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Hypocretin (orexin) neuromodulation of stress and reward pathways

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Hypocretin (also known as orexin) is a peptide neuromodulator that is expressed exclusively in the lateral hypothalamic area and plays a fundamental role in wakefulness and arousal. Chronic stress and compulsive drug-seeking are two examples of dysregulated states of hyperarousal that are influenced by hypocretin transmission throughout hypothalamic, extended amygdala, brainstem, and mesolimbic pathways. Here, we review current advances in the understanding of hypocretin's modulatory actions underlying conditions of negative and positive emotional valence, focusing particularly on mechanisms that facilitate adaptive (and maladaptive) responses to stressful or rewarding environmental stimuli. We conclude by discussing progress toward integrated theories for hypocretin modulation of divergent behavioral domains.

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Introduction

The hypocretins (also known as orexins) are two secreted neuropeptides (Hcrt1, Hcrt2) derived from the same preprohypocretin gene that bind to two G-protein-coupled receptors (HcrtR1, HcrtR2) [1–3]. Both hypocretins are expressed exclusively in the lateral hypothalamic area (LH), therefore both are referred to here as hypocretin (Hcrt). LH-Hcrt neurons are inactive during sleep, but become activated during wakefulness, likely to promote goal-oriented behavior and energy homeostasis [4,5]. Direct manipulations of LH-Hcrt neurons using *in vivo* optogenetics revealed their key role in increasing the probability of sleep-to-wake transitions through HcrtR signaling in norepinephrine (NE) neurons of the locus coeruleus (LC) [6,7••]. While LH-Hcrt neurons project widely [8], this review covers Hcrt's modulatory actions within the paraventricular nucleus of the hypothalamus (PVN), bed nucleus of the stria terminalis (BNST), central and basolateral nuclei of the amygdala (CeA, BLA), LC, ventral tegmental area (VTA), and nucleus accumbens (NAcc). Rather than providing a comprehensive summary of the literature, we focus on articles published in the last three years that examine Hcrt neuromodulation of stress-related and addiction-related phenomena.

Multiple lines of evidence identify Hcrt as a pro-stress modulator, adding complexity to the prevailing view of Hcrt as a reward-related signal. For example, intracerebroventricular (i.c.v.) Hcrt administration enhances anxiety-like behavior [9] and decreases brain reward function, reflected by increased thresholds in the classical intracranial self-stimulation (ICSS) procedure [10]. Interestingly, Hcrt's effects on the ICSS threshold are mediated by corticotropin-releasing factor (CRF), the prototypical stress neuropeptide [11•]. CRF released from the PVN activates the hypothalamic–pituitary–adrenal (HPA) stress axis, resulting in increased levels of adrenocorticotropin hormone (ACTH) and corticosterone (or cortisol; CORT). Hcrt administered i.c.v. also elevates ACTH and CORT levels [12], supporting the hypothesis that Hcrt possesses CRF-dependent anti-reward properties [11•]. Yet, an extensive literature describes Hcrt-mediated positive modulation of the mesolimbic VTA dopamine (DA) reward system. Hcrt robustly innervates the VTA [13], induces excitatory synaptic plasticity in VTA-DA neurons [14,15], and causes DA release in VTA target regions [16,17]. Reward-seeking behavior (*i.e.*, expression of conditioned place preference, operant self-administration, or reinstatement of either) is associated with activation of Hcrt neurons, and largely attenuated by systemic HcrtR blockade [18,19].

Thus, Hcrt is anatomically and functionally poised to modulate neural activity in arousal-related conditions of both negative and positive emotional valence. In reviewing the most recent findings on this topic, we discuss several mechanisms by which dysfunction of Hcrt modulation could underlie behavioral states associated with stress-related and addiction-related psychiatric disorders.

Hypocretin interactions with CRF stress pathways

Hcrt-containing efferents of the LH target the hypothalamus and extended amygdala, particularly the CRF-enriched nuclei of the PVN, BNST, and CeA [20–22]. I.c.v. infusion of Hcrt activates PVN-CRF neurons [23]

and elevates HPA hormones [12], suggesting that Hcrt directly modulates the CRF-mediated neuroendocrine output. Furthermore, the anxiolytic effects of HcrtR1 blockade are associated with reduced neural activation in the BNST and CeA [24]. Together with the CRF-dependent effects of Hcrt on the ICSS threshold described above, these data suggest that Hcrt interactions with CRF neurons of the PVN, BNST, and/or CeA are associated with anxiogenic and anhedonic states [11•]. Importantly, Hcrt-CRF interactions are reciprocal, as CRF provides excitatory input to Hcrt neurons, and Hcrt neurons undergo CRF-dependent transcriptional activation following exposure to various stressors [25].

