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Review

Physiological arousal: a role for hypothalamic systems

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Abstract. The lateral hypothalamus (LH) has long been known as a homeostasis center of the brain that modulates feeding behavior, arousal and reward. The hypocretins (Hcrts, also called orexins) and melanin-concentrating hormone (MCH) are neuropeptides produced in two intermingled populations of a few thousand neurons in the LH. The Hcrts have a prominent role in regulating the stability of arousal, since Hcrt system deficiency leads to narcolepsy. MCH is an important modulator of energy balance, as MCH system deficiency in mice leads to leanness and increased metabolism. Recently, MCH has been proposed to modulate rapid eye movement sleep in rodents. In this review, we propose a working model of the cross-talk between Hcrt and MCH circuits that may provide an arousal balance system to regulate complex goal-oriented behaviors.

Keywords. Hypocretin, melanin-concentrating hormone, arousal, goal-oriented behavior.

The lateral hypothalamus (LH) contains multiple cell types with different neurochemical profiles, including glutamate, gamma-aminobutyric acid (GABA), tyrosine hydroxylase, melanocortins, substance P, dynorphin, nesfatin-1 and cocaine and amphetamine-regulated transcripts (CART) [1, 2]. These neuronal populations form local and extensive neuronal circuits involved in hypothalamic control of food intake, energy homeostasis, vigilance state and reward. This review emphasizes the modulatory role of the hypothalamic peptides hypocretins (Hcrts, also known as orexins) and melanin-concentrating hormone (MCH) on arousal and goal-oriented behaviors.

Hcrt and MCH systems

The Hcrts are neuroexcitatory peptides produced in ~3200 neurons in the mouse brain restricted to the perifornical area of the hypothalamus (also called the posterior hypothalamus [3, 4]). In this review, we will refer to the peptides by their first-used name, the hypocretins, due to sequence similarities with various members of the incretin family, and its hypothalamic localization. The term 'orexin' refers to its orexigenic effect observed after intracerebroventricular (icv) administration of pharmacological doses in rats [4]. However, as described below, increasing evidence suggest that the main function of these peptides might be related to reward rather than appetite.

The Hcrt peptides are processed from a 130-amino-acid precursor. The C-terminal 19 residues of these two peptides – Hcrt-1 (33 residues; EPLPDCCRQKTCSCRLYELLHGAGNHAAGILTL-amide) and Hcrt-2 (28 residues; RPGPPGLQGRLQRLLQANGNHAAGILTM-amide) – share 13 amino acid identities, suggesting that the peptides have related structures and functions [3]. This region of Hcrt-2 contains a seven-amino-acid match with secretin. Hcrt-1 contains two intrachain disulfide bonds. Human Hcrt-1 is identical to the rodent peptide, whereas human Hcrt-2 differs from rodent Hcrt-2 at

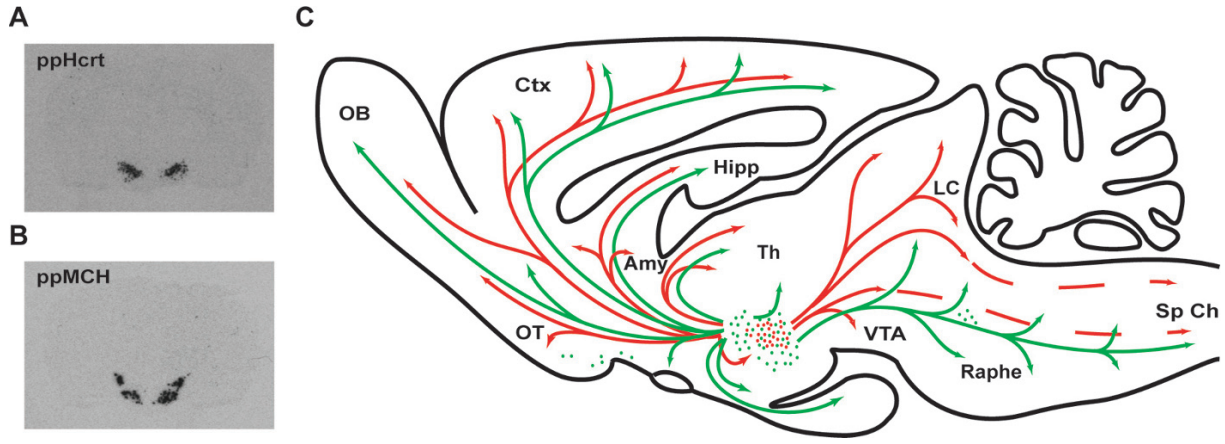

Figure 1. Neuroanatomical distribution of the Hcrt and MCH systems. (*A*, *B*) *In situ* hybridization of pp Hcrt (*A*) and ppMCH (*B*) precursor mRNA in a coronal brain section in mouse. (*C*), Schematic drawing of a saggital section through the rat brain showing the neuroanatomical organization of the Hcrt (red) and MCH (green) systems. Dots indicate the relative location and abundance of MCH- and Hcrt-expressing cell bodies. Arrows point out some of the more prominent terminal fields. Note that both systems project to the main arousal center of the brain including the LC (noradrenergic cells), histaminergic neurons of the posterior hypothalamus, cholinergic cells of the basal forebrain, serotonine-procuding neurons of the raphe, and dopaminergic neurons of the VTA. Amy, amygdala; Ctx, cortex; Hipp, hippocampus; LC, locus coeruleus; OB, olfactory bulb; OT, olfactory tubercule; Sp Ch, spinal chord; Th, thalamus; VTA, ventrotegmental area.

