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Sleep and metabolism: Role of hypothalamic neuronal circuitry

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Sleep and metabolism are intertwined physiologically and behaviorally, but the neural systems underlying their coordination are still poorly understood. The hypothalamus is likely to play a major role in the regulation sleep, metabolism, and their interaction. And increasing evidence suggests that hypocretin cells in the lateral hypothalamus may provide particularly important contributions. Here we review: 1) direct interactions between biological arousal and metabolic systems in the hypothalamus, and 2) indirect interactions between these two systems mediated by stress or reward, emphasizing the role of hypocretins. An increased understanding of the mechanisms underlying these interactions may provide novel approaches for the treatment of patients with sleep disorders and obesity, as well as suggest new therapeutic strategies for symptoms of aging, stress, or addiction.

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Introduction

Biological systems maintain stability despite dramatic environmental changes. This physiological stability, called homeostasis, is achieved through intricately interdependent biochemical and behavioral mechanisms that can be tailored for specific types of environmental changes. One of the most striking examples of these specific homeostatic responses is the combined propensity for animals to have increased wakefulness, decreased metabolism, and increased food-seeking behavior when food is scarce. Given that strong coordination is required to successfully mediate this wide range of behaviors, it is not surprising that the neural systems monitoring and regulating them overlap and share at least one critical player: the hypothalamus.

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The hypothalamus regulates homeostasis by integrating peripheral and central signals of circadian rhythms, sleep pressure, blood pressure, adiposity, caloric intake, body temperature, and water balance to affect arousal, metabolic rate and appetite. Understanding the hypothalamus's contribution to the dialogue between arousal and metabolic pathways has crucial and wide-ranging clinical significance. For example, minimizing sleep disturbances may reduce the rate of obesity and heart attack. Likewise, obesity is one of the main risk factors in the development of sleep apnea and other sleep disorders. The hypothalamus coordinates the mechanisms underlying both of these clinical observations.

Here we will review two types of mechanisms by which the hypothalamus regulates sleep and metabolism: 1) direct mechanisms and 2) indirect mechanisms. Direct mechanisms utilize direct neural connections between arousal and metabolic pathways. Indirect mechanisms utilize the modulation of stress or the reward systems to affect arousal and metabolism. First we will review how the hypothalamus regulates arousal and metabolism independently, and then we will review how the hypothalamus contributes to the integration of these pathways in the face of homeostatic change.

Hypothalamic control of arousal and metabolism

Hypothalamic regulation of sleep

The hypothalamus is subdivided into many anatomically distinct nuclei including the arcuate nucleus (ARC), paraventricular nucleus (PVN), ventromedial nucleus (VMN), dorsomedial nucleus (DMN) and lateral hypothalamic area (LHA). These nuclei send projections throughout the brain, including a dense network of intrahypothalamic connections.

Transitions between different vigilance states are thought to occur via mutual inhibition of wake- or sleep-promoting areas in brainstem and hypothalamic structures.[1] In the most simplistic model, wake occurs through the activation of wake-promoting neurons, which, in turn, inhibits sleep-promoting circuits encompassing GABAergic and glycinergic cells in the ventrolateral preoptic (VLPO) area of the anterior hypothalamus as well as GABAergic cells in the median preoptic area.[2–4] These subcortically-mediated transitions are then followed by low frequency oscillations in the neocortex, which contribute to the distinctive scalp EEG patterns characteristic of sleep.

