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# Hypocretins and the neurobiology of sleep–wake mechanisms

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## Abstract

In 1998, our group discovered a cDNA that encoded the precursor of two putative neuropeptides that we called hypocretins for their hypothalamic expression and their similarity to the secretin family of neuropeptides. In the past 15 years, numerous studies have placed the hypocretin system as an integrator of homeostatic functions with a crucial, nonredundant function as an arousal stabilizer. Here, we discuss some of the data that have accumulated over the years on the integrating capacity of these hypothalamic neurons and their role on sleep-to-wake transitions.

### Keywords

lateral hypothalamus; sleep; arousal; narcolepsy; insomnia; addiction; reward

## Introduction

In the past few years, the hypocretins (also known as orexins) have been shown to be critical components of the brain circuitry that modulates the states of vigilance (Mignot et al., 2002; Sutcliffe and de Lecea, 2002; Willie et al., 2001). Recent advances are yielding a clearer picture as to the mechanism of action of these peptides, and how they control multiple circuits to produce a coherent behavioral output. Here, I review the interactions of the hypocretinergic system with the major neurotransmitter networks and discuss the role of the neurons that contain hypocretin in integrating information that dictates the state of arousal.

## Discovery and properties of the hypocretins

Analysis of the expression patterns of subtracted hypothalamus-enriched sequences (Gautvik et al., 1996) revealed that one of these was expressed exclusively by a bilaterally symmetric structure within the posterior hypothalamus (Fig. 1). Its nucleotide sequence (de Lecea et al., 1998) encoded a 130-residue putative secretory protein (preprohypocretin) with an apparent signal sequence and three additional sites for potential proteolytic maturation. Two of the 4 putative products of proteolysis had 14 amino acid identities across 20 residues. This region of one of the peptides contained a 7/7 match with secretin, suggesting that the prepropeptide gave rise to two peptide products that were structurally related both to each other and to secretin. Thus, these peptides were named hypocretin (Hcrt) 1 and 2 to reflect their

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hypothalamic origin and the similarity to secretin, which also extends to the secondary structure (Lee et al., 1999).

Parallel work in another laboratory described the orexins as ligands of two G-protein-coupled receptors: HcrtR1 and HcrtR2 (Sakurai et al., 1998). Hcrt1 binds with equal affinity to both receptors, whereas Hcrt2 binds with preferential affinity to HcrtR2 (see Chapter 1). Immunocytochemical mapping using antisera against chemically synthesized hypocretin peptides has shown that hypocretin neurons project their terminals throughout the brain (Peyron et al., 1998). Within the synaptic terminals of these fibers, hypocretin immunoreactivity is associated with dense core secretory vesicles (de Lecea et al., 1998). Efferents of hypocretin neurons include an ascending pathway that projects to the basal forebrain, septum, and cerebral cortex; a very dense intra-hypothalamic network; and a descending pathway that connects the lateral hypothalamus with brainstem nuclei and the spinal cord (Peyron et al., 1998). Both hypocretin peptides (Hcrt1 and Hcrt2) are neuroexcitatory (de Lecea et al., 1998; van den Pol et al., 1998) and bind to postsynaptic Hcrt receptors (HcrtR1 and HcrtR2) with different selective affinities (Sakurai et al., 1998). The distribution of Hcrt fibers matches with that of the described hypocretin receptors (Marcus et al., 2001) and suggests that the hypocretins interact with multiple neurotransmitter networks involved in different functions.

## Loss of function

The studies showing that hypocretin mRNA is absent from narcoleptic brains (Peyron et al., 2000) and that Hcrt immunoreactivity is highly decreased in narcoleptic hypothalami (Thannickal et al., 2000) provide compelling evidence that the main function of the hypocretinergic system is the regulation of arousal circuits.