two residues. The non-amidated forms of the peptides are not electrophysiologically active.

MCH was originally discovered in salmon pituitaries, where it acts as a circulating hormone that lightens skin color in response to background color change or during stress reaction [5, 6]. Subsequent to its identification in fish, MCH immunoreactive-like labeling in rat brain sections led to the identification of this peptide, its corresponding gene and its tissue localization [7, 8]. Since then, MCH-producing neurons have been identified in the LH and Zona Incerta (ZI) of all the vertebrates examined so far [9–19]. In mammals, MCH is generated by enzymatic cleavage of a 165 residue precursor. The MCH intrachain disulfide bond leads to a cyclic peptide (19 residues: DFDMLRCMLGRVYRPCWQV) [20, 21]. Strong sequence similarity has been found with the somatostatin peptide family. Alternate splicing and antisense coding at the MCH locus generate additional transcripts and proteins [22–24]. Two MCH gene variants have been identified in humans [25–28].

Hypothalamic Hcrt and MCH neurons are morphologically very similar: multipolar or fusiform in shape, with two to five primary dendrites that are either smooth or sparsely invested with dendritic spines [9, 29]. In rats (~6700 neurons [30]) and humans (~50000–80000 neurons [31]) Hcrt mRNA (Fig. 1a) and Hcrt immunoreactivity are located in the perifornical area of the LH [3, 29]. In addition to their close localization to the Hcrt neurons within the LH [32], MCH-producing neurons (~12000 in the rat brain [30]) are also found in the ZI (Fig. 1b) [9, 33]. Low levels of MCH mRNA have been detected in the pontine reticular formation and the protein was localized immunohistochemically in the caudal laterodorsal tegmental nucleus of rat brain [9, 33, 34].

In the mammalian brain, both neuronal populations send parallel overlapping projections to several brain areas including the cortex, hippocampus, amygdala, nucleus accumbens, the hypothalamus itself, thalamus, ventral tegmental area (VTA), locus coeruleus (LC) and the raphe. (Fig. 1c) [3, 9, 29]. Sparse anatomical and physiological evidences support the existence of subpopulations of Hcrt or MCH neurons. Based on *c*-Fos immunocytochemistry following morphine preference and on the pattern of afferent connections, Aston-Jones and colleagues have proposed two subpopulations of Hcrt neurons related to arousal (dorsomedial hypothalamus subpopulation), consummatory behaviors and reward seeking (LH subpopulation) [35–40]. However, some discrepancies persist among those studies, as the anatomical efferents from the VTA label a sparse population of neurons distributed in both medial and lateral regions [41].

The projections of MCH-containing neurons and their colocalization with CART peptides define subpopulations of MCH cells [35, 37, 42–46].

In addition to their restricted location within the LH, both Hcrt and MCH neurons project widely throughout the brain including arousal and reward circuits. These neuroanatomical features, which classically belong to the monoaminergic systems, suggest a modulatory role of physiological arousal and goal-oriented behaviors.

Hcrts and MCH receptors

The initial orphan G-protein-coupled receptor (GPCR), Hcrtr1, binds Hcrt-1 with high affinity, but Hcrt-2 with 100- to 1,000-fold lower affinity [4]. A related GPCR, Hcrtr2, sharing 64% identity with Hcrtr1, was identified by searching database entries with the Hcrtr1 sequence and had a high affinity for both Hcrt-1 and Hcrt-2. These two receptors are highly conserved (95%) across species [4]. The mRNAs that encode the two hypocretin receptors and the receptor proteins themselves, detected by immunohistochemistry, are both enriched in the central nervous system (CNS) but have different distributions within the brain [47–49]. The distribution of Hcrt receptors is largely consistent with Hcrt axon innervation patterns.

An orphan GPCR (named SLC-1 for 'somatostatin-like receptor 1') was identified as the first MCH receptor (MCH-R1) [50–52]. *In silico* analysis of the human genome sequences identified a second MCH receptor (MCH-R2) which shares 38% identity with MCH-R1 [53–57]. However, MCH-R2 is only functional in dog, ferret, monkey and human, whereas it is absent or non-functional in mouse, hamster, rat, guinea pig and rabbit [58]. The MCH-R1 and the MCH-R2 genes are composed of two and five exons, respectively. Expression of MCH-R2 is restricted to the cortex, hippocampus, amygdala, and hypothalamus in the CNS [54, 56, 59].

