The brain hypocretins and their receptors: mediators of allostatic arousal
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The hypocretins (abbreviated ‘Hcrts’ – also called ‘orexins’) are two neuropeptides secreted exclusively by a small population of neurons in the lateral hypothalamus. These peptides bind to two receptors located throughout the brain in nuclei associated with diverse cognitive and physiological functions. Initially, the brain Hcrt system was found to have a major role in the regulation of sleep/wake transitions. More recent studies indicate Hcrts may play a role in other physiological functions, including food intake, addiction, and stress. Taken together, these studies suggest a general role for Hcrts in mediating arousal, especially when an organism must respond to unexpected stressors and challenges in the environment.

Introduction
It has been a decade since the discovery of the hypocretins (Hcrts), and during the past ten years we have learned much about their expression, structure, and function. Almost immediately after their discovery, the important role of Hcrts in maintaining wakefulness was reported in multiple species including humans [1,2,3–5]. Subsequent years have only solidified the evidence that Hcrts are both necessary to maintain and sufficient to induce wakefulness, and they are now generally considered to be ‘arousal-promoting’ peptides [6,7]. Recently, Hcrts have also been implicated in physiological functions and behaviors other than wakefulness. In this review, we provide an overview of the brain Hcrts and their receptors and survey the recent studies implicating a role for Hcrts in these diverse physiological functions. In trying to integrate these studies, we suggest that two general functions of Hcrts are to mediate wakefulness and allostatic arousal.

The hypocretins
The Hcrts were discovered independently by two groups in the late 1990s [8,9]. They consist of a pair of secreted peptides, hypocretin-1 and hypocretin-2 (Hcrt1 and Hcrt2; also known as ‘orexin A’ and ‘orexin B’, respectively). These peptides are processed from the same genetic precursor, ‘preprohypocretin’ (ppHcrt) and are expressed exclusively in the perifornical lateral hypothalamic area of the brain [8,9]. Hcrts and their receptors are also expressed in the periphery [10], but in this review we focus on Hcrts of the central nervous system.

Brain Hcrt neurons receive afferent projections from many nuclei in the hypothalamus, the allocortex, claustrum, bed nucleus of the stria terminalis, periaqueductal gray, dorsal raphe nucleus, and lateral parabrachial nucleus [11]. Hcrt neurons receive input from GABAergic, glutamatergic, and cholinergic neurons [12]. Furthermore, in vitro electrophysiology studies demonstrate that several neurotransmitters/neuromodulators excite Hcrt neurons (including corticotropin releasing factor, ghrelin, neuropeptide Y, vasopressin, and oxytocin) or inhibit Hcrt neurons (including serotonin, noradrenaline, dopamine, neuropeptide Y, and leptin) [13].

In turn, Hcrt neurons project to diverse areas of the central nervous system, including prominent projections to the noradrenergic locus coeruleus (LC), the histaminergic tuberomammillary nucleus (TMN), the serotonergic raphe nuclei, the dopaminergic ventral tegmental area (VTA), the cholinergic pedunculopontine tegmental area (PPT) and laterodorsal tegmental area (LDT), and the galaninergic ventrolateral preoptic nucleus (VLPO) [14]. Hcrt neurons also project diffusely throughout the cerebral cortex. Hcrts are excitatory peptides and therefore depolarize their effenter targets [8,9].

Taken together, these anatomical and electrophysiological studies suggest that Hcrt neurons integrate a variety of homeostatic signals from the central nervous system and periphery, and project to numerous brain regions, many of which express other neuromodulators and are capable of regulating diverse physiological functions and behaviors (Figure 1).

