Review

Sleep and metabolism: shared circuits, new connections

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Association between sleep disturbances and hormonal imbalances can result in metabolic disorders, including obesity and diabetes. The hypothalamus is likely to play a part in these pathophysiological conditions because it contains sleep-wake circuits that are sensitive to metabolic hormones, including leptin and ghrelin. Thus, shared hypothalamic circuits such as the hypocretin and melanin-concentrating hormone systems are strong candidates for mediating both sleep and metabolic imbalances. This review reveals new roles for these systems as sensors and effectors of sleep and wakefulness, and discusses their plasticity in regulating sleep and energy balance. New optical tools that remotely control neuronal circuit activity provide an effective means to understand the cooperativity of shared circuits in regulating hypothalamic functions such as sleep and metabolism.

The prevalence of sleep curtailment and metabolism-related pathologies is increasing worldwide, particularly in industrialized countries. Recent experimental evidence supports an association between sleep shortening and chronic metabolic changes that can lead to obesity and diabetes [1]. Such an association indicates the involvement of brain circuits that regulate both sleep and metabolism. This review highlights the potential role of the hypocretin (Hcrt; see Glossary), also known as orexin, system and the melanin-concentrating hormone (MCH) hypothalamic system to cooperatively provide a sensor–effector circuit of arousal that modulates energy balance based on our knowledge of their implication in sleep and metabolism physiology. Deciphering the properties of the hypothalamic neuronal circuits involved in such complex modulatory functions is now possible by using newly generated methods for remote control of neuronal activity. Such approaches will eventually result in a better understanding of brain modulation of physiological functions and the identification of new therapeutic targets.

Hypothalamic regulation of arousal and metabolism

Sleep and feeding are two mutually exclusive behaviors. In mammals, periods of starvation are accompanied by increased vigilance and sleep loss, presumably to help maximize food-seeking behavior and energetic survival [2,3]. By contrast, sleep deprivation leads to markedly increased energy expenditure and body weight loss. Both phenomena are consistent with a role for sleep in energy conservation and tissue maintenance. The past decade has seen a remarkable increase in our understanding of the basic circuitry underlying sleep-wake regulation and its metabolic modulation. In particular, the hypothalamus crucially regulates homeostatic processes including

Glossary

AgRP: Agouti-related protein (AgRP) is a neuropeptide produced by neurons located in the arcuate nucleus of the hypothalamus. It has sequence similarity with Agouti signaling peptide, a protein synthetized in the skin that controls coat color in vertebrate. Activation of AgRP/NPY neurons increases appetite and decreases metabolism and energy expenditure. It is one of the most potent and long-lasting of appetite stimulators.

Behavioral sensitization: sensitization is an example of non-associative learning in which the progressive amplification of a response follows repeated administrations of a stimulus.

CART: cocaine and amphetamine-regulated transcript (CART) encodes several peptides regulated in the striatum following acute administration of psychomotor stimulants. CART peptides are widely expressed in the central and peripheral nervous systems. CART mRNA colocalizes with POMC-producing neurons in the arcuate nuclei of the hypothalamus and has anorexigenic properties.

HCRT: Hypocretins (Hcrt), also called Orexins (Ox), are a pair of excitatory neuropeptides produced by neurons exclusively located in the LH that project widely throughout the brain. The hypocretin system is a sensor of energy homeostasis and regulates wakefulness and reward.

MCH: Melanin-concentrating hormone (MCH) is a hypothalamic peptide originally isolated from the pituitary gland of teleost fish where it controls skin pigmentation. MCH expressing neurons are located within the LH and ZI and project widely throughout the brain. In mammals MCH is involved in the modulation of feeding behavior, energy balance, locomotor activity, anxiety, depression and sleep.

Mesolimbic pathway (VTA, nucleus accumbens): the mesolimbic pathway links the ventral tegmentum area (VTA) in the midbrain to the nucleus accumbens, which is located in the striatum and is a part of the limbic system. It is one of the four major pathways where the neurotransmitter dopamine is found. The mesolimbic pathway is involved in reward and motivation. Recent research has pointed towards this pathway being involved in incentive salience rather than euphoric mood states.

Narcolepsy: Narcolepsy is a chronic neurological disorder resulting in the destabilization of the sleep-wake cycle. Major symptoms include excessive daytime sleepiness, cataplexy (loss of muscle tone) and fragmented sleep.

NPY: Neuropeptide Y (NPY) is a 36 amino acid peptide neurotransmitter found in the brain and autonomic nervous system. In mammals, NPY has been associated with a number of physiologic processes in the brain, including the regulation of energy balance, memory and learning, and epilepsy. NPY/AgRP-producing neurons in the arcuate nucleus of the hypothalamus have feeding properties.

Orexigenic/anorexigenic: a compound that stimulates (orexigenic) or diminishes (anorexigenic) appetite.

POMC: Pro-opiomelanocortin (POMC) is a precursor polypeptide with 241 amino acid residues. This precursor undergoes extensive, tissue-specific, post-translational processing that could yield as many as ten biologically active peptides involved in diverse cellular functions. In the hypothalamus, all cleavage sites can be used, giving rise to peptides with roles in pain and energy homeostasis, melanocyte stimulation and immune modulation. Mutations in the POMC gene have been associated with early onset obesity, adrenal insufficiency and red hair pigmentation.

Zona Incerta: the ZI is a subthalamic nucleus that is located medially to the internal capsule and ventral to the thalamus, and is contiguous with the thalamic reticular nucleus. This region receives widespread input from the cerebral cortex and cerebellum and sends outputs back to the cortex. Its functions, however, remain largely unknown.

