Food for Thought: Understanding the Multifaceted Nature of Orexins

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Orexins are a pair of hypothalamic neuropeptides that were discovered in the late 1990s and named initially for their ability to promote feeding. Subsequent studies have revealed the importance of orexins to a variety of physiological functions, including brown fat thermogenesis, sleep/wake cycles, physical activity, and cognition. We aim to elucidate the various roles of orexins and discuss how these multiple functions are interlinked. We explain that although the unique dual roles of orexins in increasing feeding while concomitantly elevating energy expenditure appear counterproductive, they are necessary for physiological scenarios during which simultaneous stimulation of energy expenditure and feeding occur, namely diet-induced thermogenesis and arousal from hibernation. The position of orexins at the interface between sleep/wake cycles, energy homeostasis, and environmental factors has important implications in the treatment of obesity. (***Endocrinology*** **154: 3990–3999, 2013)**

Orexin A and orexin B (also referred to as hypocretin 1 and 2, respectively) are a pair of hypothalamic neuropeptides that were discovered in the late 1990s and named initially for their ability to promote feeding (1, 2). Later studies elucidated further roles for orexins in the regulation of sleep/wake cycles (3, 4) and autonomic function, such as the regulation of blood pressure and heart rate (5). A wealth of evidence has also identified roles for orexin signaling in the mediation of autonomic control of various neuroendocrine functions involving the hypothalamic-pituitary-adrenal axis (6) and the GNHR/somatostatin-GH axis (7). These results indicate that orexin functions both centrally and peripherally through receptors located in adrenal glands (8). Of the diverse physiological functions of orexins, their effects on sleep/wake regulation have been most extensively researched. The importance of orexins to the maintenance of normal sleep/wake patterns is exemplified by the fact that the sleep disorder narcolepsy has been attributed to orexin deficiency in both animals and humans (9–11). Evidence suggests that orexin effects on feeding and sleep/wake cycles are under circadian control and influenced by photoperiod length (12–14). Further roles for orexins in behavioral traits and emotions have been described, including influences on the reward system (15), drug addiction (16), emotion (17), and alertness (18). Orexin influences over these psychological behavioral characteristics are believed to be important for maintaining vigilance during the active (awake) cycle (19). More recently, we and others have uncovered a novel role for orexins in brown adipose tissue (BAT) developmental differentiation (20, 21) and thermogenesis (22–24). BAT thermogenesis is a process by which eutherian mammals maintain a core body temperature around 37°C by producing heat in the face of a fall in ambient temperature. This process is also important during hibernation, especially to generate heat in the acute rewarming that is essential for arousal from hibernation (25, 26). The capacity for BAT thermogenesis to dissipate energy in the form of heat and its implication to obesity resistance has earned much attention since the discovery of inducible BAT in humans. Recent findings have established that BAT function (and even white adipose tissue [WAT] browning) are influenced by ultradian and seasonal triggers (27, 28) and inextricably linked to feeding

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Abbreviations: BAT, brown adipose tissue; BMPR1a, bone morphogenic protein receptor 1a; DR, dorsal raphe; LC, locus coeruleus; NPY, neuropeptide-Y; REM, rapid eye movement; SCN, suprachiasmatic nucleus; TMN, tuberomammilary nucleus; UCP1, uncoupling protein 1; WAT, white adipose tissue.

cues (29). Given orexin involvement in feeding, sleep/wake cycles, arousal, and BAT function, we propose here that the seemingly numerous and divergent actions of orexins are designed to integrate these aforementioned energetic and environmental cues to produce appropriate behavioral and physiological outcomes that are beneficial to survival during arousal from hibernation.