Narcoleptic patients with cataplexy have non- or barely detectable levels of Hcrt1 in the cerebrospinal fluid, (Nishino et al., 2000) in addition to the absence of prepro*Hcrt* gene transcripts in the hypothalamus (Peyron et al., 2000; Thannickal et al., 2000). Doberman narcoleptic dogs bear a mutation in HcrtR2, and all genetically engineered rodents with either a deletion of the Hcrt (Chemelli et al., 1999). HcrtR2 gene (Willie et al., 2003) or Hcrt cells present behavioral arrests that resemble cataplexy, the hallmark of narcolepsy. HcrtR1 KO mice do not show any overt sleep abnormality, and HcrtR2-deficient mice are less affected with cataplexy-like attacks of REM sleep compared to the mice deficient in peptide ligand that are more severely affected (Willie et al., 2003), suggesting that the altered REM sleep control in narcolepsy–cataplexy syndrome emerges from the loss of signaling through both HcrtR2-dependent and HcrtR2-independent pathways (Willie et al., 2003). These studies support a role for the Hcrt system in “lowering the arousal threshold” (Sutcliffe and de Lecea, 2002) resulting in a facilitation of wakefulness when animals are asleep.

## Hypocretin neuronal activity

Recordings of Hcrt neuronal activity in freely moving (Mileykovskiy et al., 2005) and in head restraint (Lee et al., 2005) rats revealed that Hcrt neurons fire phasically in correlation with the locomotor activity and are mostly silent during NREM and REM sleep.

Interestingly, the highest frequency of activity was found during the transitions of vigilance states and in anticipation of a reward signal. This phasic pattern of activity questioned the behavioral effects of the pharmacological experiments infusing large amounts of Hcrt peptide ligand in the brain, which would mimic, in the best possible conditions, an increase in tonic activity.

Recently, we and others have used optogenetic (Adamantidis et al., 2007) and pharmacogenetic (Sasaki et al., 2011) approaches to mimic phasic activity with millisecond resolution and determine the causal relationships between the activity of Hcrt neuronal circuit and arousal transitions. We found that direct, deep brain optical stimulation of hypocretin neurons in the hypothalamus increased the probability of transitions to wakefulness from either NREM or REM. Interestingly, photostimulation using 5–30 Hz light pulse trains reduced latency to wakefulness, whereas 1 Hz trains did not. We also asked whether Hcrt-mediated sleep-to-wake transitions are affected by light/dark period and sleep pressure. We found that stimulation of Hcrt neurons increased the probability of an awakening event throughout the entire light/dark period but that this effect was diminished with sleep pressure induced by 2 or 4 h of sleep deprivation (Carter et al., 2009). These results suggest that the Hcrt system promotes wakefulness throughout the light/dark period by activating multiple downstream targets, which themselves are inhibited with increased sleep pressure.

In contrast to the loss-of-function phenotype, overactivation of the Hcrt release has been associated with hyperarousal response associated with stress, panic disorder, and addictive behaviors (see below).

## Arousal circuits modulated by the hypocretins

### Hypothalamus

Hcrt neurons are localized in the lateral hypothalamus, an area long known as a key center for the regulation of energy homeostasis. Therefore, it was only logical that the first hypotheses about Hcrt function involved feeding and energy balance (Sakurai et al., 1998). Indeed, Hcrt neurons are connected with the main networks regulating feeding. The connectivity between NPY-positive neurons in the arcuate nucleus and Hcrt neurons has been demonstrated (Broberger et al., 1998; Elias et al., 1998). Hcrt neurons are also innervated by POMC-containing terminals. Hcrt neurons also appear to activate themselves through HcrtR2 (Yamanaka et al., 2010). Additional GABAergic input to Hcrt cells includes melanin-concentrating hormone neurons as well as neurons containing leptin receptor (Leinninger et al., 2009).

Further, hypocretin neurons are sensitive to glucose, ghrelin, triglycerides, and amino acids (Cai et al., 1999; Karnani et al., 2011; Lopez et al., 2000; Wortley et al., 2003; Yamanaka et al., 2003). In an elegant study, Hara et al. (2001) showed that genetic ablation of Hcrt neurons in transgenic mice results in obesity and hypophagia, suggesting that the balance between storage and expenditure is impaired in these mice. Together, the available data strongly suggest that the main function of the Hcrt peptides is not increasing food intake, but generating a coherent output that stabilizes brain states.

