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Hypocretins (orexins): The ultimate translational neuropeptides

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From the symposium: Neuropeptides: the diverse dialects of the nervous system

Abstract

The hypocretins (Hcrts), also known as orexins, are two neuropeptides produced exclusively in the lateral hypothalamus (LH). They act on two specific receptors which are widely distributed across the brain and involved in a myriad of neurophysiological functions that include sleep, arousal, feeding, reward, fear, anxiety and cognition. Hcrt cell loss in humans leads to narcolepsy with cataplexy (narcolepsy type 1, NT1), a sleep disorder characterized by intrusions of sleep into wakefulness, demonstrating that the Hcrt system is non-redundant and essential for sleep/wake stability. The causal link between Hcrts and arousal/wakefulness stabilization has led to the development of a new class of drugs, Hcrt receptor antagonists to treat insomnia, on the assumption that blocking orexin-induced arousal will facilitate sleep. This has been clinically validated: currently two Hcrt receptor antagonists

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are approved to treat insomnia (suvorexant and lemborexant), with a New Drug application (NDA) recently submitted to the US Food and Drug Administration (FDA) for a third drug (daridorexant). Other therapeutic applications under investigation, include reduction of cravings in substance-use disorders, and prevention of neurodegenerative disorders such as Alzheimer's disease, given the apparent bidirectional relationship between poor sleep and worsening of the disease. Circuit neuroscience findings suggest that the Hcrt system is a hub that integrates diverse inputs modulating arousal (e.g. circadian rhythms, metabolic status, positive and negative emotions) and conveys this information to multiple output regions. This neuronal architecture explains the wealth of physiological functions associated with Hcrts and highlights the potential of the Hcrt system as a therapeutic target for a number of disorders. We discuss present and future possible applications of drugs targeting the Hcrt system for the treatment of circuit-related neuropsychiatric and neurodegenerative conditions.

Key words: sleep, arousal, hypocretin/orexin, narcolepsy, insomnia, neurodegenerative diseases, drug discovery and clinical development, suvorexant, lemborexant, daridorexant.

Abbreviations: 5-$HT_{1A,\ 2C}$, serotonin$_{1A,\ 2C}$ receptor subtypes; AD, Alzheimer's disease; AN, anorexia nervosa; BDNF, brain derived neurotrophic factor; BF, basal forebrain; BLA, basolateral amygdala; BNST, bed nucleus of the stria terminalis; cAMP, Cyclic adenosine monophosphate; CB_1, cannabinoid receptor 1; CCK1, cholecystokinin receptor 1; CNS, central nervous system; CREB, cAMP response element-binding protein; CRF_1, CRF_2, Corticotropin-releasing factor receptor 1,2; CSF, cerebrospinal fluid; DORA, Dual hypocretin/orexin receptor 1/2 antagonist; DR, dorsal raphe; DSM, Diagnostic and Statistical Manual of Mental Disorders; EDS, excessive daytime sleepiness; ERK, extracellular signal-regulated kinase; ERK1/2: mitogen-activated protein kinase 3/1; FLIPR, Fluorescence Imaging Plate Reader; FDA, Food and Drug Administration (US); FTD, frontotemporal dementia; $GABA_A$, ionontropic γ-aminobutyric acid receptor A; $GABA_B$, metabotropic γ-aminobutyric acid receptor B; GPCRs, G-protein coupled receptors; $GHSR_{1a}$, $GHSR_{1b}$, growth hormone secretagogue receptor 1a,1b; Hcrt1, hypocretin 1 peptide/orexin A peptide; Hcrt2, hypocretin 2 peptide/orexin B peptide; Hcrt-Rs, hypocretin receptors; Hcrt-R1, hypocretin 1 receptor/orexin 1 receptor; Hcrt-R2, hypocretin 2 receptor/orexin 2 receptor; Hcrts, hypocretin/orexin neuropeptides; HD, Huntington's disease; hHcrt-R1, human hypocretin 1 receptor/orexin 1 receptor; hHcrt-R2, human hypocretin 1 receptor/orexin 1 receptor; HPA, hypothalamic–pituitary–adrenal; HT-29, human colon cancer cell line; ICV, intracerebroventricular; IL, infralimbic cortex; IP, intraperitoneal; IV, intravenous; KO, knockout; κOR, kappa opioid receptor; LBD, Lewy body dementia; LC, locus coeruleus; LepRB, neurons expressing leptin receptors; LH, lateral hypothalamic area; LPS, latency to persistent sleep; LS, lateral septum; MDD, major depressive disorder; $mGluR_1$, R_5, metabotropic glutamate receptor 1, 5; MND, motor neuron disease; MRN, medial raphe nucleus; mPFC, medial prefrontal cortex; NAcc, nucleus accumbens; NAMs, negative allosteric modulators; NCTXXXXXXXX, National Clinical Trial identifier number, Clinical trials.gov; NDA, new Drug Application (FDA); NREM, non-rapid eye movement sleep; NT1, narcolepsy type 1; OxA, hypocretin 1 peptide/orexin A peptide; OxB, hypocretin 2 peptide/orexin B peptide; PAM, positive allosteric