Orexin and Feeding

Orexin neurons were discovered in the lateral hypothalamus, which alluded to the functional actions of orexins on feeding, given the notable presence of various neuropeptides involved in appetite regulation in this location (1, 30). Studies have shown that central orexin injections induce feeding in rats and mice (1, 2). Conversely, central administration of orexin receptor-1-selective antagonist greatly reduced food intake in rats (31), whereas orexin-knockout and orexin neuronal-ablated mice exhibit reduced feeding behavior (32–35). Other studies suggest that orexin neurons are sensitive to energy status cues, such as glucose levels (31). Increased glucose concentrations inhibit orexin neuronal firing, reducing feeding (36), whereas decreased glucose concentrations increase firing rate of orexin-producing neurons, which promote feeding (37). This suggests that orexin-stimulated feeding can be triggered by a negative feedback mechanism signaling the need for energy consumption. Leptin, a satiety-promoting hormone, which acts centrally and peripherally, has also been shown to inhibit orexin receptor expression in the rat hypothalamus (38) as well as orexin neuronal firing rate (36, 37). Leptin is not the only appetite regulating peptide that influences orexin function. Neuropeptide-Y (NPY), melanin concentrating hormone, ghrelin, galanin, and agouti-related peptide have all been shown to modulate orexin actions on feeding behavior, suggesting that orexin functions to coordinate various elements of the appetite-regulatory system in rodents (39–41). Moreover, orexin and NPY, the most potent appetite-stimulating agent in mammals, have been demonstrated to have interdependent functions (42), through neural connections within the hypothalamus. Orexin neurons within the lateral hypothalamus have been shown to synapse with NPY neurons in the arcuate nucleus of the hypothalamus (43), whereas NPY neurons, in turn, project to, and synapse with, orexin-producing neurons in the lateral hypothalamus (43). Interestingly, the affects of orexins on NYP-induced feeding promotion are under circadian control and are greatly augmented during the active (awake) cycle (44), with only a minor effect on appetite-stimulation during the light (rest) cycle (44). Another study has shown that orexin-mediated feeding activation is greatly dependent on circadian timing (45). Given that orexin neurons are necessary for circadian control of rapid eye movement (REM) sleep (46), as well as transition between REM sleep and non-REM sleep (46), the circadian influence over the feeding functions of orexin are perhaps unsurprising, particularly in the light of strong evidence corroborating the notion that feeding behavior is governed by night/day sensory cues as well as circadian timing involving endogenous body clocks (44, 45).

Orexin on Sleep/Wake Cycles

Orexin is integral to normal sleep wake cycles to the extent that orexin neuronal loss is responsible for animal and human narcolepsy (9, 10, 47). Approximately 90% of narcoleptic patients have reduced cerebrospinal fluid levels of orexin (48). Narcolepsy is characterized by the inability to maintain vigilance during the day and demonstration of disturbed nocturnal sleep (49, 50). This often results in the insurmountable urge to sleep and an overwhelming feeling of drowsiness. Narcoleptic patients also suffer from a condition called cataplexy, in which postural muscle tone is lost, resulting in slurred speech and inability to control muscle movements (51, 52). Mouse models of narcolepsy revealed the integral nature of orexins to the consolidation of regular sleep/wake cycles (53–55). Orexin neurons originate primarily in the lateral hypothalamus and project to numerous brain regions, including the paraventricular nucleus, arcuate nucleus, locus coeruleus (LC), dorsal raphe (DR), and tuberomammilary nucleus (TMN) (56–58). In vitro studies have confirmed that these regions abundantly express orexin receptors and are important effector sites of orexins. The activity of monoaminergic neurons in the TMN, LC, and DR that secrete histamine, noradrenaline, and serotonin, respectively, is known to function in orexin-induced stimulation of wakefulness and arousal (59–61). Thus, orexin neurons are activated during wakefulness and in turn exert an excitatory influence over these wake-active neurons, maintaining their stimulation and promoting a sustained level of arousal (46). Studies have also shown that orexin neurons exert a direct chronic excitatory influence as well as indirect inhibitory influences via gamma-aminobutyric acid (GABA)-producing neurons, over cholinergic neurons in the laterodorsal tegmental nucleus and substantia nigra pars reticulate, respectively (62, 63). These findings indicate a role for both excitatory and inhibitory effects of hypothalamic orexin neurons projecting to various brain regions to illicit effects on sleep/wake regulation. As mentioned previously, orexin neuronal firing is highly gov-