In addition to the circuitry that modulates energy balance, hypocretin neurons contact several hypothalamic nuclei involved in sleep and wakefulness, including the ventrolateral preoptic nucleus (VLPO), the dorsomedial hypothalamus (DMH), and the tuberomammilary nucleus (TMN). Hypocretin neurons only account for 4% of the lateral hypothalamic input to the VLPO, which is mostly active during NREM sleep (Chou et al., 2002). The DMH is a key relay nucleus that receives input from the internal clock (Chou et al., 2003). Both hypocretin peptides excite histaminergic neurons of the TMN, probably acting through HcrtR2, and knockout mice deficient in histamine receptor 1 are impervious to hypocretin administration, suggesting that at least some of the effects of the Hcrts are caused by release of histamine and activation of postsynaptic H1 receptors (Huang et al., 2001). However, optogenetic stimulation of Hcrt neurons in histamine-deficient mice did not affect the ability of Hcrt to increase the probability of awakenings, suggesting that the Histamine is not an essential factor in this circuit.

## Locus coeruleus

The densest projection of Hcrt fibers terminate in the locus coeruleus area, the main site of noradrenergic transmission. Thus, this system was one of the first targets of the hypocretinergic system to be analyzed (Bourgin et al., 2000; Hagan et al., 1999). Noradrenergic neurons of the locus coeruleus are active during wakefulness, display low activity during slow wave sleep, are silent during REM sleep, and are thought to be critical for the alternation of the REM–NREM sleep (Pace-Schott and Hobson, 2002). Most of the LC neurons express HcrtR1 but not Hcrt2. This is important because HcrtR1-deficient animals do not have overt sleep abnormalities or cataplexy (Willie et al., 2003). Local administration of Hcrt1 in the LC increases wakefulness and suppresses REM sleep in a dose-dependent manner, and this effect can be blocked by antisera that prevent binding of Hcrt to its receptors (Bourgin et al., 2000). Application of Hcrt1 peptide to slices of the locus coeruleus increased the firing rate of noradrenergic neurons, possibly by decreasing the afterhyperpolarization current (Horvath et al., 1999).

Recent optogenetic studies have shown that a brief train of pulses is sufficient to induce an awakening (Carter et al., 2010). In particular, combinations, frequencies, and durations that led to at least 20 pulses during 5 s were deterministic in inducing an awakening. Since noradrenergic neurons in the LC contain high concentrations of HcrtR1 (Bourgin et al., 2000), it is possible that a mild, phasic stimulation of Hcrt neurons facilitates awakenings directly by depolarizing LC neurons.

## Brainstem cholinergic nuclei

The major cholinergic input to the thalamus is from the laterodorsal tegmental nucleus (LDT) and the adjacent pedunculopontine tegmental nucleus (PPT). These neurons act on the thalamocortical network to provoke the tonic activation subtending both sensory transmission and cortical activation during arousal (Steriade and Llinas, 1988). Considerable evidence has also indicated that mesopontine cholinergic nuclei also play a role in generating REM sleep, notably by stimulating the medial pontine reticular formation. Thus, cholinergic neurons in LDT and PPT, by promoting either EEG desynchronization and wakefulness or REM sleep, play a key role in regulating the vigilance state (Jones, 1991).

The wide descending hypocretinergic projection includes the mesopontine cholinergic system (Peyron et al., 1998). Moreover, HcrtR1 mRNA has been detected in these mesopontine cholinergic nuclei (Greco and Shiromani, 2001; Marcus et al., 2001; Trivedi et al., 1998). Hcrt peptides excite cholinergic neurons in the LDT (Burlet et al., 2002; Takahashi et al., 2002), and injection of Hcrt1 into the rat LDT increases wakefulness at the expense of NREM sleep (Xi et al., 2001). It has been hypothesized that the hypocretin system may coordinate activation of the entire ascending reticular activating system (see below).