Acute withdrawal following chronic drug exposure encompasses a stress-like state of hyperarousal, and withdrawal from morphine and nicotine increases transcriptional activity in Hcrt neurons of the LH, as well as CRF neurons of the PVN and CeA [26,27]. Morphine withdrawal-induced activation of the PVN, BNST, and CeA is decreased by systemic HcrtR1 blockade [27], and local HcrtR1 antagonism in the PVN reduces the behavioral expression of nicotine withdrawal [28]. These findings raise the possibility that Hcrt modulation of CRF neurons participates in the chronically relapsing, negative affective state that characterizes drug addiction. Indeed, one landmark study found that i.c.v. Hcrt mimicked the ability of stress to reinstate operant cocaine-seeking in a CRF-dependent manner [10]. Nevertheless, it remains unclear whether this form of Hcrt modulation involves CRF neurons in the PVN, BNST, or CeA.

Hypocretin modulation in the BNST and amygdala

As with the PVN, the BNST connects reciprocally with Hcrt neurons [8,29]. However, while PVN-CRF neurons are mostly glutamatergic, BNST-CRF neurons are primarily GABAergic [30], thereby offering diverse mechanisms for Hcrt modulation of stress circuits. In one recent study, slice application of Hcrt to neurons in the CRF-enriched dorsolateral BNST (dlBNST) of adult mice depressed excitatory post-synaptic currents (EPSCs) in a HcrtR1-specific manner [31]. Interestingly, these effects on excitatory dlBNST transmission mimicked those of yohimbine, the alpha-2 NE receptor antagonist known to reinstate operant alcohol-seeking through a HcrtR1-mediated mechanism [32]. In a separate study, slice application of Hcrt depolarized a subset of neurons in the posterior BNST of adult rats [33]. These effects contrast with those observed in mice [31], but are consistent with the ability of intra-BNST Hcrt to increase anxiety-like behavior *via* NMDA receptor activation [33]. Even a single subregion of the BNST can display tremendous cellular heterogeneity [30], and Hcrt's modulatory actions undoubtedly vary across cell types, highlighting the need for detailed studies of Hcrt's effects in genetically defined or physiologically defined BNST neurons.

Intra-CeA Hcrt infusion elevates anxiety levels [34], and Hcrt slice application excites 'low-threshold burst' output neurons in the medial division of the CeA [35]. Together with evidence that i.c.v. Hcrt activates CeA-CRF neurons [23], these data suggest a mechanism in which Hcrt potentiation of CeA excitability underlies a stress-like state (perhaps by releasing output neurons from inhibitory GABAergic control [34]). Elsewhere in the amygdala, Arendt *et al.* used RNA interference in adult mice to show that BLA-HcrtR2 signaling is anxiolytic rather than anxiogenic [36•]. Although this finding contrasts with the reported effects of intra-CeA Hcrt, it bears resemblance to a recent study that uncovered opposite consequences of HcrtR1 and HcrtR2 deletion on depressive-like behaviors in mice [37].

Hypocretin modulation in the LC

LC-NE neurons are a major target of Hcrt neurons, and combinatorial optogenetics revealed the necessity of LC-NE activity for Hcrt-mediated awakenings [7••]. Additionally, LC-NE neurons play a critical role in the stress response [38]. NE-induced plasticity at GABAergic synapses in the PVN regulates HPA-axis activity [39], and the relevance of BNST-NE signaling to stress and reward function has been reviewed [40]. In one recent study of Hcrt-NE interactions, Sears *et al.* used optogenetic and pharmacological approaches to demonstrate that excitatory LC-HcrtR1 signaling accentuates the strength of fear memories through downstream NE signaling in the lateral amygdala [41•]. Thus, disturbances in Hcrt modulation of brainstem NE nuclei may underlie conditions of dysregulated threat assessment *via* indirect potentiation of amygdala activity.