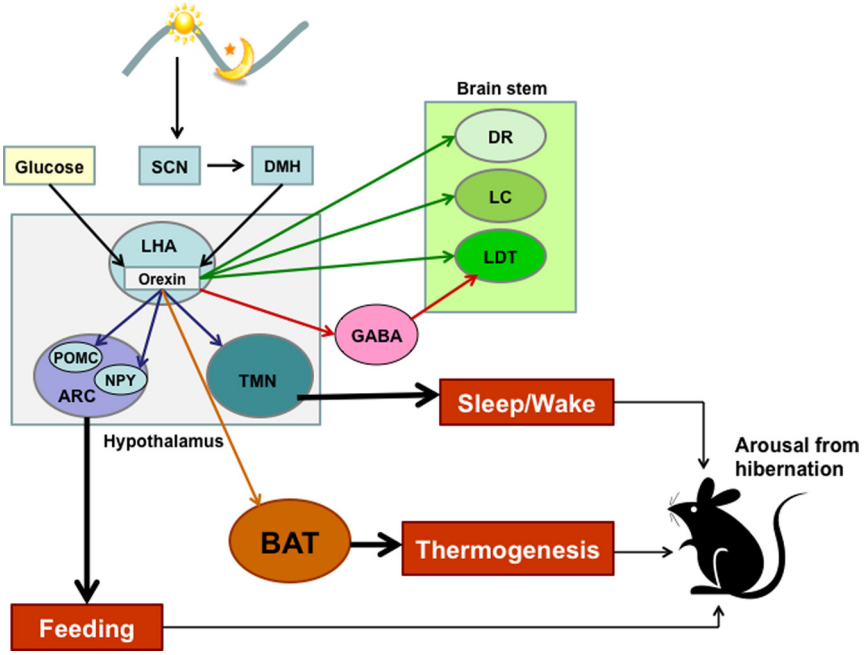

Figure 1. Circadian and photoperiodic (via SCN and dorsomedial hypothalamus [DMH]), as well as energy status, cues (glucose sensing) are received by orexin neurons. Orexin neurons, originating in the lateral hypothalamus (LHA), project to various brain regions, including the arcuate nucleus (ARC), to regulate appetite, as well as the locus ceruleus (LC), dorsal raphe (DR), and tuberomammillary nucleus (TMN) and laterodorsal tegmental nucleus (LDT), to regulate sleep/wake cycles. Orexin neurons also project to BAT to induce thermogenesis. The coordination of sleep/wake regulation, feeding, and arousal in relation to external environmental cues by orexin may be crucial for enabling arousal from hibernation.