modulator; PD, Parkinson's disease; pERK, phosphorylated (active) ERK; PKA, Protein kinase A; PK/PD, pharmacokinetic/pharmacodynamic; PL, prelimbic cortex; PO, *per os*/ by mouth; POA, preoptic area; PSG, polysomnography; PSP, progressive supranuclear palsy; PTSD, post-traumatic stress disorder; PVN, paraventricular hypothalamic nucleus; PVT, paraventricular thalamic nucleus; QRFPR, pyroglutamylated RFamide peptide receptor/GPR103; REM, rapid eye movement sleep; SON, supraoptic nucleus; SORA, selective hypocretin/orexin receptor antagonist; SORCOs, selective hypocretin/orexin receptor crossover drugs; SOREM, sleep-onset REM sleep; $T_{1/2,}$ half-life; TAAR1, trace amine-associated receptor 1; TMN, tuberomammillary nucleus; TST, total sleep time; VMH, ventromedial hypothalamus; VTA, ventral tegmental area; WASO, wake after sleep onset; WT, wild-type.

1. Hcrt/orexins: the ultimate translational neuropeptides

First discovered in the 1930's, neuropeptides are a diverse and highly interesting class of brain signaling molecules [1]. Examples include angiotensin, bombesin, bradykinin, brain-derived neurotrophic factor (BDNF), calcitonin gene-related peptide (CGRP) and amylin, cholecystokinin, cortistatin, corticotropin-releasing factor, endothelins, galanin, ghrelin, glucagon, neuropeptides S and Y, relaxin and insulin-like peptides, somatostatin, tachykinins (Substance P and neurokinins), opioids, oxytocin, vasoactive intestinal peptide (VIP) and the hypocretins (orexins). Neuropeptides have typically 5 –50 amino acids, formed from the cleavage of longer precursors ("prepro" peptides), which almost uniformly act at G-protein-coupled receptors (GPCRs). Neuropeptides commonly co-express with other neurotransmitters, but show highly distinct anatomical expression profiles. This latter feature contributes to their attractiveness for various potential therapeutic applications. However, neuropeptide receptors drug development faces challenges: 1) For some neuropeptides, the cognate receptors remain unknown. 2) Peptidomimetic approaches typically generate compounds that do not possess central nervous system (CNS) drug-like properties (e.g., poor pharmacokinetic (PK) properties and/or brain penetration and high metabolism). 3) Non-peptide agonists and antagonists with good PK properties have also proved highly challenging to discover for many neuropeptides [1]. Hypocretins represent one of the few exceptions, with a range of Hcrt-R antagonists reaching clinical development and market approval.