There are three major groups of wake-promoting neurons that promote arousal and contribute to sleep architecture: peptide-secreting neurons in the LHA of the hypothalamus, cholinergic neurons in the pedunculopontine and laterodorsal tegmental nuclei of the brainstem, and monoaminergic neurons in a variety of nuclei (primarily) in the brainstem. The wake-promoting peptidergic neurons in the LHA are Hypocretin (Hcrt)-expressing cells, which are spatially intermingled with sleep-promoting melanin-concentrating-hormone (MCH)-expressing cells. Hcrt-1 and -2 are neuro-excitatory peptides produced exclusively by a discrete group of neurons in the LHA[5] that use glutamate as their main transmitter.[6] MCH, on the other hand, is produced by a larger group of mostly GABAergic neurons in the LHA and Zona Incerta (ZI).[7] Activation of Hcrt neurons has excitatory post-synaptic effects, whereas MCH neurons are thought to have inhibitory post-synaptic effects.[8,9] Both cell types send parallel projections to the cortex, hippocampus, amygdala, nucleus accumbens (NAc), hypothalamus, thalamus, ventral tegmental area (VTA), locus coeruleus (LC) and raphe nucleus.[8,10] Recent studies have demonstrated that highly selective optogenetic stimulation of Hcrt cells decreases latency to arousal[11] and these effects are inhibited by increasing levels of sleep pressure.[12]

Groups of wake-promoting *cholinergic* neurons are located in the pedunculopontine and laterodorsal tegmental nuclei of the brainstem. Neurons in these groups have strong excitatory projections to the thalamus, but also project to the basal forebrain, the prefrontal cortex, and the LHA. The activity of these neurons is thought to play a crucial role in the onset of REM sleep, which shares many physiological features of wakefulness.[1,3]

The groups of wake-promoting *monoaminergic* neurons are found in the LC, dorsal and median raphe nuclei (DR), periaqueducal gray matter (vPAG) and in the tuberomammillary nucleus (TMN). Neurons in the LC are *noradrenergic* and they demonstrate sustained and highly regular tonic-firing patterns that are highest during wakefulness, decrease during slow-wave sleep (SWS), and virtually cease during rapid eye movement (REM) sleep.[3] Similar activity patterns are evident in *serotoninergic* neurons of the DR[13,14], *dopaminergic* neurons of the vPAG[15], and *histaminergic* neurons of the TMN in

the caudal hypothalamus.[15,16] Axons from all of these monoaminergic cell groups target the basal forebrain, cerebral cortex, prefrontal cortex, thalamus and the LHA. During wake states, Hcrt neurons excite monoaminergic neurons, which then send inhibitory feedback signals to Hcrt neurons.[1,3]

We will focus on the hypothalamic contributions to wakefulness in this review, especially the Hcrt neurons. Hcrt neurons have a significant role in metabolism as well as arousal, which makes them strong candidates for brain regions likely or able to coordinate arousal and metabolism under homeostatic pressure.

Hypothalamic regulation of metabolism

The critical role of the hypothalamus in maintaining food intake and energy homeostasis has been investigated intensively. Early work in the 1950s motivated an initial hypothesis that the VMN was "the satiety center" and the LHA was "the feeding center".[17] More recently, however, this hypothesis has been replaced by a more nuanced view that satiety is controlled by a wide network of brain regions rather than just two discrete regions, and this network acts as a coordinated entity to regulate food intake and energy expenditure. Much like sleep, hypothalamic control of metabolism is comprised of mutually inhibiting networks (Fig. 1). The appetite-promoting Neuropeptide Y (NPY) and Agouti-related protein (AGRP) neurons mutually inhibit the appetite-suppressing pro-opiomelanocortin (POMC) and amphetamine-related transcript (CART) neurons. Both sets of neurons act as sensors of the circulating hormones leptin and ghrelin. Leptin is produced by adipose tissues and promotes satiety. It inhibits NPY/AGRP neurons while activating the POMC/CART neurons. In contrast, ghrelin is an appetite-stimulating hormone. It is produced in the gut and in contrast to leptin, it inhibits POMC/CART but activates NPY/AGRP neurons.[18] Anatomically, the metabolism regulating centers in the hypothalamus are restricted to the arcuate nucleus (ARC) in the medial hypothalamus while Hcrt cells are located in the lateral hypothalamus.

Interactions between arousal and metabolic pathways

Direct pathways mediating the reciprocal effects of sleep and metabolism

Beginning with the behavioral constraint that we cannot sleep and feed at the same time, there is increasing anatomical, pharmacological and pathological evidence that sleep- and metabolic-related systems are intertwined.