In rodents, the combined distribution of the two Hcrt receptors strongly resembles the distribution of the MCH-R1 [60]. In the LC, amygdala and other brainstem noradrenergic groups, MCH-R1 mRNA distribution is similar to that of Hcrtr1 mRNA. In regions such as the septum, hypothalamus and much of the brainstem, the distribution of MCH-R1 mRNA resembles that of Hcrtr2 [60]. Some local differences exist in the distribution of the Hcrts and MCH receptors, such as the presence of MCH-R1 mRNA in CA1 through CA3 of the hippocampus, whereas HcrtR1 is found in CA1 and CA2 fields and Hcrtr2 is only expressed in CA2. The Hcrt and MCH receptors are also widely expressed in the periphery, especially in endocrine tissues including the pituitary, adrenal gland, testis, gastrointestinal tract, pancreas and pineal gland [47–49]. This perfect match between Hcrt and MCH system projections and their respective receptors suggests that both systems might modulate identical brain nuclei and thus target the same physiological functions.

Both Hcrt-1 and Hcrt-2 act through a family of GTP-binding proteins (G_q) that activate protein kinase C (PKC) and mobilization of intracellular calcium [61]. G_q-activated signaling cascades result in phosphorylation of Ca^{2+} channels, which can increase Ca^{2+} conductance and neuronal excitability. The MCH-R1 receptor could be coupled to $G_{i/o}$, G_α, G_s [52, 62–65] and activate different intracellular cascades including MAPK [66] and ERK1/2 [65, 67]. In contrast, the MCH-R2 receptor has only been reported to act through G_i [53]. Interestingly, activation of specific intracellular pathways by MCH binding on its cognate receptor might be subject to additional modulation through interactions of the receptor with cytosolic or membrane protein. Recently, using a yeast two-hybrid approach, the group of Bachner and collaborators identified several proteins (MIZIP for 'MCH-R1-interacting zinc finger protein,' periplakin and neurochondrin) which interact with the C terminus of the MCH-R1 receptor [68–70].

Consequently, modulation of receptor signaling pathways by alternative coupling of Hcrt and MCH receptors may multiply possible intracellular pathways activated by ligand-receptor binding.

Input and ouput

The LH is a central brain area that receives inputs from multiple, diverse neuronal populations. Among these are the descending projections from the limbic system, the forebrain, subsets of the hypothalamus itself (arcuate nucleus, dorso-median hypothalamus, paraventricular nuclei) and subcortical and thalamic area, whereas ascending projections come mostly from the brainstem arousal centers. In addition, several bundles of fibers including the fornix, the mamillo-thalamic tract and the medial forebrain bundle cross the Hcrt and MCH fields. Hcrt and MCH neurons are thus highly interconnected with a network of glutamatergic, GABAergic, dopaminergic and cholinergic neurons. Hcrt neurons coexpress glutamate [71] and part of the CART-expressing MCH neurons also express the glutamate decarboxylase enzyme suggesting that a subpopulation of MCH neurons are GABAergic [33, 45]. Recently, substance P has been colocalized with a few MCH neurons [72]. Glutamate [73, 74], ghrelin [75], glucagon-like peptide 1 [76], CRF [77], ATP [78], noradrenaline and carbachol (cholinergic agonist) [79] as well as cholecystokinin, neurotensin, vasopressin and oxytocin [80] exert excitatory properties on Hcrt neurons. Subpopulations of Hcrt cells are excitated by Ach [81]. On the other hand, GABA (through $GABA_{a,b}$) [73, 74, 82], glucose [74, 83, 84], serotonin (5-HT1a) [74, 85], noradrenaline (alpha 2) [75, 86], dopamine (alpha 2) [75], NPY [87], leptin [74], mAch (6%) and adenosine (A1) [88] inhibit Hcrt neurons. Accordingly, proteins detected in Hcrt neurons include

dynorphin [89], GABAA receptor epsilon subunit [90], 5-HT1a receptor [91], mu opioid receptor, pancreatic polypeptide Y4 receptor [92], adenosine A1, 2 receptor [30], leptin receptor [93, 94], the transcription factor STAT-3 [93, 94] and the neuronal pentraxin Narp [95], implicated in clustering of ionotropic glutamate receptors. Finally, acidification increases excitability of Hcrt neurons whereas alkalinization depresses it [96].