The hypocretins are two neuropeptides (Hcrt1 and 2, also called orexin A and B) derived from the same precursor (preprohypocretin), the encoding mRNA of which is expressed in a few thousand neurons, exclusively localized in the lateral hypothalamus. Hcrts bind to two GPCRs, the Hcrt receptor-1 (Hcrt-R1) and Hcrt receptor-2 (Hcrt-R2), which are widely distributed across the brain. Since its discovery, the Hcrt system is one of the most intensely studied neuroregulatory systems. A large body of evidence implicates the Hcrt system as a key player in arousal stability: 1) inactivating mutations of Hcrt-R2 result in narcolepsy with cataplexy in two breeds of dog, 2) genetic reduction of Hcrt or dual Hcrt-R1/HcrtR2 knockout in laboratory animal models such as mice causes behavioral arrests in their active period that are reminiscent of cataplexy attacks, 3) in humans, the absence of Hcrt producing cells as determined by concentration of Hcrt in the CSF, results in narcolepsy with cataplexy (Narcolepsy Type I or NT1) [2-4]. New technologies such as *in vivo* optogenetics allow

millisecond-timescale control of Hcrt neurons in animal models and demonstrate their causal role in vigilance state stability and transitions [5]. The connections of Hcrt neurons with specific brain regions allows the Hcrt system to modulate a broad network of transmitters associated with brain state transitions, alertness and reward; this highlights the therapeutic potential of both Hcrt-R agonists and antagonists for the treatment of arousal-related conditions.

At least 50 Hcrt-R1 and/or R2 selective **antagonists** have been described [6], and their features are the subject of recent reviews [6-10]. Conversely, aside from the peptides themselves, only a few non-peptide Hcrt **agonists** have been synthesized and characterized [11-13]. Nevertheless, recent studies demonstrate the effectiveness and potential of Hcrt peptide and non-peptide agonists as therapeutics for various diseases.

This review will briefly introduce the Hcrt system, examine clinically-tested compounds (suvorexant and lemborexant have been approved, whereas daridorexant is close to registration for the treatment of insomnia), discuss the central role the Hcrt system may play in the pathogenesis of select neurological disorders and how Hcrt-derived compounds can lead to therapeutic tools to treat sleep disorders, anxiety and addiction.

2. Overview of the Hypocretin System

2.1. Hypocretins and Hypocretin Receptors

Prepro-Hcrt mRNA, is exclusively expressed by neurons in the lateral hypothalamic area (LHA), which includes the perifornical, lateral, posterior, and dorsomedial nuclei of the hypothalamus [14-17]. Prepro-Hcrt mRNA encodes a single polypeptide prepro-Hcrt, which undergoes proteolytic cleavage to form Hcrt-1, a 33-amino acid residue peptide with two intramolecular disulfide bridges in its N-terminal domain, and Hcrt-2, a 28-amino acid residue linear peptide. Hcrt-1 and Hcrt-2 share 46% sequence homology [14, 15, 18]. Hcrt peptides are highly conserved among vertebrates, particularly in mammals: Hcrt-1 shares 100% sequence homology whereas Hcrt-2 differs by 1 or 2 amino acids across species [15, 19, 20].

Hypocretins activate their two cognate receptors: Hcrt-1 has equal high affinity to Hcrt-R1 and Hcrt-R2, whereas Hcrt-2 display ~10-fold selectivity for Hcrt-R2 [12, 15]. Hcrt receptor activation stimulates intracellular Ca^{2+} mobilization and other effector systems, followed by the modulation of various downstream signaling pathways [reviewed in 21, 22] resulting in neuroexcitatory activity.

---Insert Figure 1 ~ here--- Legend:

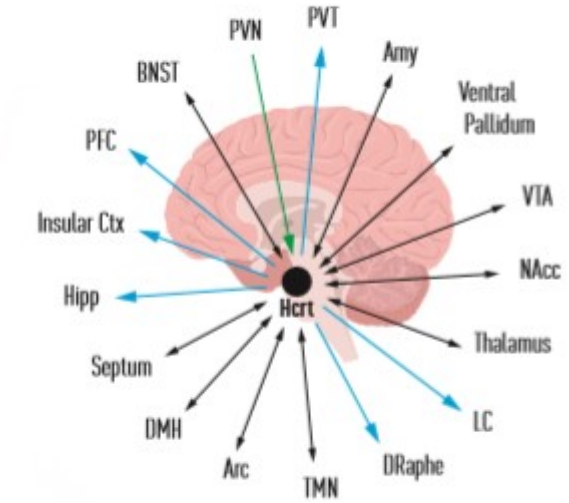

Figure 1. Hcrt neurons are located exclusively in the lateral hypothalamic area (LHA; black dots) and project widely across the brain. Hcrt-1 and Hcrt-2 interact with both receptors (Hcrt1R and Hcrt2R), Hcrt-1 with equal high affinity for Hcrt-R1 and R2, Hcrt-2 with a similar high affinity for Hcrt-R2, but lower (~10-fold) for HcrtR1. The two receptors are widely co-expressed across the brain (yellow). Some specific structures dominantly expressing **Hcrt-R1** (in pink: BLA, basolateral amygdala; BNST, bed nucleus of the stria terminalis; CeA, central nucleus of the amygdala; IC, insular cortex; LC, locus coeruleus; PVT, paraventricular thalamus; VP, ventral pallidum;). Other structures express **Hcrt-R2** (in blue: AC, anterior commissure; Arc, arcuate nucleus; BF, basal forebrain; LA, lateral amygdala; LH, lateral hypothalamus; LS, lateral septum; MS, medial septum; NAc, nucleus accumbens; POA, preoptic area; PVN, paraventricular nucleus; TMN tuberomammillary nucleus).

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3. Drug Discovery and Development:

Neuropeptide research can be approached either from discovery of the peptides themselves (e.g., by identifying possible prepro-peptide sequences with putative neuropeptide cleavage positions expressed in selected brain regions) then searching for their cognate receptors, or from the discovery of the receptors (i.e., by homology cloning, generating so called "orphan GPCRs") then searching for the unknown endogenous peptides. Neuropeptides, unlike the classical neurotransmitters, are products

of genes. Two approaches lead to the discovery of the Hcrts, their receptors and functions. In 1998 de Lecea and colleagues, in search of new potential markers of hypothalamic nuclei, described preprohypocretin as the precursor for the Hcrt1 and Hcrt2 peptides from the systematic analysis of mRNAs enriched in hypothalamic extracts [14, 23], and showed these peptides to have marked stimulatory effects. In the same year, Sakurai et al [24], utilized reporter cell lines to de-orphanize two G protein-coupled receptors (GPCRs), which they termed orexin 1 (OxR1) and orexin 2 (OxR2) receptors. They identified the endogenous peptides from hypothalamic extracts and confirmed the presence of prepro-hypocretin/orexin mRNA within hypothalamic areas implicated in feeding behaviour and demonstrated that administration of the peptides into the ventricles of rats resulted in increased food intake. The near simultaneous, independent discoveries is the reason for the two names for the same system, Hcrt and orexin. The cataplexy phenotype of Hcrt knockout mice and causative Hcrt2R mutations in canine narcolepsy, and the absence of Hcrt producing LHA neurons in human NT1 [2, 3] cemented a central role for Hcrt in arousal and sleep modulation. Together with the feeding and metabolic phenotype of Hcrt-depleted mice [25], these discoveries triggered intense drug discovery programs.

The discovery of the hypocretin system heralded a new wave of drug discovery and development unprecedented in the sleep sciences since the discovery of the $GABA_A$ receptor positive allosteric modulators in the 1960s (benzodiazepines) and the entry of the so-called Z-drugs (e.g., zolpidem, zopiclone, zaleplon) into the clinic in the late 1980s. The first clinical study with a hypocretinergic agent was reported in 2007, just 9 years after the discovery of Hcrt, with the dual Hcrt-R1/Hcrt-R2 antagonist (also known as dual orexin receptor antagonist or "DORA"), almorexant [26], from the Swiss Biotech, Actelion. This took both drug discovery as a whole and sleep research in particular by surprise, given the short timelines from target discovery to clinical evaluation. Following almorexant, several Hcrt-R antagonists have reached clinical testing, predominantly targeting insomnia. Meanwhile two DORAs, suvorexant and lemborexant have been approved by the FDA and other regulatory authorities for the treatment of insomnia, following the most extensive phase III trials in the sleep field. Other DORAs and selective Hcrt-R antagonists (SORAs) are in development for a range of CNS indications. Fundamental discoveries about the functions of Hcrt-R1 in particular, have triggered a wave of investigations on Hcrt-R1 antagonists for panic/anxiety, eating disorders and addiction/substance-use disorders. Non-peptide agonist discovery, particularly relevant for the disorder narcolepsy with cataplexy (NT1), is still in its infancy. Despite the known challenges in developing low molecular weight peptide receptor agonists, new series of potent, selective compounds have been described.