Direct effects of sleep on metabolism
Substantial evidence suggests that sleep disturbances affect metabolism. Broadly speaking, reductions in duration or quality of sleep have deleterious effects on glucose metabolism, down-regulate the satiety hormone, leptin, up-regulate the appetite-stimulating hormone, ghrelin, and
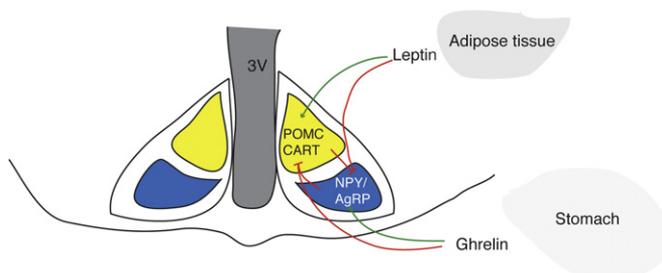

Fig. 1. Mutual inhibition in the hypothalamic regulation of metabolism. Leptin and ghrelin are secreted from adipose tissue and the stomach, respectively. Leptin activates POMC/CART cells while inhibiting NPY/AgRP cells. POMC/CART cells, themselves, also inhibit NPY/AgRP cells. Ghrelin does the opposite: it activates NPY/AgRP cells while inhibiting POMC/CART cells. The NPY/AgRP cells also inhibit the POMC/CART cells. Green lines with arrowsindicate activation and red lines with blunt endingsindicate inhibition.

increase hunger and appetite.[19,20] This is illustrated most profoundly in cross-sectional and prospective epidemiologic studies that indicate there is a strong dose-dependent effect of sleep reduction on the incidence of diabetes and obesity.[19,21]

One limitation of many human epidemiologic studies is that they do not assess sleep quality. Thus, whether short sleep in obese individuals is truly the result of behavioral sleep curtailment as opposed to reduced sleep quality (caused by sleep disorders) remains to be determined. A growing literature suggests that this distinction is important because it seems to be the quality of sleep (as measured by slow-wave sleep intensity or integrity), not just the quantity of sleep, is crucial for sleep-related processes such as memory and learning.[22] It is possible that sleep quality may be the critical factor in correlations between sleep and metabolic processes as well. Of note, sleep quality is not unrelated to sleep quantity. For example, chronic sleep loss is a state of reduced REM sleep but relatively preserved slow-wave sleep.

More specific clinical evidence that sleep is an important factor for feeding or metabolism may be derived from the fact that pathological conditions in which sleep is pathologically disturbed are consistently associated with major risk for obesity. For example, 50%–98% of people with obstructive sleep apnea (OSA), a disorder characterized by repetitive episodes of upper-airway obstruction that lead to intermittent hypoxemia and/or hypercapnia and sleep fragmentation, are morbidly obese.[19,23] Obesity is also a common symptom of narcolepsy, a sleep disorder that causes excessive sleepiness and frequent daytime sleep attacks. More specifically, Narcoleptic patients have an increased waist circumference, indicating excess fat storage in abdominal depots.[24,25] Despite lower daily food intake, patients with narcolepsy with cataplexy display significant alterations in metabolic parameters[26] and the majority of patients with narcolepsy experience symptoms of eating disorders, with an irresistible craving for food and binge eating as the most prominent features.[27]

Both OSA and narcolepsy are associated with Hcrt deficiency. In OSA, plasma levels of Hcrt-1 decrease in parallel with the severity of the disease. In narcolepsy, more than 90% of patients with narcolepsy and cataplexy lack Hcrt-1 peptide in their CSF. Postmortem studies have shown that Hcrt-secreting cells are almost undetectable in the brains of narcoleptic patients.[28,29] Comparisons with control subjects in these postmortem studies indicated that narcolepsy is more likely due to the full loss of Hcrt neurons themselves rather than just an inability to secrete the Hcrt peptide.