Van den Pol and collaborators have found that glutamate (along with AMPA and NMDA), ATP and Hcrt1 and Hcrt2 increased activity of MCH neurons [97]. Furthermore, neurotransmitters from extrahypothalamic arousal systems, including noradrenaline, serotonin, muscarin or carbachol (cholinergic agonist), inhibit MCH neurons [79, 97]. Interestingly, neuropeptide Y (NPY) has been shown to inhibit MCH neurons by pre- and post-synaptic mechanisms whereas MTII (a melanocortin agonist) and SHU9119 (a melanocortin antagonist) were without effect [97, 98]. Part of the MCH neuronal population expresses several membrane proteins including NK3 (neurokinin receptor) [72, 99, 100], CXCR4 (receptor to the chemokine stromal cell-derived factor-1 alpha) [101], leptin receptor (Ob-R, undefined subtype) [93], glutamate and GABA receptors [97, 98], adrenoreceptor (alpha2) [30], muscarinic and serotoninergic receptors and HcrtR1 and/or 2 [97, 98]. MCH neurons express a neuronal pentraxin NP1 [95].

Functional integration

As described above, the Hcrt and MCH systems are the target of arousal ascending brainstem projections and descending forebrain structures and, in turn, project reciprocally to these nuclei as well as to other brain areas [9, 29]. Thus, they are in an ideal position to integrate peripheral inputs, such as metabolic and other homeostatic afferents, and modulate efferent outputs such as goal-oriented behavior and arousal. In addition, Hcrt and MCH receptors may be coupled to different G proteins or other transmembrane proteins suggesting multimodal fine tuning of the signaling cascade activated by the selective ligands.

Activation and cross-talk between these two hypothalamic populations have a significant role in regulating arousal and goal-oriented behaviors, as detailed below.

Arousal

Concomitant discoveries of the link between narcolepsy – a chronic neurological disorder resulting in the destabilization of the sleep-wake cycle (e.g. excessive daytime sleepiness, cataplexy and fragmented sleep) – and the Hcrt system established one of the first important functions of Hcrt in arousal. Continuous recording of the behavior of mice deficient in the prepro-Hcrt gene [Hcrt knockout (KO) mice] revealed periods of ataxia, which were especially frequent during the dark/active period [102]. Electroencephalogram/electromyogram (EEG/EMG) recordings showed that these episodes were not related to epilepsy and that the mice displayed cataplexy-like attacks and their EEG traces showed episodes of direct transition from wakefulness to rapid eye movement (REM) sleep and lack of muscle tone. These features are highly reminiscent of narcolepsy. Similar observations were made in rats in which the Hcrt neurons in the LH were inactivated by saporin targeting, although in this model, cataplexy was not observed [103]. Hcrtr2 KO mice have a milder narcoleptic phenotype than the Hcrt KO animals [104]. Interestingly, Mochizuki and collaborators demonstrated that the behavioral-state instability of Hcrt KO animals is not a consequence of abnormal sleep homeostasis, poor circadian control or defective fundamental arousal systems [105]. Transgenic rats and mice depleted of Hcrt neurons (animals generated by expressing a mutant form of ataxin 3 in these cells) show a narcolepsy-like phenotype [74, 106] as well as reduced locomotor activity upon fasting [74], strongly suggesting that the Hcrt system is an important component of the brain circuit necessary to set up the arousal level depending on metabolic needs. Consistent with this hypothesis, data from recent studies demonstrated an increase of Hcrt neuron firing rate during the active wakefulness period with a strong locomotor activity and REM sleep-to-wake transitions [107, 108].

Hcrt concentrations in the cerebral spinal fluid (CSF) are tightly regulated in healthy humans, whereas most patients with narcolepsy had neither Hcrt-producing neurons nor detectable Hcrt levels in the CSF [109, 110]. Interestingly, the codistributed MCH neurons were unaffected in patients with narcolepsy [109, 110].

More recently, we found that selective photostimulation of channelrhodospin-2-expressing Hcrt neurons using optical tools increases the probability of non-REM (NREM) sleep- and REM sleep-to-wake transition latencies *in vivo* [111]. Interestingly, we observed a ~50% reduction of the latency to wakefulness transitions, suggesting that other arousal systems in the brain need to be in an 'active' state to relay the Hcrt neuronal network outputs and induce fast awakenings. Although chronic stimulation of the Hcrt neurons increases the number of sleep-to-wake transitions, it did not enhance the amount of wakefulness; further experiments using bilateral stimulation

and alternative patterns of light pulse trains should define the role of Hcrt in wakefulness maintenance. Narcolepsy is specific to a lack of function of the Hcrt system. Interestingly, the MCH system seems intact in narcoleptic patients [109], and no mutation in the ppMCH or MCHR1/2 genes has yet been found or linked to an arousal pathology. Although the cellular targets of the MCH system are well characterized, its functional role on arousal remains unclear. *c*-Fos immunodetection revealed an increase of *c*-Fos$^+$/MCH$^+$ double-positive neurons after sleep deprivation, suggesting that the MCH system is active during a sleep period occurring after prolonged wakefulness and might thus promote sleep [30, 112]. Verret and collaborators showed that during a REM sleep rebound induced by specific REM sleep deprivation in rats, a large population of MCH neurons (~60 %) is immunoreactive for *c*-Fos protein. In addition, icv infusion of the peptide in rats increases the amount of REM sleep and, to a lesser extent, NREM sleep [112]. These data suggest that the MCH system might be a counterpart to the Hcrt system in tuning arousal and behavioral state stability, but this hypothesis needs further experimental proof. MCH neurons have been found to be inhibited by noradrenaline and carbachol (a cholinergic agonist) *in vitro* [79]. Interestingly, MCH/Hcrt double-knockout animals exhibited more behavioral attack than the Hcrt KO mice, suggesting that the MCH system is important for arousal stability [113].

