefulness in elderly poor sleepers. *Sleep* 2000;23:504–510. [PubMed: 10875557]
- Marcus JN, Aschkenasi CJ, Lee CE, Chemelli RM, Saper CB, Yanagisawa M, Elmquist JK. Differential expression of orexin receptors 1 and 2 in the rat brain. *J Comp Neurol* 2001;435:6–25. [PubMed: 11370008]
- McGinty D, Szymusiak R. Keeping cool: a hypothesis about the mechanisms and functions of slow-wave sleep. *Trends Neurosci* 1990;13:480–487. [PubMed: 1703678]
- Milevskiy BY, Kiyashchenko LI, Siegel JM. Behavioral correlates of activity in identified hypocretin/orexin neurons. *Neuron* 2005;46:787–798. [PubMed: 15924864]
- Mochizuki T, Crocker A, McCormack S, Yanagisawa M, Sakurai T, Scammell TE. Behavioral state instability in orexin knockout mice. *J Neurosci* 2004;24:6291–6300. [PubMed: 15254084]
- Monda M, Viggiano A, Viggiano A, Fuccio F, de Luca V. Injection of orexin A into the diagonal band of Broca induces sympathetic and hyperthermic reactions. *Brain Res* 2004;1018:265–271. [PubMed: 15276887]
- Morrison SF. Central pathways controlling brown adipose tissue thermogenesis. *News Physiol Sci* 2004;19:67–74. [PubMed: 15016906]
- Mosko SS, Holowach JB, Sassin JF. The 24-hour rhythm of core temperature in narcolepsy. *Sleep* 1983;6:137–146. [PubMed: 6878983]
- Parmeggiani PL. Interaction between sleep and thermoregulation: an aspect of the control of behavioral states. *Sleep* 1987;10:426–435. [PubMed: 3317725]
- Peyron C, Tighe DK, van den Pol AN, de Lecea L, Heller HC, Sutcliffe JG, Kilduff TS. Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J Neurosci* 1998;18:9996–10015. [PubMed: 9822755]

- Pierangeli G, Provini F, Maltoni P, Barletta G, Contin M, Lugaresi E, Montagna P, Cortelli P. Nocturnal body core temperature falls in Parkinson's disease but not in Multiple-System Atrophy. *Mov Disord* 2001;16:226–232. [PubMed: 11295774]
- Pollak CP, Wagner DR. Core body temperature in narcoleptic and normal subjects living in temporal isolation. *Pharmacol Biochem Behav* 1994;47:65–71. [PubMed: 8115430]
- Refinetti R, Menaker M. The circadian rhythm of body temperature. *Physiol Behav* 1992;51:613–637. [PubMed: 1523238]
- Sakurai T. Roles of orexin/hypocretin in regulation of sleep/wakefulness and energy homeostasis. *Sleep Med Rev* 2005;9:231–241. [PubMed: 15961331]
- Samson WK, Gosnell B, Chang JK, Resch ZT, Murphy TC. Cardiovascular regulatory actions of the hypocretins in brain. *Brain Res* 1999;831:248–253. [PubMed: 10412003]
- Scammell TE, Saper CB. Orexin, drugs and motivated behaviors. *Nat Neurosci* 2005;8:1286–1288. [PubMed: 16189534]
- Shirasaka T, Nakazato M, Matsukura S, Takasaki M, Kannan H. Sympathetic and cardiovascular actions of orexins in conscious rats. *Am J Physiol* 1999;277:R1780–R1785. [PubMed: 10600926]
- Szymusiak R, Alam N, Steininger TL, McGinty D. Sleep-waking discharge patterns of ventrolateral preoptic/anterior hypothalamic neurons in rats. *Brain Res* 1998;803:178–188. [PubMed: 9729371]
- van den Pol AN. Hypothalamic hypocretin (orexin): robust innervation of the spinal cord. *J Neurosci* 1999;19:3171–3182. [PubMed: 10191330]
- Wang J, Osaka T, Inoue S. Orexin-A-sensitive site for energy expenditure localized in the arcuate nucleus of the hypothalamus. *Brain Res* 2003;971:128–134. [PubMed: 12691845]
- Yoshida K, Konishi M, Nagashima K, Saper CB, Kanosue K. Fos activation in hypothalamic neurons during cold or warm exposure: projections to periaqueductal gray matter. *Neuroscience* 2005;133:1039–1046. [PubMed: 15927405]
- Yoshimichi G, Yoshimatsu H, Masaki T, Sakata T. Orexin-A regulates body temperature in coordination with arousal status. *Exp Biol Med* 2001;226:468–476.
- Zepelin H, McDonald CS. Age differences in autonomic variables during sleep. *J Gerontol* 1987;42:142–146. [PubMed: 3819337]
- Zhang YH, Hosono T, Yanase-Fujiwara M, Chen XM, Kanosue K. Effect of midbrain stimulations on thermoregulatory vasomotor responses in rats. *J Physiol* 1997;503:177–186. [PubMed: 9288685]