Given the role of Hcrt in sleep disorders and the correlation between sleep disorders and metabolism, it is reasonable to hypothesize that Hcrt might play a role in metabolism. In support of this hypothesis, large doses of icv Hcrt increase food consumption in animal models (this observation inspired their alternative name of "orexins", which means "appetite" in Greek[8,30]). Moreover, depletion of Hcrt-containing neurons in animals expressing ataxin 3 under the human Hcrt promoter results in obese and hypophagic narcoleptic mice.[31,32] Furthermore, Hcrt has been shown to have indirect influences on metabolism. In contrast to wild-type animals that have increased locomotor activity during conditions of low food availability, Hcrt-deficient animals do not demonstrate increased locomotor activity. This observation hints that some of the effects of Hcrt on metabolism might manifest themselves indirectly through effects on locomotion and exercise.

A direct role for Hcrt in metabolism is supported by the anatomical connectivity between Hcrt neurons and metabolic nuclei in the hypothalamus. Immunohistochemical studies have shown that Hcrt axon terminals contact NPY and POMC-positive neurons in the ARC of rats. Electrophysiological studies in slice preparations demonstrate that Hcrt neurons functionally regulate NPY, POMC and glucose-responsive neurons in the ARC and VMH in a manner reciprocal to leptin.[33] Whole-cell patch-clamp recordings demonstrate that Hcrt application suppresses the spontaneous firing of POMC neurons.[34] Behaviorally, REM-deprived rats have increased hypothalamic prepro-Hcrt mRNA levels and increased NPY expression[35], and eat 25–35% more food after 72 h of deprivation.[35] Thus, there is clear anatomical and functional evidence that Hcrt neurons interact directly with metabolic pathways.

It is important to clarify that although most evidence suggests that sleep disruption correlates positively with obesity, some studies have shown the opposite relationship between sleep and metabolism, with sleep deprivation correlating with increased energy expenditure.[36] Therefore it has been suggested that sleep "resets" metabolism and energy expenditure rates in the brain rather than simply regulating metabolism and energy expenditure in one direction, which gives it a particularly

effective and flexible control of energy expenditure in the face of changing environmental pressures.[36,37]

Taken together, there is strong evidence that sleep, especially the lack of sleep, impacts metabolism and food consumption, and Hcrt cells are likely to play a role in this interaction. However, there is still much to learn about the biological basis of how the coordination of sleep and metabolism occurs (Fig. 2).

Direct effects of metabolism on sleep

Just as changes in sleep can affect metabolism, metabolic changes can have profound effects on sleep architecture. Certain foods of high caloric content are believed to promote sleep, whereas others disturb sleep. In humans, a study that measured the influences of food intake on sleep found a significant correlation between late-night-snack intake and sleep patterns.[38] Similar effects have been observed in rodents. In particular, it is well-documented that when animals have access to food only during their normal sleep cycle, they adjust their sleep onset to match the food availability.[39] Recent data have proposed the dorsomedial hypothalamus is the putative anatomical site of the food-entrainable circadian pacemaker, as the expression of the circadian clock gene mPer oscillates in this region only under restricted feeding.[40]

Hypothalamic pathways, particularly those including Hcrt and MCH neurons, provide a potential link to explore the impact of metabolism on sleep. This is emphasized by the fact that the primary downstream cellular effects of NPY are on Hcrt cells.[41] NPY reduces spike frequency and hyperpolarizes the membrane potential of Hcrt neurons in mouse hypothalamic slices. These effects of NPY are mediated by multiple pre- and post-synaptic mechanisms and attenuate voltage-dependent calcium currents mainly via Y1[42] and Y2 receptor subtypes.[43,44] Inline with these observations, Hcrt-induced feeding (mentioned above) could be blocked with Y1 receptor antagonists.[42]