Hypocretin modulation in the VTA

VTA-DA neuron burst firing is sufficient to produce conditioned reward [42], and Hcrt neurons project strongly to the VTA [13], forming appositional contacts with DA neurons [43]. VTA-DA neurons demonstrate increased EPSC amplitudes and firing rates upon Hcrt application, and display HcrtR1-dependent excitatory synaptic plasticity following cocaine exposure. The precise mechanisms underlying these forms of modulation have been reviewed elsewhere in detail [14,15]. Intra-VTA infusion of Hcrt increases DA efflux in the prefrontal cortex and NAcc, suggesting that Hcrt modulation of the VTA promotes appetitive responding and motivation for salient environmental cues *via* enhanced DA receptor signaling [16,17]. An expansive literature describes the contributions of Hcrt neurons and HcrtR signaling to reward-related behavior [18,19]. Here, we simply highlight a few recent examples that add to the growing understanding of how Hcrt modulation of

the VTA fits within the context of brain reward function and drug-seeking behavior.

Srinivasan *et al.* found that intra-VTA HcrtR antagonism reduced lever-pressing for 20% alcohol, and replicated previous findings by demonstrating that Hcrt increased the firing rate of putative VTA-DA neurons, indicating that Hcrt promotes alcohol-seeking by increasing VTA-DA excitability [44]. Mahler *et al.* reported the necessity of simultaneous Hcrt and glutamate transmission within the VTA for cue-induced reinstatement of operant cocaine-seeking [45], and Morgan *et al.* established that prenatal nicotine exposure enhances Hcrt innervation of VTA-DA neurons, hinting at a developmental role for Hcrt in drug-induced mesolimbic plasticity [43].

Hypocretin modulation in the NAcc

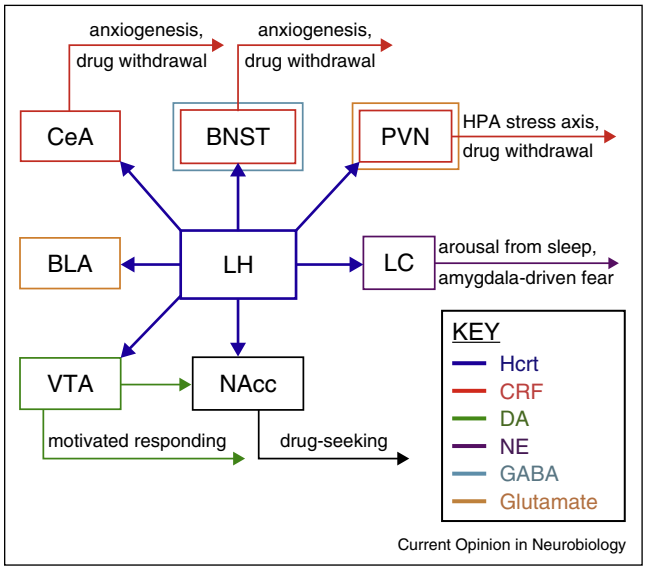
In addition to modulating DA activity at the level of the VTA, two new studies examine how Hcrt inputs to the NAcc could further amplify reward-related effects associated with striatal DA release. Mori *et al.* described a subpopulation of neurons in the NAcc shell that displayed a synergistic increase in firing rate upon combinatorial application of Hcrt and DA, relative to Hcrt alone [46]. Patyal *et al.* used voltammetry in NAcc shell slices to reveal a glutamate-dependent mechanism in which Hcrt enhanced DA levels following phasic, but not tonic, patterns of electrical stimulation [47]. Together with data implicating NAcc-HcrtR signaling in stress-induced reinstatement of morphine conditioned place preference [48] and cue-induced reinstatement of nicotine-seeking [49], these findings identify the NAcc (particularly the shell) as an additional site through which Hcrt could promote reward-based behavior *via* augmentation of DA release from VTA terminals.

Conclusions

Recent studies on Hcrt modulation have identified diverse mechanisms that point toward both pro-stress (PVN, BNST, CeA, LC) and anti-stress/pro-reward (BLA, VTA, NAcc) behavioral consequences (Figure 1). Future investigation of the discussed pathways may shed light on clinical findings that highlight Hcrt's involvement in the response to both negative and positive motivational situations. For example, Hcrt-deficient patients display impaired amygdala activation following exposure to conditioned aversive stimuli, yet human amygdala Hcrt levels are elevated during laughter and affiliative social interactions [50••,51]. Below, we discuss potential explanations for the complex and occasionally bi-directional effects of Hcrt neuromodulation on emotional behavior.