There are a number of detailed reviews on Hcrt-R pharmacology and drug discovery (e.g. [9, 27-31] and see the book "Behavioral Neuroscience of Orexin/Hypocretin" [32]). Here we provide an overview of the current state of hypocretinergic drugs in clinical development. Clinically explored ligands and key references are summarized in Table 1.

3.1 Antagonists

3.1.1. Dual Hcrt-R1/Hcrt-R2 antagonists (DORAs)

Almorexant was the first orally active, brain-penetrant DORA described [26] and has provided a wealth of preclinical and clinical findings, with approximately 20 clinical studies reported to date; Almorexant did not progress to registration due to liver enzyme changes unrelated to Hcrt-Rs [33]. Almorexant increases total sleep time (TST) in preclinical species and humans and was the first DORA to be evaluated in insomnia patients [34]. Clinical trial polysomnography (PSG) data shows the increased TST is mainly due to a dominant enhancement of REM sleep [35]. In addition to the first hypnotic proof of concept, preclinical and clinical studies with almorexant provided the first evidence that DORAs, and by extension Hcrt-R2 antagonists, do not impair memory, and have limited interaction with or potentiation of the effects alcohol or other drugs of abuse. DORAs preserve the ability to wake after dosing, unlike benzodiazepines and z-drugs [36-40] as reported in various species [41-43]. These are distinct advantages in the development of new, improved hypnotics.

Subsequent DORAs investigated in clinical studies include MK-4305/suvorexant [44], SB-649868 [45], MK-6096/filorexant [46, 47], E-2006/lemborexant [48, 49], ACT-462206 [33, 50] and ACT-541468/daridorexant [31, 51]. Except for ACT-462206 for which testing in subjects with insomnia has not been reported, all DORAs induce and maintain sleep in healthy volunteers and insomnia patients (Table 1).

SB-649868 was tested in male healthy volunteers, in situational insomnia and primary insomnia [52-54]. Enhancement of REM is particularly evident with SB-649868 which produced sleep-onset REM (SOREM; i.e. REM sleep within 15 min of persistent sleep) in ~15% of patients in an insomnia study [54]. Receptor kinetic studies show SB-649868 dissociates from the Hcrt-R1 more slowly than from the Hcrt-R2 receptor, i.e., effectively becoming an Hcrt-R1 antagonist with time, which may underlie this effect [55]. SB-649868 has been discontinued, likely due to safety reasons (see Clinical trials.gov, National Clinical Trial (NCT) identifier number NCT00534872).

Suvorexant (Merck's MK-4305, Belsomra® [44, 47]) gained FDA approval in 2014 and was subsequently approved in Japan and Australia. It has been tested across a wide range of preclinical species (rats, mice, dogs and non-human primates) with highly consistent evidence for sleep induction and maintenance. Its clinical development encompasses the largest and longest studies conducted with a hypnotic ([56-60], reviewed in [29, 61]). Suvorexant is generally well-tolerated and, like almorexant, does not impair cognition [62]. PSG analyses also indicate a dominant effect on REM sleep in comparison to NREM [35]. Somnolence, as may be expected for a hypnotic, is the most common side effect [29]. Nightmares, hallucinations, and muscle weakness have also been reported in some subjects.