The direct connection between Hcrt neurons and metabolic factors is also illustrated by the fact that Hcrt neurons are sensitive to glucose, ghrelin, and leptin.[9,31] Hcrt-positive cells show leptin receptor (ob-r) immunoreactivity[45], and prepro-Hcrt mRNA expression is down regulated in ob/ob mice.[46] In agreement with these findings, rats fasted for 48 h had increased concentrations of Hcrt mRNA and increased c-fos immunoreactivity in Hcrt-expressing cells.[45]

The sleep-promoting MCH neurons are also affected by metabolic state. MCH was originally discovered in fish as a metabolism-related factor and further study revealed that MCH is involved in regulating food intake in mammals as well.[47,48] MCH expression changes in states of altered energy balance, such as fasting and obesity.[48] MCH levels increase after fasting, and activation of MCH neurons reduces energy expenditure. Chronic infusion or transgenic expression of MCH stimulates feeding and increases adiposity, whereas targeted deletion of MCH or its receptor (MCHR1) leads to decreased body
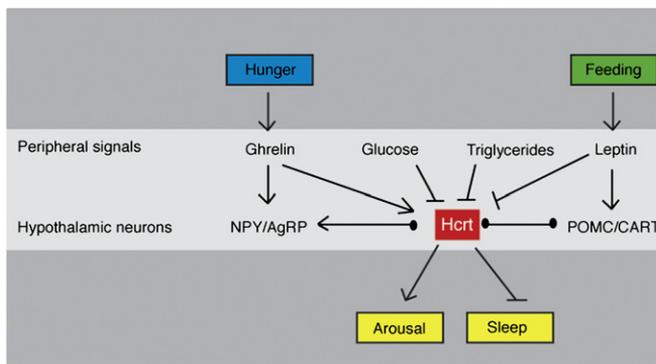

Fig. 2. Direct interactions between sleep and metabolism. This simplified diagram illustrates the major anatomical and functional interactions between hunger, feeding, and sleep. Excitatory relationships are indicated by arrow heads while inhibitory interactions are indicated by blunt endings. Circular endings indicate unspecified relationships.

weight and fat mass and resistance to diet-induced obesity.[49] Mice that over-express MCH or MCHR1 exhibit weight gain compared to control animals, and MCHR1 is a central target of leptin signaling and appears to be a mediator of insulin resistance.[50]

Further important evidence for direct connections between sleep and metabolism comes seren-dipitously from studies of aging. Normal aging is accompanied by changes in sleep quality, quantity, and architecture. Specifically, there is a measurable decrease in the ability of the healthy elderly to initiate and maintain sleep, accompanied by a decrease in their proportion of deeper, more restorative NREM and REM sleep.[51] Normal aging is also accompanied by metabolic changes. Healthy elderly have decreased total energy expenditure, reduced organism mass, and specific changes in the metabolic rates of individual tissues. Thus, ageing provides an ideal context for studying the interaction between sleep and metabolism. The hypothalamus plays a critical role in both of these age-related changes, particularly via the Hcrts and AgRP.

A detailed analysis of expression of Hcrt at different ages in rats demonstrated that the number of Hcrt neurons increases during maturation and then significantly decreases during the period of 8–24 months.[52] In mice, the age-related decrease in the number of Hcrt neurons becomes evident between 400 and 800 days, and declines at a rate of approximately 1 neuron/day.[53] Hcrt innervation also changes with age. Excitatory Hcrt innervation of the noradrenergic LC[54] and specific nuclei of the BF are compromised as organisms grow older.[55]

Aging is often accompanied by changes in food intake regulation. During the middle half of life many humans increase their body mass and develop glucose intolerance. However, later in life many suffer from being underweight, commonly known as "anorexia of elderly". Studies have shown a strong correlation between Hcrt deficiency and age-related glucose intolerance and insulin resistance.[56] Insulin signaling through Akt, a member of the protein kinase B family of intracellular signaling molecules, was disrupted in peripheral tissues of middle-aged (9-month-old), but not young adult (2–3-month-old), Hcrt knockout mice fed a normal chow diet. Basal levels of hypothalamic Akt phosphorylation are abnormally elevated in Hcrt knockout mice at every age studied, and insulin stimulation fails to increase Akt phosphorylation. Similar abnormalities are observed with respect to another signaling pathway, GSK3beta phosphoryla-tion, in the hypothalamus and peripheral tissues of middle-aged Hcrt knockout mice.[56]