## The basal forebrain

The majority of neurons in the magnocellular basal forebrain are wakefulness active with highest discharge activity during wakefulness and a marked reduction in activity just before and during the entry to NREM sleep. A variety of basal forebrain structures receive a moderate hypocretin innervation. Infusion of hypocretin peptides into the medial septal area significantly increases wakefulness (Espana et al., 2001). Infusion of Hcrt1 in slices shows a strong and direct excitatory effect on the cholinergic neurons of the basal forebrain. Interestingly, these effects are mediated through HcrtR2 which are those lacking in narcoleptic dogs. Interestingly, some studies have linked hypocretin secretion to the processing of beta amyloid and the progression of Alzheimer's disease. Since cholinergic neurons in the basal forebrain are among the first to be affected in this disorder, it is possible that the hypocretins excite cholinergic neurons that release acetylcholine in the cerebral cortex and thereby contribute to cortical arousal.

## The VTA/NAcc reward circuit

Ventral tegmental area (VTA) contains cell bodies of dopaminergic neurons projecting to the nucleus accumbens, amygdala, hippocampus, and prefrontal cortex. Defined as the mesocorticolimbic dopamine system (Albanese and Minciacchi, 1983), these neurons are critically implicated in brain mechanisms of reward, reinforcement, and emotional arousal (Wise and Rompre, 1989). Their activity has been closely correlated to the availability of primary rewards such as food, water, and sexual behavior (Schultz, 1998). The mesolimbic dopamine system, which is an established component of the reward system, receives glutamatergic input from cortical structures including the medial and occipital prefrontal cortex and amygdala, GABAergic inputs from striatal sources, and cholinergic input from the brainstem (Wise, 2002). Hypocretin activity may mimic lateral hypothalamic self stimulation, activating the LDT/PPTg nuclei and subsequently increase the activity of dopaminergic neurons in the VTA (Wise, 2002). In addition of the hypothetical indirect activation of VTA dopaminergic neurons by Hcrt via the LDT/PPT brainstem nuclei, Hcrt neurons directly excite dopamine fibers in the VTA (Fadel and Deutch, 2002; Korotkova et al., 2003; Uramura et al., 2001) and that the VTA dopaminergic system is critically involved in hypocretin-induced hyperlocomotion and stereotypy (Nakamura et al., 2000). Hcrt appears to increase glutamatergic excitability in VTA synapses (Borgland et al., 2006, 2009). Lastly, hypocretin-immunoreactive fibers and receptors are present in the nucleus accumbens (Peyron et al., 1998), and Hcrt peptides modify the response to glutamate and GABA in this nucleus (Martin et al., 2002). We and others (Boutrel et al., 2005; Harris et al., 2005) demonstrated a functional association between Hcrt activation and relapse of drug-

seeking behavior, suggesting that Hcrt activation increases the allostatic load that may develop into pathological hyperarousal associated with compulsivity and addictive behaviors.

## The HPA axis

Hcrt peptides interact with autonomic, neuroendocrine, and neuroregulatory systems (Date et al., 2000; Hagan et al., 1999) and have recently been shown to be mediators of the stress response (Ida et al., 2000). Thus, the hypocretinergic system has been associated with increased sympathetic tone (Samson et al., 1999). Immunocytochemical studies have shown long descending Hcrt-containing axonal projections from the lateral hypothalamus to the spinal cord (van den Pol, 1999). Innervation of the intermediolateral column and lamina 10 suggests that the Hcrt may participate in the sympathetic and parasympathetic components of the autonomic nervous system. Indeed, injection of an agonist for the Hcrt1 receptor increases heart rate, blood pressure, cerebral blood flow, and renal sympathetic activity in awake animals (Samson et al., 1999; Shirasaka et al., 2002), as well as gastric secretion (Takahashi et al., 1999).