erned by light/dark cycle, with highest frequency of neuronal activity in the dark (active) cycle of rodents (64). Furthermore, studies employing dark-pulse activation studies have revealed the necessity of orexin neurons for daily resetting of the internal circadian clock as a means to synchronize sleep/wake states with night/dark cycles, actions which involve both hypothalamic and suprachiasmatic nuclei (SCN) elements (65). Consistent with these observations is the finding that orexin levels in the cerebrospinal fluid peak during the dark period and decrease during the light period in rodents. Thus, orexin may be a key player in the consolidation of sleep/wake cycles with external environmental cues. Interestingly, in addition to disturbed circadian control of sleep/wake patterns, narcoleptic patients exhibit reduced appetite and consume considerably less calories per day than normal individuals (66, 67). The ability of orexins to govern both appetite and sleep/wake regulation possibly has an important physiological function. For example, during periods of food scarcity, rodents respond by maintaining a longer awake period, thereby disrupting the normal circadian pattern of sleep/wake cycle regulation (68, 69). This adaptive response to reduced food availability in rodents is absent in orexin neuron-ablated mice (36). Therefore, increased orexin neuronal activity may promote wakefulness and vigilance in rodents, an adaptive response aimed at increasing foraging and alertness in an attempt to support food seeking (70–72). To this end, orexin neurons also influence food anticipatory behavior (73). In fact, daily food restriction entrains an adaptive anticipatory locomotor activity rhythm and entrains a molecular oscillator that is distinct from the central clock located in the SCN (46). Thus, peaks in orexin neuronal activity shift from night to the period during which food is restricted, indicating that orexin neurons are activated to promote food seeking. Moreover, orexin neuron-ablated mice show reduced expression of genes integral to the regulation of the food-entrainable oscillator, such as brain and muscle aryl hydrocarbon receptor nuclear translocator (arnt)-like protein 1 and neuronal Per-Arnt-Sim (PAS) domain protein 2 (46). The dorsomedial hypothalamic nucleus, which has been implicated as a site for food-entrainable oscillation (74), has neurons that project to orexin neurons, indicating that orexin may mediate circadian control of feeding behavior (75). Thus, by influencing both sleep/wake regulation and feeding in a manner closely associated with circadian and endogenous body clock cues, orexin actions are crucial for orchestrating advantageous physiological responses that greatly improve chances of survival. Paradoxically, although orexin promotes feeding, orexin-knockout mice display obesity, despite a considerable hypophagia (53, 76). The mechanistic basis for the obesity in narcoleptic individuals is not well understood. Orexin has been shown to induce energy expenditure by promoting physical activity (77), whereas recent observations have shown that it also increases energy expenditure by influencing BAT thermogenesis. The lack of both these components of energy expenditure is likely to contribute to the obesity associated with narcolepsy.

Orexin and BAT Function

BAT is specialized for energy expenditure. Although WAT contains a single large lipid droplet, few mitochondria and a limited vascular network, BAT contains multiple lipid droplets, numerous mitochondria, and a dense capillary

network (78–83). BAT mitochondria uniquely express uncoupling protein 1 (UCP1), an inner mitochondrial membrane protein that uncouples ATP synthesis from oxidative phosphorylation, liberating energy in the form of heat (83). Thermogenesis is activated by central stimulation of sympathetic nervous system neurons, which innervate BAT and secrete noradrenalin. Noradrenalin-induced activation of β-3 adrenergic receptors on BAT leads to a signaling cascade resulting in lipolysis. The consequent release of free fatty acids activate UCP1-driven mitochondrial uncoupling (25). β-3 adrenergic receptor stimulation also induces UCP1 mRNA and protein upregulation (25). The efferent cues for activation of sympathetic nervous system neurons include changes in ambient temperature and diet (25). This thermogenic capability is beneficial to small rodents, because it allows for the maintenance of body temperature during exposure to cold as well as increasing body temperature during arousal from hibernation (25). Rodents also use the energy wastage properties of BAT to resist weight gain when exposed to high-fat diet or increased caloric load, a process known as diet-induced thermogenesis (84–86). Diet-induced thermogenesis involves the increase in gene and protein expression for UCP1 as well as the recruitment of brown adipocytes, which increase both basal and β-3 adrenergic receptor-stimulated energy expenditure (85, 86). In light of its potential to combat obesity in the presence of high-fat feeding, much attention has been afforded to BAT since the much publicized emergence of functional BAT in adult humans (87, 88). Previously, BAT was long thought to be evident only in rodents, deteriorating in amount and activity with age (89). The contribution of BAT to whole-body energy expenditure in humans is substantial. It has been estimated that maximally stimulated BAT can account for almost one quarter of daily metabolic activity (90), a prospect that has triggered an abundance of studies documenting therapeutic advances in the combat of obesity through BAT pharmacological manipulation (91–93). Our own studies have identified orexin as an integral component of the brown fat-regulatory circuit (20, 21). The first clues that brown fact thermogenesis may be compromised came through studies in the orexin-knockout model. When exposed to acute cold, orexin-knockout mice exhibited hypersensitivity to cold. About 25% of orexin-knockout mice die within the first 6–8 hours of cold exposure due to hypothermia, and those who survive exhibit poor adaptability to cold (21), a phenotype that is also shared by orexin receptor 1-deficient mice. Measurements of metabolic rates in chow and high-fat diet conditions provided direct support for thermogenic dysfunction in orexin-deficient mice. Rodents with intact orexin signaling elevate their metabolism by 14% relative to chow-fed control cohorts, whereas animals lacking orexin fail to do so, suggesting an impairment in diet-induced thermogenesis. Histological and transcriptional analysis revealed that BAT of orexin-knockout mice exhibited a developmental differentiation defect. This contention is based on several observations. Firstly, brown adipocytes in orexin-knockout mice expressed high levels of preadipocyte markers. Secondly, these brown adipocytes had fewer mitochondria. Thirdly, proteins that drive BAT thermogenic function, such as peroxisome proliferator-activated receptor gamma 1/2 (PPAR-γ1/2), peroxisome proliferator-activated receptor gamma coactivator 1-alpha/beta (PGC-1α/β), and UCP1, were poorly expressed. Finally, orexin was able to potently induce brown adipcoyte differentiation in C3H10T1/2 mesenchymal stem cells, HIB1b brown preadipocytes, and primary brown preadipocytes isolated from C57BL6 wild-type mice (20). Together, these observations suggest that orexin is an extracellular regulator of brown fat thermogenic function and that failure of thermogenic mechanism induces obesity in orexin-knockout mice.