The hypocretin receptors
Both Hcrt peptides bind with different affinities to two Hcrt receptors, hypocretin receptor 1 (Hcrttr-1 – also...
called ‘OxR1’) and 2 (Hcrtr-2 – also called ‘OxR2’) [8\*\*,9\*\*]. Hcrt-r1 binds Hcrt1 with high affinity and binds Hcrt2 with 100–1000-fold lower affinity [9\*\*,15]. Hcrt-r2 has a high affinity for both Hcrt1 and Hcrt2 (Figure 2). The Hcrt receptors are located on postsynaptic terminals in a pattern consistent with the anterograde projections of hypocretin neurons described above (Figures 1 and 2) [6\*\*,8\*\*,9\*\*,14\*]. Hcrt-r1 mRNA is detected within the

The brain hypocretins and their receptors, Hcrt1 and Hcrt2 are both spliced from the same genetic precursor, Preprohypocretin. Hcrt1 binds with high affinity to both Hcrt receptors, while Hcrt2 only binds with high affinity to Hcrt-2. Hcrt receptors are differentially expressed throughout the brain, with Hcrt-r1 expressed in the LC and LDT/PPT, and Hcrt-r2 expressed in the TMN. Modified from [14*].
hypothalamus, the LC, the cerebral cortex, and several brainstem nuclei. By contrast, Hcrt-r2 mRNA is expressed in cholinergic nuclei in the brainstem, the ventral tegmental area, and TMN, as well as overlapping expression with Hcrt-r1 in the hypothalamus. Partially due to a lack of specific antagonists (Box 1), little is known about the distinct functions of Hcrt-r1 and Hcrt-r2. However, Hcrt-r2 knockout animals, but not Hcrt-r1 mice, show narcolepsy, supporting a prominent role for this receptor in arousal stability.

The crucial role of hypocretins in arousal stability

Extensive evidence demonstrates that Hcrt system causes the sleep disorder narcolepsy in mice, dogs and humans [1*,2*,3–5]. Most human narcoleptics have decreased levels of Hcrt in their cerebrospinal fluid, and postmortem analysis reveals a reduction of Hcrt neurons in human narcoleptic brains [4,5]. Interestingly, the Hcrt system is also necessary for normal emergence from general anesthesia [16*]. Intracerebroventricular (i.c.v.) injection of Hcrt1 and/or Hcrt2 increase the time spent awake and decrease the time spent in slow-wave and REM sleep in a variety of vertebrate species [17,18]. Furthermore, artificial stimulation of Hcrt neurons using a light-activated cation channel, channelrhodopsin-2, increases the probability of transitions from sleep to wakefulness during both slow-wave and REM sleep [19**]. Thus, there is now solid evidence that Hcrt are necessary to maintain and sufficient to induce wakefulness.

Other potential functions of the hypocretin system

Hcrt are implicated in many physiological functions other than maintaining wakefulness. For example, the alternate name of Hcrt, ‘orexins’, was designated because i.c.v. infusion of Hcrt increased food intake in rodents [9**]. These results are now considered to be an indirect effect of the wake-promoting effects of Hcrt, but this is still an active area of investigation. Microinjection of Hcrt into the arcuate nucleus stimulates orexigenic GABAergic neurons and inhibits anorexigenic POMC-expressing neurons. Hcrt also inhibit neurons in the ventromedial hypothalamus, an established satiety center [20]. Thus, Hcrt act in a reciprocal manner to the satiety hormone leptin in important energy-homeostatic regions of the hypothalamus.

Recently, an exciting role for Hcrt has been established in reward-seeking and addiction. Activation of Hcrt neurons is correlated with cues associated with drug and food reward. Stimulation of Hcrt neurons or micro-injection of Hcrt1 into the VTA or ventricles reinstates previously extinguished drug-seeking behaviors, and these effects are blocked by a Hcrt-1 antagonist [21**,22**]. These seminal studies have sparked a rapidly growing body of research that repeatedly confirms Hcrt modulate reward processing [23*].

Stimuli that increase arousal/wakefulness also often increase stress and anxiety. Therefore, the ability of Hcrt to promote wakefulness suggests that these peptides may play a role in increasing the behavioral and physiological hallmarks of stress. In support of this hypothesis, i.c.v. injection of Hcrt1 elicits many stress-related behaviors [17,24*]. Increased Hcrt activity is also correlated with a variety of stress-related autonomic processes, such as elevation of mean arterial blood pressure, heart rate, oxygen consumption, and body temperature [25–27]. Moreover, Hcrt fibers project to corticotropin releasing factor (CRF) neurons within the paraventricular nucleus (PVN) [28*,29], neurons that activate the hypothalamus–pituitary–adrenal (HPA) axis organismal response to stress. Bath application of Hcrt1 elicits depolarization...
and increased spike frequencies in these CRF cells [28*]. This evidence suggests that Hcrts may interact with central CRF systems to activate the HPA axis and other stress-related processes.