Hcrt neurons are modulated by adrenergic input (Hajszan et al., 2002). Moreover, centrally administered orexin/Hcrt activates HPA axis in rats (Kuru et al., 2000; Nakamura et al., 2000), induces plasma ACTH and corticosterone (Ida et al., 2000; Kuru et al., 2000; Malendowicz et al., 1999) and *c-fos* mRNA in the parvocellular division of the PVN. In addition, glucocorticoids modulate hypothalamic hypocretin mRNA expression (Stricker-Krongrad et al., 2002), suggesting that this system could constitute a sensitive key relay for mediating stress behavior. Interestingly, hypocretin receptors have been detected in adrenal gland: HcrtR1 is expressed in the cortex of the normal human adrenal gland (glomerulosa, fasciculata, and reticular zones) and HcrtR2 is located in the medulla (epinephrine and norepinephrine cells) (Blanco et al., 2002; Lopez et al., 1999), and addition of Hcrt to adrenocortical cultures stimulates norepinephrine release (Nanmoku et al., 2002). However, the origin of the ligand that would bind to Hcrt receptors in the periphery is unclear.

Further supporting the role of the hypocretins in the activation of the HPA axis, Ida et al. (2000) have shown that icv administration of the alpha helical CRF antagonist blocks Hcrt-induced grooming behavior. Also recently, Espana et al. (2002) have shown that mild stress increases *c-fos* immunoreactivity in hypocretin positive neurons in the perifornical area. Interestingly, the effect of Hcrt on the stress response appears to be specific and finely regulated, since *in vitro*, Hcrt1 inhibits CRF-induced ACTH release via a pertussis toxin-sensitive mechanism, but does not affect baseline levels of ACTH, or release of LH, PRL, or FSH from the pituitary (Samson and Taylor, 2001). These data, and our own preliminary evidence showing that hypocretin neurons are activated by CRF, suggest that the hypocretinergic system is an important component of the neural circuitry modulating the stress response. Along this line, the hypocretin system may also be involved in hyperarousal associated with panic and posttraumatic stress disorder, as silencing Hcrt neurons prevent panic disorder in rats, and some human patients with panic disorder show elevated levels of Hcrt in CSF (Johnson et al., 2010),

## The hypocretins as an integrator circuit in arousal

The anatomical localization and functional connectivity of Hcrt neurons reveals a prominent role in homeostatic control of physiological switches. Data from multiple laboratories have shown a very diverse set of classical and peptide transmitters as well as metabolites that modulate Hcrt activity. Recent data showing phasic activity of Hcrt in correlation with goal oriented behaviors, locomotor activity, and behavioral state transitions also suggest that these neurons provide physiological signals in a changing environment and prepare other neuronal circuits to adapt to new situations. Following Cannon's original concept of homeostasis, Hcrt neurons act as integrators that convey possibly conflicting physiological signals into a coherent output to other effector systems, which include norepinephrine neurons for arousal transitions and dopaminergic neurons of the mesocorticolimbic system to engage in rewarding activities (Fig. 2). The deconstruction of hypothalamic circuits using opto and pharmacogenetic methods will undoubtedly reveal new ways of integration that underlie complex behaviors.

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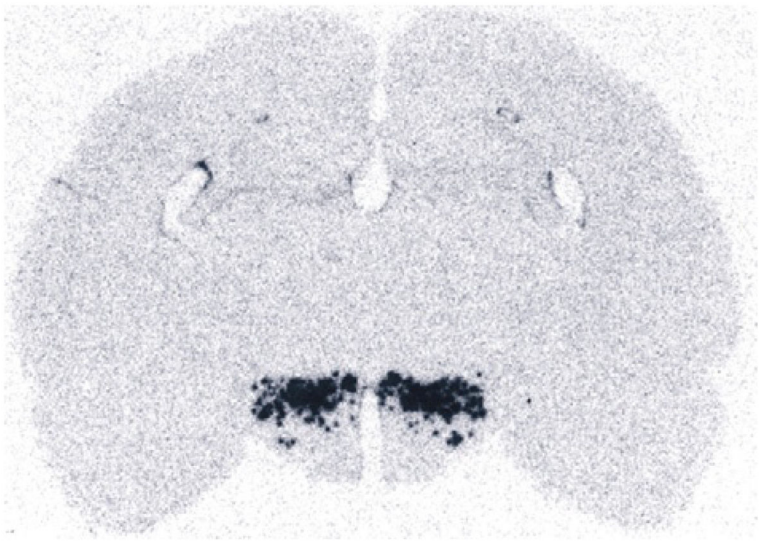