Our results showed that orexin receptor-1 signaling was integral to the effects of orexin on brown adipocyte recruitment through a G-protein coupled-receptor (GPCR)-mediated signaling cascade (94–98). Knockdown of orexin receptor 1 in C3H10T1/2 and HIB1b cells lines attenuates orexin-induced brown adipocyte differentiation (20). These studies indicate that at least a part of orexin actions of BAT function occurs independently of neural connections. In this regard, orexins are similar to bone morphogenic protein-8b (BMP8B) which has been found to elicit central as well as peripheral affects on BAT via bone morphogenic protein receptor 1a (BMPR1a) signaling (99). Interestingly, orexin-induced brown fat differentiation involved BMPR1a receptor signaling through mothers against decapentaplegic (SMAD) and P38 MAPK phosphorylation, a well-established pathway for adipogenesis. Orexin actions on brown adipocyte differentiation were disrupted by antagonism of BMPR1a signaling. Thus, cross talk between the BMP and orexin pathways may be important for peripheral activation of BAT function. A recent study has identified an important role for central orexin in sympathetic activation of BAT thermogenesis (24), through a direct orexigenic projection from the lateral hypothalamus to the raphe palidus (24). Studies have also shown that orexin activation of BAT thermogenesis is potentiated by the central injection of the antipsychotic drug risperidone (100). Risperidone displays molecular antagonism of both serotonin and dopaminergic receptors, suggesting that monoaminergic neurons might be involved in orexin control of BAT thermogenesis in addition to orexin control of feeding, as mentioned previously. It is noteworthy, given the known relevance of serotonin to stress (101), that

orexin has been shown to be essential for stress-induced thermogenesis in mice (102). Orexin control of adipose tissue function appears to involve both central and peripheral actions and use various brain regions in an effort to regulate energy expenditure according to multiple efferent cues (103–105). It remains to be elucidated whether enhanced orexin signaling plays a role in the recruitment of brown adipocytes in WAT, a phenomenon termed "browning." However, on account of orexins aforementioned functional modulation by circadian events as well as its recently discovered regulation by photoperiod (13, 106), it is noteworthy that BAT function and indeed WAT browning have been shown to be under circadian (107–109) as well as photoperiodic control (28, 110).