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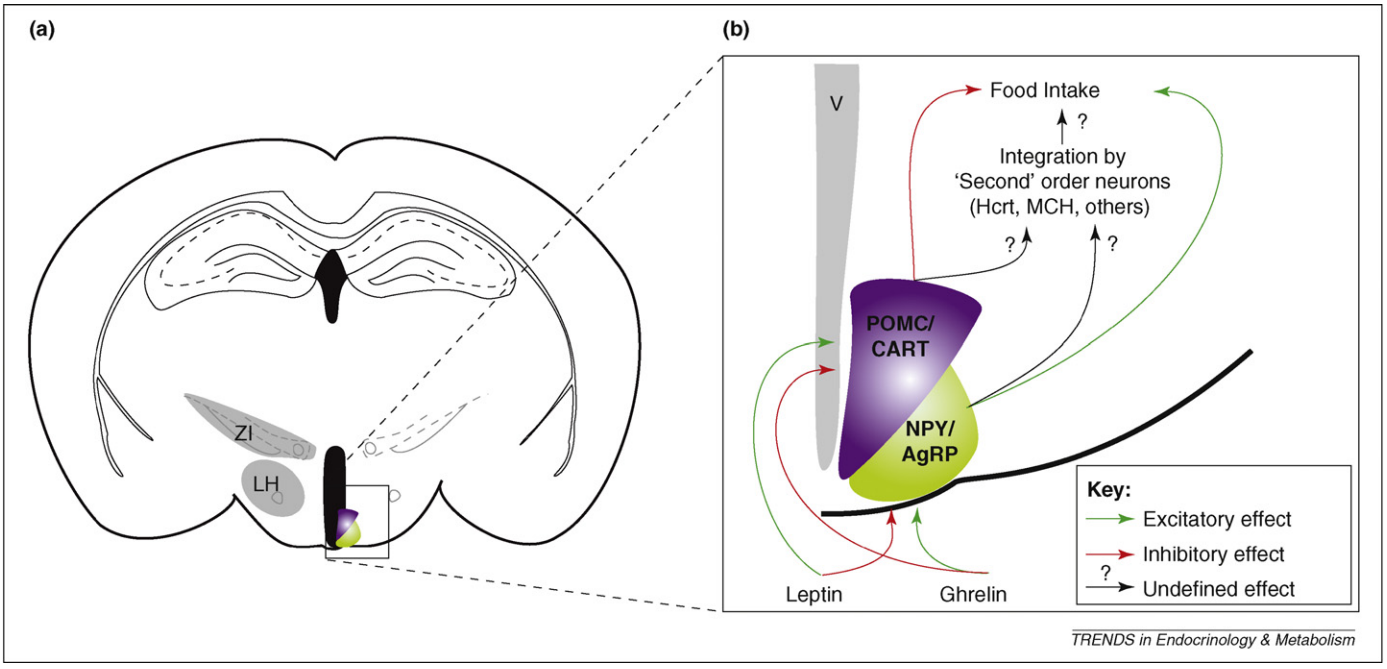

Figure 1. Hypothalamic circuits regulate energy homeostasis. **(a)** Schematic drawing of a coronal section through the entire rat brain. The lateral hypothalamus (LH) and the Zona Incerta (ZI) are shown. The arcuate nucleus is highlighted by the box. **(b)** Magnification of the arcuate nucleus of the hypothalamus showing NPY/AgRP and POMC/CART neurons. Peripheral signals (leptin and ghrelin) directly regulate the activity of arcuate nucleus through the median eminence, which lacks a brain–blood barrier. Leptin inhibits NPY/AgRP neurons and activates POMC/CART neurons, whereas ghrelin has opposite effect [8]. These neurons project to mutiple brain regions, including the hypothalamus, where the signal is further processed and integrated into coherent feeding behavior. V, third ventricle.

temperature, energy and stress through subtle adjustments of autonomic and endocrine functions. Recent work indicates that it also contains neuronal circuits that modulate specific behaviors including the sleep-wake cycle and feeding, two behaviors associated with basic homeostatic mechanisms [4].

The neural underpinnings of the sleep-wake cycle involve interactions between sleep-promoting areas, such as the anterior hypothalamus, and arousal systems located in the posterior hypothalamus, the basal forebrain and the brainstem [5,6]. Arousal is a state of heightened cortical responsiveness to sensory inputs mediated by the activation of the ascending reticular formation originating in the brainstem; it is accompanied by an increase of physiological activity (postural tone, cardiac and respiratory rhythms) [7]. During sleep, GABAergic and glycinergic cells located in the ventrolateral preoptic (VLPO) area of the anterior hypothalamus are activated and inhibit wake-promoting circuits of the posterior hypothalamus, basal forebrain and brainstem to control sleep onset and maintenance [5,6]. During sleep, the alternation between rapid-eye movement (REM) and non-REM (NREM) sleep is controlled by multiple neuronal populations located in the hypothalamus, basal forebrain and brainstem [5]. Behavioral transitions to wakefulness involve the reactivation of wake-promoting neurons, which, in turn, inhibit sleep-promoting circuits [5]. Thus, sleep-wake cycles are accompanied by different brain states that influence behavior and cognition.