Figure 1.

Orexin KO mice have slightly higher Tb despite lower LMA. The average hourly Tb (top panel) of orexin KO mice is similar to that of WT mice, except it is slightly higher late in the dark and light periods. Orexin KO mice have less LMA (middle panel) than WT mice especially during the dark period, but the diurnal pattern is still apparent. Orexin KO mice have normal hourly amounts of wakefulness (bottom panel). The data are presented as hourly means $\pm$ S.E.M of 7 orexin KO mice and 8 WT littermates. Grey shading represents the dark period. * $p < 0.05$; ** $p < 0.01$ compared with WT mice.

Figure 2.

Orexin KO mice have less variable Tb and very fragmented sleep-wake behavior compared to WT mice. Upper panels: When viewed in 5 min bins, a typical WT mouse has a long episode of wakefulness at the beginning of the dark period followed by many episodes of wakefulness in an ultradian pattern. Large variations in Tb and LMA parallel these wake episodes. Lower panels: A typical orexin KO mouse has very short wake episodes at all times, and Tb is less variable than in WT mice. LMA is low even during the dark period.

Figure 3.

The ultradian rhythms of Tb, LMA, and wakefulness are reduced in orexin KO mice. Cosine fitting analysis demonstrates that WT mice have clear ultradian rhythms with peak periods of 1.5 to 2 hours during the light period. Orexin KO mice have much smaller rhythms that do not differ statistically from chronologically randomized data. * $p < 0.05$; ** $p < 0.01$ compared to the randomized data.

Figure 4.

During spontaneous sleep, the Tb of orexin KO mice decreases less than normal. During the dark (left panels) and light (right panels) periods, Tb drops substantially during sleep in WT mice, but the decrease is much smaller in orexin KO mice. Before they enter a sleep episode, orexin KO mice are less consistently awake and have less LMA. The data series for orexin KO mice is shorter during the dark period because they rarely sleep for longer than 20 min during this interval. * $p < 0.05$; ** $p < 0.01$.

Figure 5.

The Tb of orexin KO mice is elevated even at the end of a sleep episode. After 15 min of wakefulness, the Tb of orexin KO mice rises to a normal level during the dark period. In the light period, the Tb of orexin KO does not rise to the level of WT mice. Orexin KO mice are less consistently awake during the dark period and have less LMA during the dark and light periods. The data series for orexin KO mice are slightly short because they do not maintain long episodes of sleep or wake. * $p < 0.05$; ** $p < 0.01$.

Figure 6.

The Tb of orexin KO mice is high even during intense recovery sleep. After 8 hr of total sleep deprivation (SD), both WT and orexin KO mice have long, consolidated episodes of sleep. Tb initially falls rapidly in both groups, but orexin KO mice then stabilize at a higher Tb. Bottom panels: During the first 20 min of the recovery period (a), orexin KO mice have more awakenings lasting 40–150 seconds than WT mice, but later (b), both groups have a similar pattern of awakenings. * $p < 0.05$; ** $p < 0.01$.