Orexins and Hibernation

Orexins induce both feeding and energy expenditure. They are the only neuropeptides known to have these seemingly paradoxical actions. Generally, endogenous agents that affect both appetite and energy expenditure ensure the sustenance of an inverse relationship between the two (111). For example, NYP, a potent appetite stimulator, increases appetite while reducing energy expenditure (112). On the other hand, proopiomelanocortin (POMC) represses appetite while concomitantly elevating energy expenditure (113). On the face of it, functional coordination of alternating cyclical episodes of elevated feeding and increased energy expenditure appears to be energetically logical, because it would be counterproductive to mobilize fuel for energy and consume energy at the same time. Thus, coordinated energy balance regulation through neuropetides with differing effects on energy intake and expenditure seems to have evolved as a means to prevent costly energy wastage (114, 115). There are physiologically beneficial natural scenarios, however, where increased energy expenditure and increased food intake occur simultaneously, and this is where orexins seemingly paradoxical actions on appetite and energy expenditure may be illuminated. Aside from diet-induced thermogenesis, where elevated energy expenditure is a response to increased calorie and fat consumption, the two also occur simultaneously during arousal from hibernation (116). Hibernation is a period during which mammals living in seasonal climates enter a sustained energy saving mode of inactivity characterized by lower metabolism and reduced body temperature (116, 117). In order to maintain temperature during hibernation, in which feeding is impossible, mammals have to rely upon BAT thermogenesis (118), which is triggered by both cold and shorter photoperiods, to prepare for hibernation. A recent study has shown that although humans do not hibernate, they have maintained the same environmental cues for BAT activation. In addition to cold, shorter photoperiods lead to enhanced BAT thermogenesis in humans (119). During hibernation in rodents, BAT increases in size and raises its thermogenic and lipolytic activity (26, 120, 121). BAT thermogenesis is also critical for arousal from hibernation, where body temperature rises rapidly to terminate hibernation (122–124). The importance of brown adipose thermogenesis to arousal is exemplified in a UCP1-deficient mouse model, in which animals fail to enter and exit coordinated periods of temporary hibernation known as torpor, and arousal. This lack of BAT thermogenic capability results in animals using some 60% extra calories to accommodate sufficient arousal from hibernation compared with animals with efficient BAT function (125). In animals who potentially survive months without feeding, relying heavily upon stored reserves that were accumulated in the previous summer, this could mean death. A recent study suggests that orexin directly mediates arousal from hibernation through stimulating BAT thermogenesis (126). Orexin antagonist inhibited TRH-increased core body temperature and subsequent arousal from hibernation in Syrian hamsters. The precise mechanisms involved in orexin mediation of arousal from hibernation remain to be fully explored, and it is uncertain whether known pathways involved in orexin mediation of arousal from sleep are also important for orexin-induced arousal from hibernation. Certainly, the elevated core body temperature that accompanies orexin-induced arousal from sleep has been shown to involve the increased firing rate of noradrenergic neurons (127), possibly involving the TRH system (128–130). Enhanced responsiveness of BAT to adrenergic stimulation has also been documented to be pivotal to BAT-driven arousal from hibernation (131), and it remains to be elucidated whether peripheral orexin actions are involved in this process. The study in question investigated BAT responsiveness to adrenergic stimulation during arousal in the Syrian hamster (131). But given that exposure to shorter days stimulates BAT thermogenesis in preparation for cooler winter months in the Syrian hamster, it is unlikely that exposure to longer days, during arousal in the spring, is responsible for the same induction of BAT activity. Melatonin, the release of which is positively correlated with exposure to shorter photoperiods, has been shown to increase BAT thermogenesis in winter months (120). However, other factors must be responsible for the increased BAT activity during arousal from hibernation during spring months where rodents are exposed to longer photoperiods. We propose that it is orexin that is responsible for the mediation of arousal from hibernation in rodents. In a recent study, Tupone et al (24) showed that