Appetite is regulated by the interaction between metabolic and hormonal signals and the central nervous system. The hypothalamus regulates energy homeostasis (i.e. food intake and metabolism) by sensing circulating hormones including leptin and ghrelin through the median eminence, and integrating autonomic, endocrine and environmental signals into coherent goal-directed behaviors [4] (Figure 1). Leptin is a satiety hormone produced by adipose tissues that inhibits arcuate neurons that coexpress neuropeptide Y (NPY) and agouti-related peptide (AgRP), and activates proopiomelanocortin (POMC) neurons that also coexpress cocaine- and amphetamine-related transcripts (CART). Ghrelin is an appetite-stimulating hormone from the gut that has the opposite effect [8]. Thus, activation of POMC/CART and NPY/AGRP neurons induces anorexigenic and orexigenic properties, respectively. Arcuate neurons project to the lateral hypothalamus (LH), where additional signal processing results in coherent metabolic (food intake and metabolism) and behavioral (sleep or wakefulness) outputs. Consequently, hormone-sensitive neurons of the arcuate nucleus (NPY/AgRP- and POMC-expressing cells) have been coined 'primary order' or 'sentinel' neurons in regard to their hierarchical position in integrating peripheral signals, whereas 'second order' neurons include cells in the medial region and LH cells producing Hcrt, MCH, CART, corticotropin-releasing factor (CRF) and endocannabinoids [8]. Finally, leptin also increases energy expenditure, possibly via increased thermogenesis, whereas ghrelin decreases locomotor activity and, thus, promotes energy conservation [1].

Sleep-wake perturbations and metabolic consequences

Sleep duration and the length of a sleep-wake cycle are inversely correlated with metabolic rate – defined as the amount of energy expended when at rest in a neutrally temperate environment in the post-absorptive state – across species. This indicates that availability of metabolic fuels conditions sleep architecture (i.e. NREM and REM sleep-bout duration, quality and occurrence, and length of

the sleep-wake cycle) [1,3]. For instance, mice have a higher metabolic rate than humans and a shorter sleep-wake cycle (a few minutes versus ~1.5 h). In rodent studies, total and partial sleep deprivation is invariably accompanied by increased activity and stress, and weight loss that results in a negative energy balance (a condition in which less energy, i.e. food, is taken in than is expended in metabolism, resulting in a decrease in body weight), even though food intake increases [1,3,9]. Importantly, altered levels of metabolic hormones (decreased leptin and increased ghrelin), activation of hypothalamic orexigenic pathways and alteration of metabolic gene expression (e.g. NPY) have all been linked to alterations in sleep architecture [3,10]. Epidemiological studies have shown a strong association of short sleep duration with lower leptin and higher glucose and ghrelin levels [1,11,12]. Such peripheral signals activate NPY/AgRP neurons and inhibit POMC/CART neurons [8] that result in a net feeding signal. This might be responsible for the higher body mass index and increased incidence of type 2 diabetes reported after extended sleep perturbation [3]. However, mechanisms of causality in the association between self-reported short sleep duration and type 2 diabetes need further experimental confirmation in humans [3].

Hcrt and MCH: distinct populations, shared circuits, new functions

Description

Hcrt and MCH peptides and their cognate receptors are highly conserved across species. Hcrt-1,2 are neuroexcitatory peptides that are produced in approximately ~6700 neurons in the rat brain [13], restricted to the LH [14]. In mammals, MCH [15,16]-producing neurons (~10 000–12 000 cells in the rat brain [13]) are mainly restricted to the LH and Zona Incerta (ZI) and define a distinct neuronal population intermingled with Hcrt cells [17]. Interestingly, Hcrt and MCH neurons send parallel projections to the cortex, hippocampus, amygdala, nucleus accumbens, hypothalamus, thalamus, ventral tegmental area (VTA), locus coeruleus and raphe [2,17], indicating integrative roles. Although the release dynamics of these peptides remain unclear, Hcrts bind to two specific Hcrt receptors (Hcrt-1R and Hcrt-2R) that result in increased neuronal excitability [2]. This review focuses on the rodent MCH system in which the peptide binds to MCH receptor 1, or MCH-R1 (MCH-R2 is only functional in dogs, ferrets, monkeys and humans), resulting in inhibitory and excitatory intracellular cascades [17]. Importantly, activation of Hcrt neurons have postsynaptic excitatory properties, whereas MCH neurons are thought to have the opposite effect [2,18].

Effector functions in sleep and metabolism

The physiology of Hcrt and MCH systems in regulating the sleep-wake cycle, energy homeostasis, feeding, locomotor activity, reward and motivation [2,17,19] reflects their anatomical organization with cell bodies restricted to the LH and ZI, and widespread axonal projections. In turn, they are the target of multiple neuronal cell types located throughout the brain (reviewed in Refs [2,18]).

Hcrt-expressing neurons have a major role in stabilizing the sleep-wake cycle; deficiency in the Hcrt system has been linked to narcolepsy in humans [20], dogs [21] and mice [22]. In addition, circadian fluctuation of Hcrt-1 within the cerebrospinal fluid is maximal during the wakefulness period, while Hcrt peptide infusion promotes wakefulness [2]. It was thus proposed that Hcrt facilitates arousal by setting the arousal threshold [23]. Accordingly, increased arousal and alertness in response to acute stress might result from a lower arousal threshold. This hypothesis was supported by recent studies showing that Hcrt neurons are in close contact with CRF, a neurotransmitter that initiates the central response to acute stress. Indeed, Hcrt neurons express CRF-$R_{1,2}$ receptors and directly contact CRF-containing fibers, thereby showing increased firing rates in response to CRF [24]. Hcrt neurons could integrate a CRF-mediated stress response into a 'hyperarousal' output signal that activates the arousal centers of the brain including the cortex, the posterior hypothalamus, the locus coeruleus and the VTA.