Previous efforts to integrate the disparate behavioral profiles of Hcrt resulted in a 'divergent efferent hypothesis,' based on the apparent stress-responsiveness *vs.* reward-responsiveness of Hcrt neurons in the dorsomedial and perifornical hypothalamic nuclei *vs.* the lateral

Figure 1



LH-Hcrt neurons project widely, including to the PVN, BNST, CeA, BLA, LC, VTA, and NAcc. Recent evidence implicates Hcrt modulation of CRF neurons of the PVN, BNST, and CeA in the negative affective states associated with anhedonia, drug withdrawal, HPA-axis activation, and anxiogenesis. Primarily glutamatergic *vs.* GABAergic phenotypes of PVN-CRF *vs.* BNST-CRF neurons provide diverse mechanisms for Hcrt modulation of stress circuitry. Preliminary evidence implicates BLA-HcrtR2 signaling in anxiolysis rather than anxiogenesis. Hcrt innervation of LC-NE neurons participates in sleep-to-wake transitions, and strengthens fear memories *via* subsequent LC-NE release in the amygdala. Hcrt modulation of excitatory synaptic plasticity in VTA-DA neurons is well-studied. Together with potentiation of DA release at the level of the NAcc shell, Hcrt may promote motivational responding and drug-seeking behavior *via* enhanced DA receptor signaling.

division of the LH. Specifically, Harris and Aston-Jones suggested that a stress-reward dichotomy could be explained by the differential strength of Hcrt projections from these two subpopulations to the LC *vs.* the VTA [52]. However, Gonzalez *et al.* performed a rigorous analysis of the anatomical locations of LC-projecting and VTA-projecting Hcrt neurons and failed to find a pattern consistent with that proposed by the divergent efferent hypothesis [53]. Nevertheless, the LH is a particularly heterogeneous structure, and functional segregation of Hcrt neuron subpopulations using viral-assisted circuit mapping is theoretically feasible with optical and chemical genetic tools.

An alternative theory is that the stress response, drug reward, and seeking behavior are all linked to increased Hcrt tone simply because these states are associated with enhanced levels of arousal. Yet, the concept of Hcrt as a pan-arousal modulator remains controversial. Rather, some have suggested that Hcrt acts specifically in response to environmental challenges to facilitate transitions between contrasting behavioral states [1], or even

that Hcrt displays selectivity for precise emotional domains such as excitement and anger, rather than wakefulness *per se* [50••].

A further parsimonious explanation is that Hcrt peptides (Hcrt1 and Hcrt2) produce behavioral specificity *via* their differential actions at Hcrt receptors (HcrtR1 and HcrtR2), which are expressed in varying ratios across diverse cell types [1]. Apparent differences in the effects of Hcrt1 *vs.* Hcrt2 on VTA-DA neurons have been documented [15,44], and emerging data provide an intriguing basis for the hypothesis that HcrtR1 and HcrtR2 signaling oppositely modulate anxiety-like and depressive-like behaviors [36,37]. Such a theory also predicts that varying magnitudes of Hcrt release could initiate divergent behaviors in a concentration-dependent manner, based on the relative affinities of Hcrt peptides for their receptors.

Finally, Hcrt modulation of behavior is unlikely a static phenomenon, and may be particularly sensitive to experiential effects. For example, region-specific modulation of neural circuits by Hcrt could promote appetitive *vs.* aversive responding depending on an individual's prior experience with stress or addictive substances. The observation that repeated stress flips the hedonic valence of intra-NAcc CRF treatment [54•] highlights the powerful potential of environmental factors to interact with biological systems that are capable of divergent behavioral responses. The application of these ideas to the study of Hcrt could be especially valuable, given the remarkably broad range of Hcrt's anatomical projections and modulatory outcomes described herein.

In summary, continued study of Hcrt modulation of stress and reward pathways (particularly Hcrt interactions with CRF, DA, and NE systems) may provide critical insight into the relationship between HcrtR signaling and behaviors relevant to psychiatric disease symptoms. Differential effects of Hcrt on plasticity of glutamatergic (PVN, BLA, VTA) and GABAergic (BNST, CeA, VTA) circuitry will be especially important in understanding persistent Hcrt-induced adaptations related to stress and reward function. Recent methodological advances now allow detailed interrogation of precise neural subpopulations, and we anticipate significant progress toward understanding the complex and diverse roles of Hcrt modulation with future application of these tools.

Conflict of interest statement

Nothing declared.

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By quantifying brain region-specific peptide levels in human patients *in vivo*, this article describes the relationship between Hcrt and specific behavioral states, highlighting elevations in Hcrt during positive emotions and social interactions.

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