**Fig. 1.**

The first image of the hypocretin system, as reported by Gautvik et al. (1996). The picture shows an autoradiograph of an *in situ* hybridization of clone 1D4 corresponding to a gridded hypothalamic subtracted cDNA library.

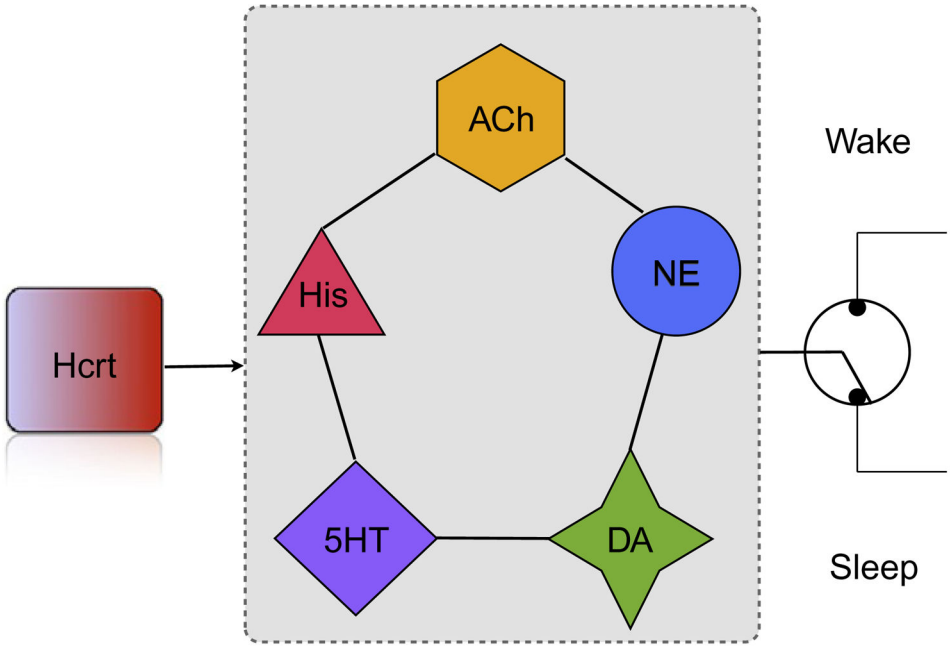


**Fig. 2.**

Multiple lines of evidence suggest that the Hcrt system integrates multiple variables including metabolite concentration and limbic tone to produce a coherent output to arousal systems. Each of these arousal systems contribute in different ways to sleep-to-wake transitions. For instance, optogenetic experiments suggest that noradrenergic neurons in the locus coeruleus (NE) are strong effectors of arousal systems, as only a few action potentials are sufficient to induce an awakening (Carter et al., 2010). In contrast, histamineric neurons in the tuberomammilary region of the posterior hypothalamus (His) do not appear essential to elicit transitions (Carter et al., 2009) but contribute to the length of the wake bout. Serotoninergic neurons have been proposed as gates to REM sleep, whereas dopaminergic and cholinergic systems have different effects on cortical excitability and affect different frequency bands. Coordination of these systems by Hcrt neurons is essential for arousal stability, and narcolepsy with cataplexy may be the result of chaotic signaling during transitions.