In rodents, the Hcrt system mediates stress-induced reinstatement of cocaine-seeking behavior [25], the development of opioid drug dependence and the expression of physical withdrawal signs [26]. In addition, Hcrt neurons express μ- and k-opioid receptors, and their activation is linked to preferences for cues associated with drug and food reward, and also naltrexone-precipitated withdrawal (reviewed in Ref. [19]). By contrast, Hcrt KO mice exhibit dramatically attenuated morphine withdrawal symptoms, morphine-induced place preference and hyperlocomotion [19]. These behavioral consequences might result from an increased sensitivity to glutamate in VTA synapses, as was recently shown *in vitro* [27].

In addition to its regulatory function of brain reward pathways activated by artificial drugs, the Hcrt system is a major component of natural reward associated with feeding behavior. In rats, Hcrt increases motivation for palatable food, possibly through opioid or dopamine signaling [19], and the lack of a functional Hcrt system impairs the elevated arousal associated with fasting [2]. Thus, Hcrt-mediated modulation of brain reward pathways is likely to be responsible for Hrct's acute orexinergic effect. Hcrt-1,2 peptides were also termed *Orexins-A,B* because of their orexigenic properties when infused into the rat brain ventricles at pharmacological doses [28] (Figure 2a). Although central administration of Hcrt-1 increases the metabolic rate in rodents [2], its role in feeding behavior remains unclear. First, continuous intracerebroventricular (icv) infusion of Hcrt-1 in rats for seven days has no effect on daily food intake, body weight, blood glucose, total cholesterol or free fatty acid levels [2]. Second, although fasting increases *Hcrt* mRNA expression in the rat hypothalamus, Hcrt-1 peptide accumulation in the cerebrospinal fluid during fasting does not exceed concentrations observed during the wakefulness period in rat [2]. Third, Hcrt KO mice eat less compared to weight- and age-matched littermates [2], and Hcrt neuron-ablated mice (Orexin/ataxin-3 transgenic), which would be expected to be lean because of the absence of Hcrt peptides, instead develop late-onset obesity with modest genetic background-dependent hypophagia and hypolocomotion [2]. Collectively,