Goal-oriented behaviors

The elaboration of goal-oriented behavior requires an appropriate level of arousal. Such behavior can be elicited by natural (food, thirst, sex) or artificial (drug) stimuli. Depending on those physiological needs, specific circuits are likely to support arousal. Here, we will focus on Hcrt and MCH system modulation of food intake, energy homeostasis and drug addiction. Icv administration of either Hcrt-1 or Hcrt-2 increased short-term food consumption in rats [4], sheep [114] and goldfish [115, 116]. Furthermore, rats that had been deprived of food for 48 h showed increased concentrations of hypocretin mRNA and peptides in the hypothalamus. The Hcrt system also influences, and is influenced by, primary energy homeostasis circuits [117]. For example, Hcrt neurons are sensitive to glucose [83], leptin [74], triglyceride [118] and carbon dioxide [96] concentrations. However, other findings suggest that the Hcrts are not critical players in food intake behavior, but rather play roles in increasing arousal and motivation levels allowing feeding to take place. Continuous administration of Hcrt-1 for 7 days in rats does not significantly alter daily food intake, body weight, blood glucose, total cholesterol or free fatty acid levels [119], suggesting that many Hcrt effects may be limited to short-term, immediate stimulation of feeding behavior consequent to the increased duration of wakefulness or locomotor activity. In addition, Hcrt KO mice show modest differences in food intake, and Hcrt-ataxin 3 mice, which would be expected to be lean, show obesity and hypolocomotion [106], an effect dependent on diet and genetic background [120]. Furthermore, during fasting, Hcrt-1 accumulation in the CSF does not exceed concentrations observed during the waking period [121]. All these data suggest that some of the food uptake effect may result from arousal rather than direct feeding pressure. An alternative hypothesis is that a functional Hcrt system is necessary for heightened arousal during food craving and/or food intake. This concept has been highlighted by the recent discovery of the implication of the Hcrt system in drug addiction.

Hcrt neurons project to reward systems of the brain, including the VTA, nucleus accumbens and prefrontal cortex. Hcrt neurons receive input from the limbic system and express the μ-opioid receptor [122]; hence their response may be directly related to morphine and naltrexone. Hcrt neurons are highly responsive to morphine and their activation is linked to preferences for cues associated with drug and food reward and also naltrexone-precipitated withdrawal [122, 123]. mRNA levels of ppHcrt and μ-opioid receptor increase after precipitated withdrawal [122, 124]. The levels of dopamine and its metabolites in the nucleus accumbens are markedly increased by an injection of Hcrt1 and Hcrt2 into the VTA [125]. Infusion of Hcrt1 in the brain ventricles elevates intracranial self-stimulation (ICSS) thresholds, indicating that the peptide decreases brain reward function, in a similar way as stress [126]. Moreover, infusion of Hcrt reinstates extinguished drug-seeking behavior, an effect prevented by blockade of the Hcrt1, noradrenergic and corticotropin-releasing factor receptor systems. Accordingly, Hcrtr1 receptor antagonist blocks footshock-induced reinstatement of previously extinguished cocaine-seeking behavior, leading to the conclusion that the Hcrt system is a gate in driving stress-mediated drug-seeking behavior [126]. Hcrt KO mice exhibit dramatically attenuated morphine withdrawal symptoms [122] and morphine-induced place preference and hyperlocomotion [125]. *In vitro* application of Hcrt-1 induces potentiation of N-methyl-D-aspartate receptor (NMDAR)-mediated neurotransmission via a PLC/PKC-dependent insertion of NMDARs in dopamine neuron synapses of the VTA, an effect occluded by an Hcrtr1 antagonist [127, 128].