At least in rodents, ageing is associated with the down-regulation of hypothalamic peptides other than hypocretin that stimulate food intake as well, while expression of anorexigenic peptides are left unchanged over the life span. The expression of NPY and of NPY receptors are highly suppressed in the hypothalamus of old rats, and the increase of NPY mRNA after fasting is severely blunted in old as compared to young rats. Similar reductions, although of lower magnitude, are reported for other orexigenic compounds such as AgRP. Intracerebroventricular (icv) administration of Hcrt-1[57] and NPY stimulates food intake in young and adult rats, but have no effects at any dose in old rats. Ghrelin increases food intake in a dose-dependent manner in all ages, but its effects are still reduced with advancing age.[58] Hypothalamic mRNA levels of anorexigens such as CART (Hypothalamic cocaine- and amphetamine-regulated transcript), on the other hand, do not change with ageing.[59] In fact, MCH KO mice are resistant to aging-associated increases in body weight and insulin resistance.[60]

Taken together, data from multiple laboratories converge on the idea that sleep and metabolism affect each other and are often "tuned" together. This tuning is likely mediated by Hcrt and MCH hypothalamic neurons that integrate metabolic- and vigilance-related inputs to generate a physiolog-ical and behavioral output that are appropriate for a given environment. The impact of age on metabolism and sleep and its correlation with changes in hypothalamic circuitry not only emphasizes the impact of the hypothalamus on both pathways, but also highlights the need to appreciate how stages of life might impact clinical interventions for sleep disorders and/or obesity.

Indirect pathways mediating interactions between sleep, metabolism, stress and reward

The role of the hypothalamus in the stress response and its effect on metabolism

Adaptation to cumulative stress exposure, or allostasis, is an important homeostatic mechanism with strong survival advantages.[61] Two key components of allostasis are changes in sleep–wake cycles and changes in metabolism. As many people know from their own experience, stress can affect how

much and how well one sleeps, as well as how much and how well one eats and metabolizes. Supporting this observation, stress hormone levels correlate positively with decreased sleep duration, obesity, incidence of disruptive metabolic syndromes[62], and are elevated in a wide range of eating disorders ranging from stress-induced binging to stress-induced anorexia.[63] The effects of stress on sleep are most evident in pathological conditions such as posttraumatic stress disorder (PTSD) in which people encounter traumatic memories in their dreams disrupt that overall sleep patterns.[64]

In rodents, stress reduces the duration of both slow-wave sleep (SWS) and REM sleep.[65] Sleep quality, measured by changes in EEG power (such as reduced delta power), is also affected by stress.[66] The magnitude and efficiency of these changes varies according to the type of stress (restraint, electrical foot shock, forced swimming, cold)[65], its timing relative to the sleep episode[67], and the strain of mice tested.[66]

Stress also affects metabolism in rodents, but the nature of the relationship is often very complex and varies across conditions and circumstances. For example, immobilization stress leads to a decrease in POMC mRNA 1 h after the stress, but an increase in POMC mRNA 4 h after the stress.[68] In addition to post-stress duration, the duration of stress exposure itself as well as the type of stress exposure can affect metabolism as measured by NPY, leptin, and ghrelin levels, but the combination of environmental variables characterizing the specific type and duration of stress defines the unique direction and mechanism of the effect.[68–72]

The stress response, in general, is mediated by several pathways. The HPA axis is an important part of the stress response, and involves release of corticotropin-releasing factor (CRF) from the paraventricular hypothalamic nucleus. CRF causes the production of adrenocorticotropic hormone (ACTH), which in turn elicits the release of stress hormones from the adrenal gland.