In addition to food intake, addiction, and stress, Hcrts have also been implicated in rodent models of attention [30] and male sexual behavior [31*]. Hcrts have also been hypothesized to play a role in the symptoms of Parkinson’s disease [32], schizophrenia [33*,34], and depression [35,36]. In sum, studies of the Hcrt system have progressed far beyond the initial discovery of the involvement of Hcrts in sleep and wakefulness. These studies beg the question: How can Hcrts play a role in such a diverse arsenal of behaviors ranging from wakefulness to food intake, addiction, stress, vigilance, and even sexual behavior? Below, we provide a preliminary answer to this question.

### Hypocretins: regulators of arousal and allostasy

The role of the hypocretin system in promoting wakefulness is often described as a role in ‘arousal.’ Generalized arousal is marked by increased motor activity and heightened responsiveness to sensory and emotionally salient stimuli [37*,38–40]. Less often emphasized, however, is that arousal systems are involved in much more than just regulating sleep/wake cycles, such as the vigilance, anxiety, and symptoms of many psychiatric disorders [41]. Importantly, brain structures implicated in generalized arousal, including the reticular formation of the medulla and pons, midbrain, and the paraventricular, dorsomedial, and lateral hypothalamic nuclei [42], receive projections from Hcrt neurons. We propose that if Hcrts can modulate this arousal network, they are also likely able to modulate behaviors orchestrated by this network. By appreciating the role arousal is known to play in such behaviors studied outside the sleep field, investigators may be able to make increasingly novel yet specific hypotheses about the function of Hcrts in non-sleep behaviors. For example, recent reports that Hcrts modulate behavior in murine models of depression [35,36] is understandable and even anticipatable in the face of years of psychiatric research showing that arousal processing is impaired in humans with depression [43].

Hcrts seem to have their greatest influences when arousal is needed to regulate basic homeostatic pressures like hunger, anxiety, or the drive for sex. Therefore, we propose that Hcrts are particularly important for allostasis. In contrast to homeostasis, allostasis maintains stability at levels outside the normal range and is achieved by varying the internal milieu to match perceived and anticipated environmental demands [44*,45*]. For example, consider a recent study testing the effects of calorie restriction on stress and depression [46*]. Work linking stress and depression shows that

Box 2 Unresolved questions about the hypocretin system

- Are there functional subdivisions within Hcrt nuclei? It has been suggested that there are at least two discrete functional populations of Hcrt neurons: a lateral population playing a role in food intake and addiction, and a more medial population playing a role in arousal and stress [53]. Future studies are needed to test this hypothesis.
- Do the two Hcrt receptors differentially regulate distinct physiological functions and behaviors? Are they both necessary for regulating a behavior, or is a single receptor sufficient?
- Do Hcrt neurons promote wakefulness by projecting to many sites in the brain, or just a few key populations of neurons? Several models of sleep/wake circuitry, such as the flip/flop model of sleep, suggest that Hcrt enhances an awake state by projecting to other arousal centers such as the LC, TMN, and dorsal raphe nuclei [7*]. However, lesions of these nuclei do not lead to a robust phenotype and normal wakefulness is maintained, even when all these nuclei are ablated in the same animal [54*]. Thus, the postsynaptic sites necessary to mediate the action of Hcrt neurotransmission are still unclear.
- What allostatic pressures are necessary or sufficient to drive Hcrt-mediated arousal? How do environmental pressures translate into activation of the Hcrt system?
Conclusions
In the ten years since their discovery, we have learned much about the brain Hcrt system. Indeed, the role of Hcrts in promoting wakefulness is indisputable. This review suggests a framework for thinking about a general role for Hcrts in other behaviors as well. While more research is needed to elucidate the precise functions of Hcrts, perhaps the role of the Hcrt system will only be fully appreciated in the context of organismal homeostasis and allostatics. With sophisticated new imaging and optogenetic technologies, the next ten years will no doubt contain continued advances in our understanding of this fascinating brain arousal system (Box 2).