Among the putative roles of MCH neurons, their involvement in feeding behavior, energy homeostasis and body weight are by far the best documented. Chronic intracerebral injection increases food intake during the hours following injections, without modifying the amount of ingested food for 24 h, or body weight. In addition, both ppMCH mRNA and MCH peptide increase after fasting as does MCHR1 mRNA [129]. PpMCH KO mice were found to be lean and hyperactive [130–132] whereas mice overexpressing MCH develop mild obesity and hyperphagia [133]. MCH-R1 KO mice are lean (in contrast to Hcrt KO) but hyperphagic, probably because of their increased nocturnal locomotor activity and consequent increased metabolic rate [131, 134, 135]. Accordingly, transgenic mice depleted of MCH neurons also develop late-onset leanness [136]. Interestingly, deletion of the MCH system in the leptin-deficient obese (ob/ob) mouse (double KO animals) dramatically reduced body fat with no alteration of their characteristic hyperphagia compared with ob/ob animals. Instead, leanness was secondary to a marked increase in energy expenditure, highlighting the importance of the MCH system in downregulation of energy expenditure [131]. Finally, leptin has been shown to depress MCH gene expression in a series of obese leptin-deficient animal models (ob/ob mouse, fa/fa rat, yellow agouti mice in which animals develop overfeeding) [129]. At the cellular level, glucose dose-dependently enhances the electrical excitability of MCH neurons by inducing depolarization and increasing membrane resistance, but has the opposite effect on the electrical activity of Hcrt cells [83]. As a result of this participation in food intake behavior and energy homeostasis, a plethora of MCHR1 antagonists have been developed in recent years [137, 138]. In contrast to the Hcrt system, MCH neurons are not activated during acute and chronic morphine treatment, or following naltrexone-induced withdrawal [122, 139]. However, one cannot rule out that better temporal resolution tools (single unit recording, optical stimulation/inhibition) might help in finding short-lasting activity changes (burst vs tonic firing) that are invisible with low temporal resolution methods (for example, *c*-Fos immunodetection). Interestingly, MCH injection in the nucleus accumbens has been shown to induce food intake whereas an MCH-R1 antagonist has the opposite effect [140]. These data highlight the importance of brain reward mechanisms in food intake and energy homeostasis. Furthermore, MCH-R1 KO mice fail to develop behavioral (locomotor) sensitization to cocaine and do not exhibit any sign of cocaine conditioning during a saline challenge [141]. MCH-R1 may contribute to the neurobiological mechanisms of conditioned cocaine-induced psychomotor effects and, to a lesser extent, those subserving acute pharmacological cocaine action.

Control of state boundary and arousal

Experimental studies across species described in this review demonstrated that the Hcrt and MCH systems are two important components in the control of state boundary and arousal.

What is physiological arousal in mammals? Arousal is a state of heightened alertness/responsiveness to sensory inputs mediated by the ascending pathways, which is accompanied by an increase of physiological activity (postural tone, cardiac and respiratory rhythms) mediated mainly by the reticular formation [142]. Thus, being aroused implies that the animal is awake, but to be awake does not necessarily mean to be aroused. The arousal centers of the brain are composed of nuclei producing the neurotransmitters acetylcholine (ACh; basal forebrain and pontine tegmentum), norepinephrine (NE; LC), dopamine (DA; VTA), serotonin (5-HT; raphe) and histamine (posterior hypothalamus) [143]. The ascending projections of the reticular formation separate into a dorsal (through the thalamus) and a ventral (through the VTA, lateral hypothalamus and basal forebrain) pathway before diffusely innervating the cortex (Fig. 1). The descending reticular formation projects directly to the spinal cord. The ascending pathway is responsible for cortical arousal, and the increase in physiological activity during arousal (postural tone, cardiac and respiratory rhythms) is mediated mainly by the descending pathways to motor neurons and activating autonomic systems [144].

Neurons producing NE (LC), 5-HT (raphe), histamine (tuberomamillary nucleus) or Hcrts (LH) fire during waking with behavioral arousal, decrease firing during slow-wave sleep (SWS) and cease firing during REM sleep [145]. Interestingly, much evidence shows that these neuron populations are involved in arousal and are also important neural substrates of natural (sex, food) and artificial (drugs) reward seeking [123, 126, 146–148]. Accordingly, Hcrt- and putatively MCH-producing neurons might be important systems involved in the fine-tuning of physiological arousal during reward-seeking behaviors as hypothesized in the model described below.

Model

Growing evidence from a combination of cellular, molecular and behavioral techniques indicates that the Hcrt system provides an alarm signal to arousal centers, possibly by lowering the arousal threshold