Expansion of disease indications for suvorexant have reached far beyond primary insomnia, a sleep disorder classification discontinued after DSM-IV. Presently over 45 clinical trials are listed on clinicatrials.gov. These explore suvorexant for insomnia in different types of patient populations: elderly, adolescents, hypertension, Alzheimer's disease (AD), hot flashes, substance use disorders, Parkinson's disease (PD), fibromyalgia, bipolar disorder, trauma, chronic obstructive pulmonary disorder, type-2 diabetes, shift work and progressive supranuclear palsy (PSP). In adsdition, suvorexant is being/has been clinically explored for delirium, amyloid beta kinetics in AD, opioid abuse and withdrawal, use of cocaine, nicotine and recreational sedatives, post-traumatic stress disorder (PTSD), alcoholism, obstructive sleep apnea, restless leg syndrome, panic disorder and augmentation of antidepressant responses in major depressive disorder (MDD).

Suvorexant shows beneficial effects in delirium [see 63], it improves TST without negative effects on cognition in probable AD patients with insomnia [64], highly relevant for this patient population given the memory-impairing effects of most other hypnotics. Suvorexant also improves sleep and is well tolerated in insomnia in the elderly [65] and hypertensive patients [66].

The backup compound for suvorexant, filorexant (MK-6096), produces similar effects in patients with primary insomnia to those described for suvorexant [67], including dose-dependent next-day somnolence, despite a shorter pharmacokinetic terminal half-life (3–6 h) compared to suvorexant (12-19 h). It must be noted that most of the early DORAs (e.g., almorexant, suvorexant, SB649868 and filorexant), are characterised by very slow dissociation rates from their receptors, which result in sustained receptor occupancy and may explain their prolonged duration of action, in addition to their long PK half-lives [see: 27, 55, 61, 68]. Perhaps due to lack of differentiation from suvorexant, the development of filorexant has been stopped.

Lemborexant (E-2006, Dayvigo®) is the second hypocretinergic agent to reach registration, both in Japan and the US. Like for suvorexant, phase II and III studies were extensive (1006 subjects) and show improved sleep onset and maintenance in insomnia patients [69, 70]. The main clinical endpoints were subjective sleep onset latency, subjective total sleep time and subjective wake after sleep onset, whereas effects on sleep architecture were not reported. Unlike most DORAs, lemborexant is somewhat selective for Hcrt-R2 over Hcrt-R1 in functional pharmacology assays, although it is nonselective in receptor binding assays [48, 71]. Another point of distinction from other DORAs is lemborexant's more rapid binding and dissociation from Hcrt receptors compared to the typically slow binding kinetics of other DORAs [55, 71]. This may help to explain the improved next morning function of patients taking lemborexant relative to other hypnotics (including DORAs), despite the slow PK of this compound [half-life:17-19 h; 72, 73]. A terminal half-life of up to 50 hours has been reported, which may explain lemborexant's dose-dependent increase in next morning somnolence in up to 10% of the patients. Lemborexant was superior to zolpidem on both middle-of

the night and morning postural instability in older adults, an important benefit in this patient group[49].

ACT-462206 [50] which was investigated in Phase 1 studies, but stopped [33, 74], provided a valuable chemical starting point that led to the discovery of ACT-541468 / daridorexant [51, 74]. Daridorexant (Idorsia Ltd), currently in Phase III, is a highly potent DORA, developed based on pharmacokinetic/pharmacodynamic (PK/PD) modeling to improve both onset of action and clearance commensurate with an 8 h sleep period in humans, and to provide an increase in NREM and REM similar to natural sleep, as seen in preclinical species [31, 51]. Clinical studies support a relatively rapid PK (T_{max}: 0.8-1.0 h) and improved clearance compared to other DORAs ($T_{1/2}$: 8.5-9.8 h), with dose-dependent improvements in wake after sleep onset (WASO) and latency to persistent sleep (LPS) in adult and elderly insomnia patients [75, 76]. Several Phase III trials are in progress. Daridorexant was submitted for registration and the FDA accepted the New Drug Application (NDA) submission in March 2021.