central orexin injection increases BAT thermogenesis but only when the animal is cold initiated. In other words, orexin per se is insufficient to induce BAT thermogenesis but rather contributes to the potentiation of cold-induced BAT activation. Although these findings appear to be at odds with the aforementioned study detailing the role of orexin in arousal from hibernation in the Syrian hamster (126), it is plausible that the hibernating hamster, with a lower body temperature that is a key characteristic of hibernation in rodents (116), represents an animal with an initial activation of BAT (similar to the cold-initiated rat in the above mentioned study). Certainly, hibernating animals have increased sympathetic outflow to BAT (25). One important point in the study of orexin-induced BAT activation from the context of hibernation and arousal from hibernation is that not all rodents hibernate. Although most studies that have addressed the role of orexin in BAT function and sleep/wake regulation have employed the use of mice and rats, these are not the ideal model to study mechanisms that are governed by circadian and photoperiodic events. Studies in the Djungarian hamster, which does not hibernate but enters a more temporary state of reduced metabolism and body temperature known as torpor, have indicated that orexin may not be involve in rewarming from torpor, although the study in question analyzed orexin mRNA expression and did not investigate orexin neuronal activity (132). Therefore, future studies using animal models where hibernation occurs, such as the Syrian and Siberian hamsters, would be most relevant in investigating the role of orexin in BAT-induced arousal from hibernation.

Integration of Diverse Functions During Arousal

Orexin influences over other factors besides thermogenesis are also critical to arousal from sleep and hibernation in rodents. Feeding is of paramount importance in the immediate period after arousal (133). For successful foraging to occur, physical activity must be increased, appetite should be stimulated, and cognitive function and alertness must be maintained (116). Orexins play a critical role in each of these individual functions and therefore are in a uniquely situated to integrate and consolidate these various physiological actions to sustain arousal. Orexins affect appetite, physical activity, and BAT as outlined above but also improve cognitive function, fear conditioning, and foraging success (134, 135), activities that are vital to enabling survival during and immediately after arousal from hibernation (116). Moreover, impairment of orexin signaling with age, driven by reduced orexin neurons and lowered circulating orexin levels (136), has been shown to be responsible for the cognitive decline that accompanies neurodegenerative diseases, such as Alzheimer's disease (136, 137), which also raises the intriguing question of whether orexins are involved in the well-documented decline of BAT with age (89). Certainly, aging in humans is associated with impaired thermoregulation (138), coupled with circadian desynchrony (139), and impaired energy homeostasis (140).

It remains to be determined whether rodent BAT studies are relevant to human metabolism. Although BAT has been found in humans, it is localized in regions that are distinct from rodents (87, 88). Moreover, a recent study has shown clear differences in gene expression profile of human BAT compared with that of rodents (141). The direct and precise determination of the contribution of BAT thermogenesis to total daily energy expenditure in humans remains elusive, and the developmental origins of human BAT are even more obscure. Another concern with current information regarding BAT activity in humans is that current methods rely on the use of positron emission tomography-computer tomography (PET-CT) technology that have proved unreliable and with various shortcomings compared with BAT analysis in rodents (142). Nevertheless, the sizeable attention given to BAT studies in both rodents and humans is thoroughly justified based solely on its therapeutic promise.

In conclusion, behavioral and physiological arousal from hibernation involves the integration of various individually reported functions of orexins. Orexins stimulate feeding as well as BAT thermogenesis while at the same time triggering an increased physical activity and cognitive function consistent with the support of alertness, exploratory, and feed-seeking behavior. The integration of these often theoretically contradictory functions is not well understood. However, it is plausible that orexin, a multifaceted neuropeptide with physiologically diverse actions, is at the heart of this coordination (Figure 1). Orexins have the unique capability to increase energy expenditure and feeding simultaneously in response to environmental and energy status cues. Although we have attempted to shed light upon the "raison d'être" behind this apparent paradox, there can be no enigma as to the potential therapeutic impact of this concept given that drugs aimed at curtailing the appetite drive to combat obesity have failed in the long term (143) and that inability to refrain from hyperphagia and hypercaloric feeding is the major barrier to staving off weight gain and obesity in individuals with a predisposition to obesity (144). Our discussions also bring to the fore the important concept of timing (night vs day) and seasonal (winter vs summer) considerations in studies assessing therapeutic interventions to treat obesity.

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