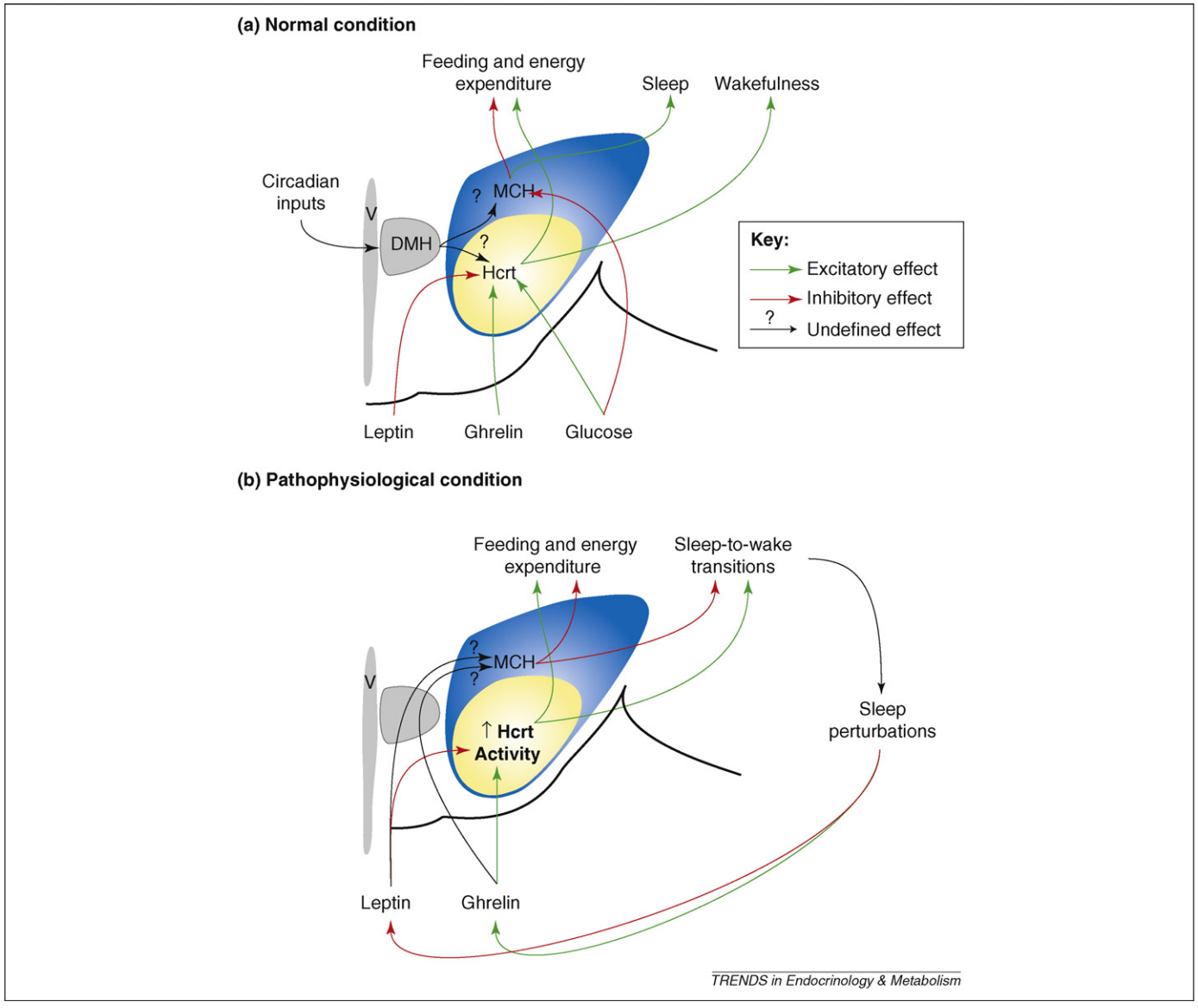

Figure 2. Hcrt and MCH systems as a sensor of arousal. Hcrt and MCH systems regulate energy homeostasis and sleep-wake cycle in normal and pathophysiological conditions. **(a)** Hcrt neurons are inhibited by leptin and activated by ghrelin and glucose. Eventually, activation of the Hcrt system promotes arousal and increases metabolism. By contrast, MCH neurons are inhibited by glucose, and the action of leptin and ghrelin on these cells remains unknown. Both Hcrt and MCH systems received indirect circadian inputs that are integrated together with metabolic signals to modulate sleep and metabolism. Thus, activation of the Hcrt system promotes arousal and energy expenditure, whereas activation of the MCH system dampens metabolism and might promote sleep. DMH, dorso-median hypothalamus; V, third ventricle. (a) Chronic short sleep perturbation induces metabolic changes, including lower leptin levels and increasing ghrelin levels, that directly increase the activity of the Hcrt system (arousal sensor). This increase in Hcrt activity promotes consummatory behaviors (food or drug), energy expenditure (via higher locomotor activity and metabolic rate) and sleep-to-wake transitions. Consequently, activation of the Hcrt system inhibits sleep (by promoting sleep-to-wake transitions [44]) and energy conservation. Triggering activity of the Hcrt neuronal circuit by circulating metabolic factors might, then, result in a positive feedback loop, worsening existing sleep perturbation symptoms. Inhibition of this loop upon sleep pressure (a consequence of sleep demand) might decelerate this positive feedback to stabilize the sleep-wake cycle. Although the role of the MCH system in this pathophysiological model is still unclear, it might decrease energy expenditure and stabilize sleep (by promoting sleep) by modulating Hcrt neuron activity and brain circuits involved in sleep and metabolism.

these data indicate that activation of the Hcrt system can increase hedonic feeding.

Among the numerous roles for MCH neurons, their involvement in feeding behavior and energy homeostasis are by far the best documented (reviewed in Ref. [17]) (Figure 2a). MCH has acute short-term orexigenic properties; in fact, the MCH system is upregulated after fasting. Mice overexpressing MCH develop mild obesity and hyperphagia, whereas genetic inactivation of MCH neurotransmission (MCH KO and MCH-R1 KO) and ablation of MCH neurons in mice leads to leanness, hyperactivity and increased metabolic rate [17]. Interestingly, genetic deletion of the MCH system in leptin-deficient (*ob*/*ob*) obese mice does not alter hyperphagia of *ob*/*ob* animals but induces a dramatic reduction in body fat secondary to a marked increase in energy expenditure [17]. These studies collectively demonstrate that activation of the MCH system decreases energy expenditure.

Based on their neuroanatomical features, Hcrt and MCH systems are thought to have opposite functions in the sleep-wake cycle. The MCH system is thought to be a sleep-promoting system [13,29,30]. MCH neurons are activated after sleep deprivation, indicating a role for the MCH system in promoting sleep [13,29,30]. In addition, icv infusion of

MCH peptide in rats increases the amount of REM sleep and non-REM sleep [29]. Consistent with this hypothesis, MCH-R1 KO mice show disinhibited locomotor activity in response to psychomostimulants (drugs, including amphetamine and cocaine, that act on the brain to stimulate locomotor activity), indicating an inhibitory role on locomotor activity and energy expenditure. In contrast to the hypothesis that MCH is a sleep-promoting factor, MCH-R1 KO mice present a mild hypersomniac phenotype during the spontaneous sleep-wake cycle and after total sleep deprivation [31]. Thus, complementary studies are needed to define the precise role of the MCH system in the sleep-wake cycle and drug-induced locomotor activity.

As demonstrated for the Hcrt system, MCH might affect brain reward circuits to regulate energy homeostasis. The MCH system modulates behavioral responses to psychostimulants by acting on the dopaminergic system. Amphetamine, cocaine and a dopaminergic receptor 1 agonist cause enhanced behavioral sensitization in MCH KO and MCH-R1 KO mice [32–35], possibly via upregulating dopaminergic, noradrenergic [32,36] or glutamatergic neurotransmission [37]. However, in contrast to the Hcrt system, MCH neurons are not activated during acute stress, during acute and chronic morphine treatment or after naltrexone-induced withdrawal [26,37]. Interestingly, MCH delivery to the nucleus accumbens stimulates feeding, whereas an MCH-R1 antagonist has the opposite effect [37]. This latter study demonstrates that MCH modulation of energy homeostasis relies, at least in part, on modulation of brain reward pathways.

In conclusion, the hypothalamic Hcrt and MCH systems share common brain circuits regulating sleep, wakefulness, metabolism and motivated behaviors for natural (food) and artificial (drug) rewards. According to the current hypothesis, Hcrt and MCH systems have antagonistic function of sleep and metabolism, with Hcrt-system activation promoting wakefulness and inducing energy expenditure. The resulting cortical arousal then facilitates physical activity and alertness, and precipitates reward-seeking behaviors. By contrast, the MCH system promotes energy conservation and sleep, possibly by inhibiting arousal centers of the brainstem and posterior hypothalamus or by activating sleep-promoting neurons of the anterior hypothalamus [13,29,31]. However, this hypothesis requires further investigation.