ORN-0829/TS-142 [77] is a DORA non-selective for Hcrt-R1 *vs.* Hcrt-R2 in functional pharmacology assays. It was designed with similar principles in mind to daridorexant, namely the generation of a hypnotic for the treatment of insomnia with a short onset of action and a relatively short half-life in humans in order to reduce next day side effects. Seven clinical trials (four completed) are listed on clinicaltrials.gov at present, but no peer-reviewed clinical data have been reported.

3.1.2 Hcrt-R1 antagonists

Initial drug discovery efforts investigated the role of Hcrt-R1in feeding and metabolism with the antagonist SB-334867 [78, 79]. SB-334867 was also explored in sleep, demonstrating that Hcrt-R1 blockade could prevent the suppression of REM sleep induced by intracerebroventricular (ICV) delivery of Hcrt-1 [80]. Despite this, subsequent studies with Hcrt-R1 antagonists such as SB-408124 [81], GSK1059865 [82], ACT-335827 [83], Johnson & Johnson's compound 56 [84] and JNJ-54717793 [85] provided consistent evidence that Hcrt-R1 antagonism alone does not affect sleep-wakefulness [83, 84, 86-88]. The combination of Hcrt-R1 and R2 antagonists or Hcrt-R2 gene knockout (KO), however, illustrates a role for Hcrt-R1 in sleep architecture, suggesting that Hcrt-R1 blockade contributes to REM sleep disinhibition under such circumstances [86, 88, 89]. Rather than targeting sleep disorders, and consistent with the expression profile of Hcrt-R1 in structures such as the locus coeruleus (LC), bed nucleus of the stria terminalis (BNST), amygdala and cingulate cortex, recent Hcrt-R1 antagonists research focuses on anxiety disorders, including panic disorder, PTSD, eating disorders and substance abuse/addiction [reviewed in 6, 90, 91].

To date, a number of Hcrt-R1 selective antagonists have been described in the scientific and patent literature (reviewed in [6, 30], see also [92]). Clinical data of Hcrt-R1 drug candidates have been

published for ACT-539313 [Idorsia Pharmaceuticals Ltd, Switzerland; 93, 94] – the first report of a Hcrt-R1 antagonist in humans and JNJ-61393215 [Janssen Research & Development LLC; 95]. ACT-539313 was tested for PK/PD, single ascending dose safety and tolerability and drug-drug interaction potential [93, 94]. A Phase 1 study of JNJ-61393215 provided positive proof of concept in CO_2-induced panic in male healthy volunteers [95].

3.1.3 Hcrt-R2 antagonists

MK-1064 [96] is the first clinically tested selective Hcrt-R2 antagonist, to demonstrate that Hcrt-R2 antagonism alone is sufficient to promote sleep in humans [97]. In healthy volunteers, MK-1064 increased TST and sleep efficiency and decreased LPS and WASO. Interestingly, although the development of DORAs has shown animals to be highly predictive of sleep architecture effects in humans, REM sleep effects with MK-1064 dominated those of NREM in humans, unlike the responses in animals which showed effects on REM to be more modest than for NREM. Human PK data showed significant plasma levels of MK-1064 were observed 16-24 hours post administration. Overall, Gotter and colleagues concluded that Hcrt-R2 antagonists may have limited advantages over DORAs, at least with regard to sleep architecture as assessed with the acute dosing of MK-1064. Development appears to have stopped. Interestingly, radiolabeled [^{11}C]MK-1064 was developed for positron emission tomography imaging of Hcrt-R2 receptors, which would be of great value to estimate target engagement in clinical trials, but clinical data have yet to be reported [98].