In this stress response the hypothalamus is a major player, since CRF is secreted from the hypothalamus. However, in addition, it has been proposed that the effects of stress on sleep and metabolism are at least partially mediated by Hcrt circuitry.[1,7–9] As might be expected if this hypothesis is true, the Hcrt system is anatomically and functionally linked to the stress system.[1,8,9] Hcrt cells project to many CRF-responsive brain regions including the noradrenergic LC, the PVN, the bed nucleus of the stria terminalis, and the central amygdala.[73,74] Functionally, icv administration of Hcrt activates the HPA axis as measured by plasma ACTH and corticosterone levels.[75–77] Likewise, injection of a Hcrt-1 receptor agonist increases heart rate, blood pressure, cerebral blood flow and renal sympathetic activity in awake animals.[78] Highly relevant to the clinical effects of stress on sleep and metabolism, stress circuitry also anatomically regulates the Hcrt circuitry. CRF neurons project to Hcrt-expressing cells[73] and excite them via the CRF-R1 receptor. Foot shock, restraint stress, or cold-exposure are all able to excite Hcrt cells as measured by increased c-fos expression.[73,79,80] Finally, the activation of Hcrt cells following stress is severely impaired in CRF-R1 knockout mice.[73]

As this highly interconnected anatomy makes evident, the stress and arousal systems continuously influence and feedback on each other. One well-studied example of this is the mechanism by which stress in response to sleep deprivation affects sleep. Sleep deprivation activates several stress-related pathways including the hypothalamic-pituitary-adrenal (HPA) axis, other parts of the sympathetic nervous system, and inhibits parasympathetic nervous activity.[81–83] In doing so, it indirectly affects arousal and metabolism. In other words, sleep-deprivation evokes activation of the stress circuitry, which then feeds back on sleep-related circuitry again.

Put together, these data demonstrate that the stress, arousal, and metabolism systems overlap and are highly intertwined, and Hcrt cells seem to be at the junction of all three systems both functionally and anatomically. This suggests that it will be important to monitor the outputs and interactions of all three systems when clinically treating sleep or metabolic disorders, and the Hcrt system might be a promising target for pharmaceutical interventions in complex situations in which stress is combined with sleep or metabolic disruptions.

The role of the hypothalamus in reward and its effects on hedonic feeding

Although sleep and feeding have distinct biological functions, both sleep and feeding are intensely regulated by brain hedonic systems. Hcrt cells are ideally suited both functionally and anatomically to mediate this regulation.

Reward is thought to be mediated predominantly by limbic structures of the mesolimbic brain system. Key players in this system are the ventral tegmental area (VTA), amygdala, and nucleus accumbens (NAc). Dopamine is one of the primary neurotransmitters these brain regions use to code for reward.

At least two of these reward-related regions – the VTA and amygdala – project to Hcrt cells as demonstrated by retrograde tracing.[41,84] Likewise, Hcrt cells project to many reward centers, including dopaminergic cells in the VTA[85,86] and the NAc. In addition, the anatomical connections between Hcrt neurons and reward centers have functional relevance. Hcrt-1 has been shown to increase the firing rate –and in some cases cause burst firing – of VTA dopamine neurons in rat brain slices.[87,88]

Hcrt-1 administration also transiently enhances excitatory post-synaptic currents[89] in dopamine VTA neurons, and a 5-min application of Hcrt-1 changes the levels of the NMDA receptors in the VTA. The latter effect can be blocked by the Hcrt-1 antagonist SB 334867, emphasizing the direct involvement of Hcrt-1 in this process.