The Hcrt system: a sensor of arousal and metabolic changes

Recent studies in mammalian species, including human, reported that chronic short sleep resulted in lower leptin levels, increased glucose and ghrelin levels and higher risk of developing obesity and type 2 diabetes [3,9,11,38]. Because activity of Hcrt and MCH neurons can be regulated by metabolic factors, it is possible that the Hcrt system, in combination with the MCH system, acts as a sensor (i.e. measures or detects a changing condition, from neurotransmitters, neuromodulators or metabolic factors, and converts it into an output signal) of arousal state. Therefore, these systems could provide the hypothalamus with a local circuit that integrates sleep-wake cycle and metabolism into a coherent behavior.

As shown in Figure 2a, decreased circulating leptin levels and increased glucose and ghrelin levels directly modulate Hcrt activity by increasing Hcrt neuronal excitability [2,39–41]. Sensitivity of Hcrt neurons to triglycerides [2], carbon dioxide and pH [42] further support the hypothesis that Hcrt neurons are sensors of metabolic factors. Thus, enhanced Hcrt neuronal activity could promote feeding behavior, arousal and increased energy expenditure (via higher locomotor activity and metabolic rate), acting in part via activation of NPY cells and indirect inhibition of POMC cells [2,43], activation of arousal centers in the brain [2] and increasing sympathetic tone [2]. Furthermore, increased Hcrt-system activity might result in instability of the sleep-wake cycle because selective stimulation of Hcrt neurons *in vivo* increases the probability of NREM and REM sleep-to-wake transitions [44]. Interestingly, hormonal changes occurring after alteration of sleep architecture might lead to hyperexcitability of Hcrt neurons, mirroring the effects of prolonged wakefulness periods [45]. Further, acute and chronic leptin deficiency induces synaptic and axonal plastic changes in the Hcrt [46], NPY and POMC arcuate neurons [47], as does ghrelin on synaptic inputs to VTA neurons [48]. Thus, triggering activity of the Hcrt circuits by circulating metabolic factors would then result in a positive feedback loop, which could worsen existing sleep perturbation symptoms. Prolonged wakefulness and extended periods of sleep perturbation inevitably increase sleep demand through the accumulation of adenosine [49] and disinhibition of sleep-promoting circuits such as GABAergic/glycinergic cells of the VLPO [50]. This accumulation of sleep pressure might decelerate the positive feedback loop and stabilize the sleep-wake cycle.

Supporting data for this hypothesis come from clinical and experimental studies on the behavioral and metabolic consequences of an absence of Hcrt function. An absent Hcrt system in narcoleptic patients leads to a fragmented sleep-wake cycle and decreased caloric intake, despite increased body mass index owing to a low basal metabolism and unaltered leptin levels [51,52]. Hcrt-deficient narcoleptic mice show severe sleep and wake fragmentation with mild obesity and reduced locomotion, feeding, drinking and energy expenditure [2,53]. More importantly, these animals are unable to stabilize wakefulness appropriately during fasting periods [2]. Collectively, these data indicate that the Hcrt system acts as a sensor of internal state (metabolism and arousal) in promoting arousal. In turn, the MCH system might decrease activation of this arousal sensor circuit by dampening energy expenditure [17] and promoting sleep.

Sleep and metabolism regulation through reward pathways of the brain

Alternative brain pathways regulating sleep and metabolism include the mesolimbic pathway, the activation of which is associated with motivation, reward and incentive salience. Dopaminergic cells are sensitive to metabolic factors and have been identified recently as crucial brain regulatory circuits of energy homeostasis [48,54,55]. Direct administration of leptin into the VTA decreases food intake [55], possibly through modulating dopamine release in the

nucleus accumbens [54], whereas VTA administration of ghrelin has the opposite effect [48]. Furthermore, leptin reduces the firing rate of VTA neurons, whereas ghrelin and Hcrt have the opposite effect [19,48,55,56]. In addition, dopamine is necessary for appropriate occurrence and electroencephalogram spectral quality of REM sleep during the sleep-wake cycle [57], and dopaminergic neurons of the VTA change their firing patterns during REM sleep [58].

This indicates interplay between sleep- and wake-promoting circuits and reward pathways of the brain to regulate brain states. Interestingly, Hcrt and MCH might interact with brain reward pathways where their cognate receptors are expressed [2,17] to modulate arousal and food-seeking behaviors [19,37]. This suggests a pathophysiological role for these circuits in patients with Night Eating Syndromes (NES), Nocturnal Sleep-Related Eating Disorder (NS-RED) and narcolepsy who present sleep perturbation associated with night feeding [1,52].

Next-generation tools for manipulating neuronal circuit activity

Defining the Hcrt system as a sensor of arousal highlights several issues that need to be addressed at the single-circuit level. Common methods for controlling neuronal circuit activity include non-specific chemical or electrical lesion approaches of a specific brain area to suppress its functional activity. Pharmacological methods include injecting inhibitory or excitatory agonists to target specific cell types that express the receptor of interest and to avoid inadvertent neuronal activation/inhibition that occurs with electrical stimulation. Ultimately, genetic targeting enables cell-type specific modifications from gene insertion and gene mutation (knock-in) to gene deletion (knockout) and progressive cellular ablation (e.g. ataxin-3-transgenic animals). Major problems of these techniques include lack of spatial, cellular and temporal resolutions and compensatory mechanisms that might occur in knockout animals when a gene is lacking from the early step of the development. Conditional gene knockouts and gene expression knockdown have partly overcome these concerns.