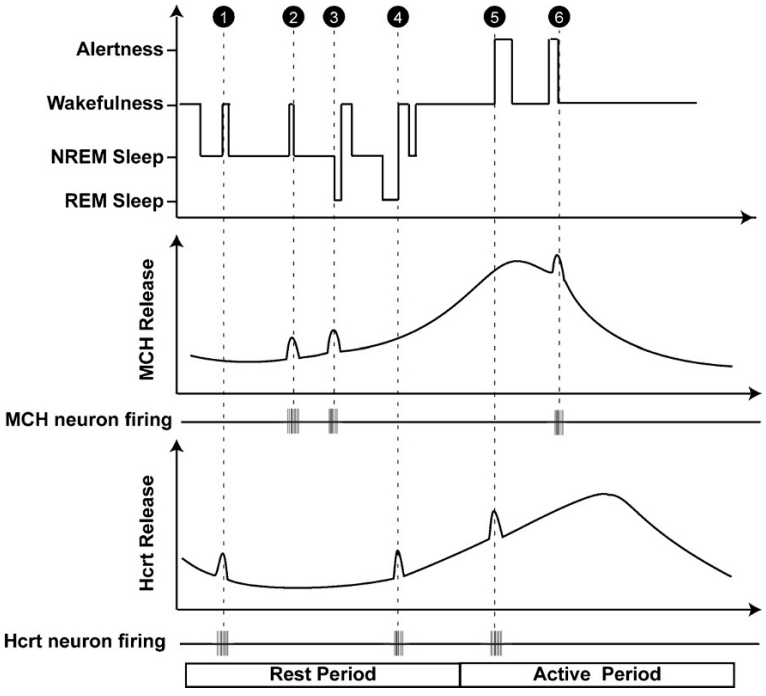

Figure 2. Model of Hcrt and MCH regulation of vigilance state and arousal across the light/dark cycle. Schematic representation of the circadian (slow) release of Hcrts and MCH peptides, on which is superimposed transient release of the peptides during phasic [burst or sustained firing during a short (~1 min) period] activity of these cells. Firing of Hcrt neurons during the rest period (=low CSF content of Hcrt and MCH) would promote short awakenings from NREM and REM sleep (transitions 1 and 4), whereas MCH tonic release would promote sleep (transitions 2 and 3). During the active phase (=high CSF content of Hcrt and MCH), sustained activity of Hcrt neurons would lead to an alert state. In contrast, MCH tonic release would dampen activity and energy expenditure. This balance could provide a coherent output that modulates goal-oriented behaviors.

[149]. As described above, pharmacological infusions of Hcrt1 in several rat brain areas induce sustained wakefulness [150] and inactivation of the Hcrt system results in altered behavioral state boundaries [102, 109, 110, 151]. A recent study has shown that triple lesions of basal forebrain cholinergic neurons, histaminergic neurons and noradrenergic LC neurons result in sleepiness during transition between rest to active periods, suggesting that combined activation of these cells groups by Hcrt maintains wakefulness [152]. Hcrt neurons are active during REM sleep-to-wake transitions, active waking (i.e. exploratory behaviors, grooming), consummatory behaviors and alertness elicited by external stimuli such as sound. Importantly, they are mostly silent during quiet wakefulness, SWS and REM sleep onset [107, 108]. Finally, selective stimulation of the Hcrt neuronal network using optogenetics increased the probability of sleep-to-wake transitions [111]. Altogether, these data suggest that the activity of the Hcrt system is linked to alertness. Interestingly, the Hcrt system is an important relay of hypothalamic response to stress-including acute stress [77] and stress-induced reinstatement of cocaine-seeking behavior [126].

Few experimental studies have reported a possible role for MCH in arousal. Pharmacological administration of MCH induces REM sleep and NREM, and MCH neurons are active during a REM sleep rebound [112]. Although MCH neurons are mostly inactive *in vitro* [97], MCH neuronal activity across the sleep-wake cycle or during goal-oriented behaviors is unknown.

It has been proposed that Hcrt and MCH systems act as two counterparts on the sleep/wake balance [112, 153, 154]. Interestingly, Hcrt1 peptide extracellular and CSF contents follow a circadian variation in rat [121, 155, 156], monkey [157] and human [158]. MCH mRNA follows a parallel circadian variation in different species [159–161], and it is expected that MCH peptide release follows a similar pattern. These circadian variations might be the result of direct or indirect inputs from the suprachiasmatic nucleus (SCN) to the Hcrt and MCH circuits [162], as lesions of the SCN suppress the daily rhythm of Hcrt1 content in the CSF [156].

Therefore, we hypothesize that release of Hcrts or MCH might induce specific behavioral transitions depending on the circadian time and the physiological needs. Hcrt and MCH net function would be the result of a superimposed slow circadian (tonic) and a fast (phasic) acting rhythm of peptide release. The increase of Hcrt (and possibly MCH) concentration in the CSF during the active phase might be a direct consequence of increased neuronal activity. Alternatively, it could indirectly result from a prolonged persistence of the peptides in the extracellular space during this period. In contrast, phasic release of the peptide would correspond to a transient release of the peptide in the synaptic cleft after a burst mode activity. As shown in Figure 2, release or persistence of the Hcrts and MCH peptides in the brain displays a circadian pattern with a peak during the active phase of the animals. A transient release of peptides from tonic activity of the cells could be superimposed on