The immediate effect of Hcrt-1 on the NMDA-mediated responses is specific, as Hcrt-1 does not affect responses evoked via the AMPA receptors in the VTA. With longer exposure, however, AMPA receptors can be affected as well as demonstrated by an increase in AMPA receptor-mediated responses and the number of receptors expressed.[64]

Studies of the effects of Hcrt on reward systems *in vivo* have revealed that Hcrt-1 infusions in the VTA of rodents increase extracellular dopamine levels in the prefrontal cortex (PFC) and in the shell of the NAc, but not in the NAc core region.[90–93] This specificity of the Hcrt effects has functional relevance as these regions differ in their cellular composition, receptor expression, connectivity and they differentially affect behavior and addiction.[94]

Hcrt infusions also significantly increase activity in small- to medium-sized dopamine neurons in the caudomedial VTA[93] as measured by c-fos immunoreactivity, and the majority of these fos-activated neurons project primarily to the PFC and NAc shell.[89,93] Inline with these findings, morphine-induced increases in dopamine levels are blocked in Hcrt-deficient mice.[90]

One indirect mechanism by which Hcrt neurons might affect reward is via the amygdala. The amygdala is believed to contribute to detecting the salience of reward.[95] Hcrt cells have reciprocal connections with the amygdala[96], and these connections are thought to be responsible for the phenomenon of narcoleptic animals having sleep attacks when highly-desirable food is present.[97] The amygdala has also been shown to be preferentially active in human narcoleptic patients as measured using functional MRI.[98] This connection between hypothalamic circuits and the amygdala may have broad impacts on reward as well as on stress pathways, and requires further investigation.

The interaction between reward and metabolism systems is mediated by many hypothalamic peptides in addition to Hcrt. For example, a high concentration of NPY immunoreactivity corresponding with the distribution of NPY containing terminals has been detected in the NAc[99], and NPY and POMC-expressing neurons project to the amygdala.[99] Furthermore, dopaminergic cells are sensitive to metabolic cues. Direct application of ghrelin to slice cultures modulates the activity and synaptic organization of VTA neurons in addition to causing an increase in food intake in intact animals.[100,101] Leptin reduces the firing rate of VTA neurons whereas ghrelin and Hcrt have the opposite effect.[88,101,102] The effects of leptin on the dopaminergic and GABAergic neurons of the VTA include phosphorylation of the signal-transducer-and-activator-of-transcription-3 (STAT3) pathway.[103] Immunohistochemistry for pSTAT3 combined with retrograde tracing showed leptin-responsive VTA neurons projecting to the NAc.[102] Behaviorally, direct administration of leptin into the VTA decreases food intake.[102] Furthermore, blockade of AMPA and kainate glutamate receptors in the medial part of the NAc resulted in a pronounced feeding response in rats, which was completely inhibited by concurrent infusion of the GABAA agonist muscimol into the LHA.[104]

In sum, the organism uses the reward system to motivate behaviors that will assure adequate sleep and nutrition. In return, the reward system can influence the set point of the hypothalamus, causing it to orchestrate physiological responses and behavior according to hedonic value rather than to biological needs. Understanding the mechanisms underlying this relationship between reward, arousal, and metabolism may help inform our understanding of natural unhealthy behaviors such as overeating, and may also help us understand some pathological behaviors, such as cravings associated with addiction.

Summary

Biological organisms have heavy bidirectional links between their sleep and metabolism systems to help them maintain homeostasis in changing or limited environments. Recent evidence suggests that the hypothalamus, especially hypothalamic Hcrt cells, plays a critical role in coordinating the homeostatic outputs of these systems. We have reviewed evidence that:

- There is a tight relationship between sleep and metabolism in both humans and rodents.
- Hypothalamic nuclei involved in sleep and metabolism are anatomically and functionally connected with each other as well as with other arousal and metabolic centers.
- Hcrt cells in the hypothalamus have direct and possibly causal roles in behaviors that require coordination of wakefulness, metabolism, and feeding.
- Changes in Hcrt anatomy and functionality over the life span are paralleled by changes in metabolism and sleep.
- Stress, sleep, and metabolism have profound circular effects on each other, and many of these effects might be mediated by Hcrt.
- Reward systems strongly modulate sleep and metabolic systems, and Hcrts might determine the "set point" that mediates hedonic influences on sleep and feeding.

Learning more about how sleep and metabolism interact might inform clinical treatments for a wide variety of arousal-related disorders and symptoms while simultaneously providing great insight into how organisms evolved to withstand complex, dynamic environments.

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