By contrast, newly developed optical tools to manipulate membrane potential of defined cell populations overcome the lack of specificity and low temporal resolution of previous approaches. Such techniques are classified by their molecular mechanisms and have different temporal and spatial resolutions (reviewed in Refs [59,60]). They include caged neurotransmitters (glutamate, GABA and ATP), which become uncaged when exposed to ultraviolet light and activate (glutamate) or inhibit (GABA) target neurons within different temporal resolutions (from a few milliseconds to 1 s). Modified glutamate and potassium channels enable activation and inhibition of neurons by exogenous light-sensitive molecules (agonist or blocker) tethered to the channel. Light-sensitive receptors (ChARGe and RO4) have low temporal resolutions (from seconds to minutes) and require retinol to activate (ChARGe) or inhibit (rhodopsin RO4 coupled to inhibitory ionic channel) neurons. In comparison, light-sensitive cations channels (Channelrhodopsin-2, VChR1 [61]) and ionic pumps (Halorhodopsin) are used to activate and inhibit, respectively, cells in which they are expressed upon optical stimulation at specific wavelengths (473 and 580, respectively) and have millisecond temporal resolution. The development of optogenetic technology (i.e. based on the use of ChR2 and Halorhodopsin, or NpHR) (Box 1) recently has enabled us to selectively probe the function of the Hcrt system in behavioral state transitions *in vivo* using the light-gated cation channel Channelrhodopsin-2 [44]. This optical tool is a major technical advance that permits researchers to probe single circuits within the intricate hypothalamic network with high temporal resolution and cell-type specificity.

Hcrt neurons represent only 20% of all the neurons in the LH, so electrical stimulation of this region would activate many additional circuits, resulting in uninterpretable data. By contrast, optogenetics can deconstruct the connectivity and plasticity of different hypothalamic circuits (e.g. Hcrt, MCH, NPY, POMC, and so on) and their sensitivity to leptin and ghrelin with unprecedented cellular resolution (Figure 3a,b). The possibility of inhibiting single action potentials in cells targeted with NpHR also opens new questions about neuronal encoding of information. Whereas electrophysiological recordings of neurons tagged with fluorescent markers have generated valuable information about the cellular properties of hypothalamic cells, optogenetic methods enable bimodal modulation (stimulation and inhibition) of the entire cellular population. Combining optogenetic stimulation or inhibition with voltage-sensitive dyes and calcium sensors will

Box 1. Optogenetic probing of hypothalamic circuits

The hypothalamus consists of multiple neuronal subtypes with excitatory, inhibitory and modulatory properties, each of which has separate roles in hypothalamic functions (e.g. NPY/AgRP, POMC/CART, Hcrt and MCH) [62]. Despite current identification of the circuitry that affects the sleep-wake cycle and metabolism, all of the evidence supporting a role for these circuits stems from brain lesion, electrical stimulation, pharmacological, gene-deletion, knockdown or *in vivo* single-unit recording studies.

To understand the contribution of specific circuits to the physiology of the hypothalamus with better spatial and temporal resolution, we recently used optogenetics ('opto' for optical stimulation and 'genetics' for genetically targeted cell types [63]) to manipulate Hcrt neuron activity *in vitro* and *in vivo* [44]. When expressed in a genetically targeted neuronal population, the light-sensitive proteins Channelrhodopsin-2 (ChR2) from *Chlamydomonas reinhardtii* [64] and the halorhodopsin (NpHR) from *Natronomonas pharaonis* [65] enable bimodal modulation of electrical signals (activation or inhibition) with millisecond timescale precision (reviewed in Ref. [60]). ChR2 is a monovalent cation channel that allows Na^+ ions to enter the cell after exposure to ~470 nm blue light, whereas the NpHR is a chloride pump that activates upon illumination with ~580 nm yellow light [60]. Their fast temporal kinetics (millisecond timescale) make it possible to drive reliable trains of high-frequency action potentials *in vitro* and *in vivo* using ChR2 [60] or suppress single action potentials within high-frequency spike trains using NpHR [65]. A fiber-optic-based system was developed that is suitable for delivering light *in vivo* to both superficial and deep brain structures [44,66,67].

Genetic targeting of ChR2 or NpHR into defined classes of hypothalamic neurons enable circuit-specific neuromodulation and avoid inadvertent stimulation of neighboring neurons as occurs with electrical stimulation [68]. This approach has been used successfully for optically interrogating intact neural circuits and specific behavior [44,67,69].