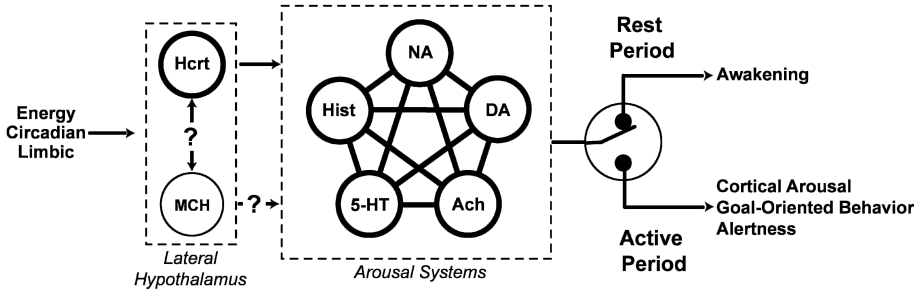

Figure 3. Schematic interactions of hypothalamic Hcrt and MCH systems on arousal centers of the brain. The Hcrt and MCH systems are the targets of multiple physiological stimuli including direct and indirect external (circadian, social interaction) and internal signals (metabolic status, limbic inputs, reward). After integration of those signals, the hypothalamic Hcrt and MCH neurons would modulate the activity of the arousal centers of the brain which would lead to a fine-tuning of arousal as described in Figure 2. Hcrt neuronal projections to these arousal centers, which in turn project back onto Hcrt cells, would be an important brain circuit subserving awakening, cortical arousal and alertness depending on the circadian timing. According to the model proposed here, Hcrt neurons would promote wakefulness or arousal by direct and indirect modulation of arousal center activity. Although the MCH system is thought to promote sleep, its mechanism of action with LH and other cell populations within the brain remains to be determined. Ach, acetylcholine; DA, dopamine; Hist, histamine; NA, noradrenaline; 5-HT, serotonin.

this slow circadian variation (Fig. 2, transitions 1 to 6). Thus, fast release of Hcrts during NREM or REM sleep would induce transitions to wakefulness during the rest period, [107, 108, 111] (Fig. 2, transitions 1 and 4, respectively), most likely by excitation of arousal centers such as the histaminergic cells of the tuberomamillary nuclei [163] and the noradrenergic neurons of the LC [150]. Waking events would often be short-lasting due to strong inhibition of brain arousal centers [154, 164] and low level of Hcrts during this period. Reciprocally, during the rest period, MCH tonic release might promote sleep [30, 79, 112] by reducing the latency of Wake-to-NREM sleep or NREM-to-REM sleep transitions (Fig. 2, transitions 2 and 3, respectively). This might be achieved by MCH-mediated inhibition of arousal centers of the brain targeted by the MCH system. However, it is still unknown if MCH is able to inhibit hypothalamic and extra-hypothalamic arousal centers. Interestingly, MCH-producing neurons are inhibited by norepinephrine, serotonin, and Hcrt *in vitro* [97].

During the active period, high levels of Hcrts could participate in wakefulness maintenance, whereas tonic release of the peptide may induce transitions to a 'hyperarousal' state (Fig. 2, transition 5). Such a state would occur during sustained (locomotor) active goal-oriented behavior driven by motivation toward natural [108, 148] and artificial [123, 126, 128] rewards. Circadian or phasic release of MCH during this period might have an opposite effect on Hcrt function by decreasing energy expenditure [131, 134] and promoting transitions to a quiet wake state by inhibiting arousal center activity (Fig. 2, transition 6).

The lack of fluctuating Hcrt peptide levels across the sleep-wake cycle in narcolepsy would lead to the absence of counterbalance to the sleep-promoting systems, such as the GABA- and glycine-producing cells of the ventero-lateral preoptic area and MCH-expressing neurons of the LH, leading to an unstable sleep-wake balance. This would result in unexpected switches to wakefulness during the rest period and an inability to promote or maintain alertness required for goal-oriented behaviors during the active period [153, 154, 165]. Accordingly, the lack of a functional Hcrt system in rodents leads to decreased rewarding effect and hyperlocomotion induced by morphine [125] and an inability to maintain locomotor activity associated with food-seeking behavior during fasting [74]. Furthermore, behavioral arrests seen in mice, dogs and narcoleptic patients are often elicited by emotional and cognitive stimuli such as food reward and social interactions [166].

This theoretical framework integrates the different combinations of Hcrt and MCH system activities across the light-dark cycle. Although the functional role of MCH in arousal remains to be characterized, the Hcrt system promotes arousal by modulating the activity of arousal centers of the brain such as the LC, raphe, VTA, posterior hypothalamus and basal forebrain (Fig. 3). The Hcrt peptides depolarize MCH neurons *in vitro* [97] but the actions of MCH on the Hcrt neurons remain unknown.

Consequently, Hcrt and MCH circuits are two important modulatory systems of the brain that integrate multiple converging inputs including circadian inputs, metabolic status and reward. A combination of cellular and molecular technologies with high spatial and temporal resolutions detecting and inducing neural network activity such as optogenetics and imaging calcium- and voltage-sensitive dyes with genetically modified animals will undoubtedly shed light on the modulatory function of the Hcrt and MCH systems. Future studies on the cross-talk between these two neuronal circuits and their temporal dy-

namics will provide further insight into arousal mechanisms and neuropsychiatric disorders linked to motivation.

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