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References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

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16. Kelz MB, Sun Y, Chen J, Cheng Meng Q, Moore JT, Veasey SC, Dixon S, Thornton M, Funato H, Yanagisawa M: An essential role for orexins in emergence from general anesthesia. Proc Natl Acad Sci U S A 2008, 105:1309-1314. This study demonstrates that Hcrt neurons are necessary for the normal emergence from unconsciousness after administration of the common anesthetics isoflurane and sevoflurane. This demonstrates that emergence from anesthesia depends on sleep/wake circuitry and that the Hcrt system is a key player.
19. Adamantidis AR, Zhang F, Aravanis AM, Deisseroth K, de Lecea L: Neuronal substrates of awakening probed with optogenetic control of hypocretin neurons. Nature 2007, 450:420-424. Optogenetic technology is applied to the Hcrt system, showing that stimulation of Hcrt neurons is sufficient to increase the probability of an awakening event during slow-wave-sleep or REM sleep. This effect is blocked in Hcrt KO mice and in the presence of a Hcrtr-1 antagonist, demonstrating that Hcrt peptides, and not other neurotransmitters, are necessary for the wake-promoting effects of Hcrt neurons.
These studies were first to demonstrate an important role for the Hcrt system in reward seeking behavior, an active area of current addiction research.


An excellent review on the recent research on the role of Hcrt in reward seeking behaviors.


A review that surveys evidence that Hcrt neurons play an important role in regulating the increased arousal associated with the response to environmental stressors.


This study presents anatomical and electrophysiological evidence that Hcrt neurons receive excitatory input from CRF-containing neurons and that this circuit may mediate the increase in arousal associated with the stress response.


This interesting study demonstrates that Hcrt are necessary for the normal increase in arousal associated with male sexual behavior. Furthermore, electrophysiological evidence suggests that Hcrt activate the mesolimbic dopaminergic system, and that this circuitry may facilitate the arousal for natural rewards, such as sex.


This chapter outlines evidence suggesting the Hcrt system to identify other brain systems responsible for psychiatric dysfunction.


This theoretical paper provides evidence for the existence of a generalized arousal system in the CNS, and then proposes an operational definition for how to monitor this system. It describes the neuroanatomical, neuropsychophysical, and genomic mechanisms underlying general arousal, differentiating it from specific arousal. It also raises the interesting idea that specific arousal systems can conflict, such as when hunger leads an animal to wake up during its circadian sleep cycle.


This study provides evidence to support the hypothesis that allostatic mechanisms can explain drug relapse, a behavior coincidentally instigated by Hcrt system activation.


This recent study nicely illustrates how some functions of the Hcrt system might only be detectable in the presence of allostatic pressure. In the example under investigation, hunger activates the Hcrt system, which in turn counteracts the depressive effects of chronic stress.


This study provides another example of how some functions of the Hcrt system can only be observed when allostatic pressure is applied, again in the form of hunger.


This review highlights progress by the pharmaceutical industry in pharmacologically targeting the Hcrt system for possible treatments in sleep and other psychiatric disorders.


This study describes an Hcrt receptor antagonist that targets both Hcrt receptors, can be orally administered, readily crosses the blood-brain barrier, and reversibly blocks Hcrt function in vivo.
52. Zeitzer JM, Nishino S, Mignot E: The neurobiology of hypocretins (orexins), narcolepsy and related therapeutic interventions. *Trends Pharmacol Sci* 2006, 27:368-374. This review highlights methods of stimulating arousal in patients with dysregulation of the Hcrt system, either by trying to stimulate Hcrt neurons or Hcrt levels, or by pharmacologically targeting other brain arousal systems.


54. Blanco-Centurion C, Gerashchenko D, Shiromani PJ: Effects of saporin-induced lesions of three arousal populations on daily levels of sleep and wake. *J Neurosci* 2007, 27:14041-14048. Three populations of neurons that receive heavy afferent projections from Hcrt neurons are lesioned in the same animal in order to determine their necessity in the sleep/wake cycle. Daily levels of sleep and wake are relatively normal, suggesting that Hcrt’s wake-promoting effects are due to additional or altogether different nuclei within the brain.