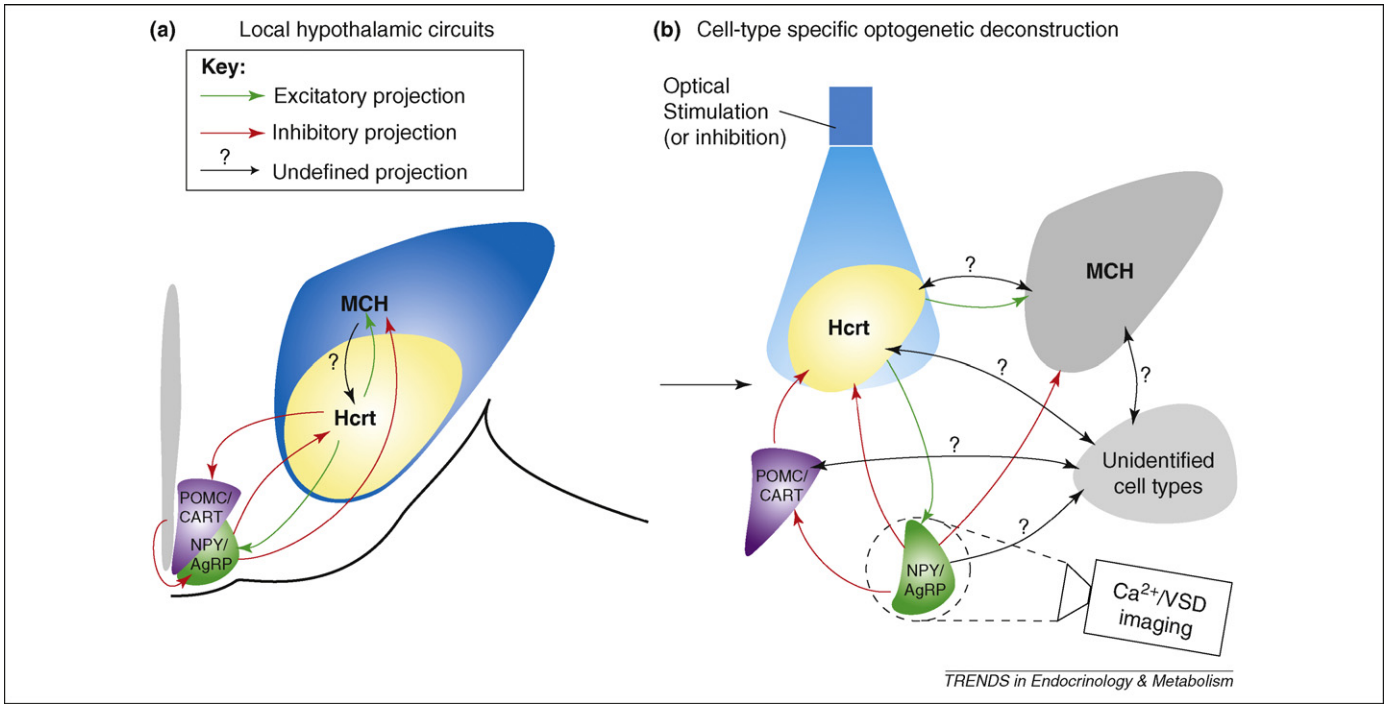

Figure 3. Optical deconstruction of local hypothalamic circuits. **(a)** Schematic drawing of a coronal section through the rat showing hypothalamic neuronal populations involved in sleep and metabolism. Hcrt and MCH neurons are the targets of projections from multiple brain areas [18], including NPY/AgRP and POMC/CART neurons of the arcuate nucleus. Although the interplay between hypothalamic nuclei in regulating energy homeostasis remains unclear, NPY/AgRP neurons inhibit Hcrt and MCH neurons. In turn, Hcrt activates NPY/AgRP cells and inhibits POMC/CART neurons. Hcrt peptides increase the firing rate of MCH neurons; however, the effect of MCH on Hcrt cells remains unknown. Eventually, activation of the Hcrt system promotes arousal and increases metabolism, whereas the MCH system dampens energy expenditure and might promote sleep. **(b)** Complex hypothalamic circuits can be functionally deconstructed with high temporal and spatial resolution using optogenetics. Optogenetic stimulation/inhibition of genetically targeted cells (e.g. Hcrt, as shown in the figure) avoids inadvertent activation/inhibition of neighboring cells (e.g. gray neuronal populations, as shown in the figure). Combination of optogenetics with imaging of fluorescent calcium sensors (Ca^{2+}) or voltage-sensitive dyes (VSD) of identified neuronal populations (e.g. NPY/AgRP, as shown in figure) in brain slices will reveal synaptic function and plasticity associated with metabolism and arousal. In addition, bath application of metabolic factors (leptin, ghrelin and glucose), variation of environmental parameters (temperature, pH and CO_2) and animal models of neurological disorders (narcoleptic mice, *ob/ob* obese mice, and so on) might be used to mimic pathophysiological conditions.

enable all-optical interrogation of neuronal circuit function and plasticity associated with behavior [60] (Figure 3b). Eventually, all-optical approaches will define the precise dynamics and functions of hypothalamic circuits in arousal and compulsive consummatory behaviors. Importantly, this will improve our understanding of a variety of neuropsychiatric disorders, from sleep to consummatory disorders.

Summary and perspectives

In this review, we proposed that Hcrt and MCH systems provide sensory functions of arousal and metabolism that are integrated with additional physiological inputs (circadian, motivation and environment) into coherent goal-oriented behaviors. The Hcrt system acts as a sensor of arousal through its sensitivity to circulating metabolic factors (leptin, ghrelin and glucose), in addition to its effector signals of arousal and reward. Although it requires further investigation, the MCH system dampens energy expenditure [17] and might promote sleep. We hypothesize that plasticity of this functional circuit results in a positive feedback loop between Hcrt sensory and effector properties that might be responsible for the metabolic disorders associated with sleep perturbation. Optical tools now enable functional deconstruction of neuronal circuits with unprecedented spatial and temporal resolution relevant to *in vivo* physiological conditions. The synergy between such optical tools and more classic techniques will, undoubtedly, define the role of hypothalamic circuits in regulating sleep and wakefulness according to homeostatic processes, stress, motivation and reward.

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