JNJ-10397049 [99] provided the first demonstration of the hypnotic actions of Hcrt-R2 antagonism in animal models [86, 87]. Further chemical derivation resulted in JNJ-42847922/MIN-202/seltorexant, a high-affinity antagonist with 100-fold selectivity for Hcrt-R2 over Hcrt-R1 [100, 101]. Seltorexant which is currently in Phase III, produces proportional increases in NREM and REM in animal models [35]. PK properties are ideal for sleep induction, with rapid absorption (T_{max}: 0.5-1.5 h) and a $T_{1/2}$ of 2-3 h [102]. In a Phase II study in subjects with insomnia, seltorexant showed a favorable safety profile, increased TST, reduced WASO and LPS, and like MK-1064, enhanced REM sleep [103]. Seltorexant is being investigated in patients with major depressive disorder and co-morbid insomnia [104, 105]: it improves sleep and depressive symptoms over 28 days of dosing, and improves NREM delta power, a feature not replicated by other hypnotics [105]. A Phase IIb study (NCT03227224) reported clinically relevant reductions in depressive symptoms in MDD patients with suboptimal responses to antidepressants with adjunct seltorexant, and more so in patients with sleep disturbances [106]. Further clinical studies are ongoing as posted in clinicaltrials.gov (NCT04532749, NCT04533529).

3.2 HcrtR Agonists

Hypocretin replacement therapy

The loss of Hcrt-producing neurons in narcolepsy with cataplexy (NT1) naturally recommends the development of a Hcrt replacement therapy or Hcrt-R agonists for this devastating disorder, and other disorders with excessive daytime sleepiness (EDS). There are more than 240 clinical trials listed for the treatment of NT1 and/or EDS, although the vast majority of the drug candidates are stimulants (such as modafinil and analogues), antidepressants or other non-hypocretinergic hypnotics (sodium oxybate or histamine receptors inverse agonists). Other drug candidates in clinical trials include histamine H_3 receptor antagonists, Thyrotropin-releasing hormone analogues, solriamfetol, $GABA_A$ receptor allosteric modulators, R-baclofen, adenosine receptor antagonists, Prostaglandin DP1 antagonists, and TAAR1 agonists [107].

Although animal data suggested that intranasal Hcrt-1 administration would be effective in alleviating narcolepsy symptoms [108, 109], human clinical trials with intranasal Hcrt-1 have not demonstrated efficacy in patients with EDS and NT1, most likely due to insufficient brain exposure (see [107, 110-113][114][115]. There are no clinical reports of successful Hcrt-2 treatment in NT1 or EDS, presumably because of the anticipated short half-life of Hcrt-2.

However, Hcrt-1 or Hcrt-2 replacement/supplementation, remains a promising therapeutic avenue for the treatment of narcolepsy, EDS, hypersomnias and perhaps cognitive impairments related to aging, neurodegenerative diseases, and sleep deprivation. Since the peptides do not readily cross the blood-brain barrier, oral or intravenous (IV) administration routes are excluded. Intrathecal administration of Hcrt-1 is effective in treating cataplexy in narcoleptic Hcrt-KO mice [116], and may be a viable option in human narcolepsy, although it would require a combination of surgery and delivery device implantation.

Selective Hcrt-R2 Agonists

The development of non-peptide low molecular weight, bioavailable and brain penetrant Hcrt-R2 agonists is appealing, since the wake-promoting effects of Hcrt are largely driven by activation of Hcrt-R2, as defined by hypnotic effects of Hcr-R2, but not Hcrt-R1 antagonists (see above) and since Hcrt-R2 blockade is essential and sufficient for the sleep-inducing effects of DORAs.

YNT-185 [12] is a full Hcrt-R2 agonist which increases the firing rate of histaminergic neurons in the tuberomammillary nucleus (TMN) in animals, thereby mimicking the effects of Hcrt on these neurons [11, 117]. In wildtype (WT) mice, YNT-185 increased wakefulness and decreased NREM sleep in a dose-dependent manner, whether applied ICV, intraperitoneally (IP) or orally (PO). In Hcrt-KO mice, YNT-185 increased wakefulness and the latency to REM and decreased the number of SOREM events. YNT-185 also blocked morphine-induced sedative effects in rats [118] and alleviated chemotherapy-induced anorexia in mice [119], similar to Hcrt-1 [120, 121].

TAK-925 is a low molecular weight highly selective Hcrt-R2 agonist [13]. TAK925 increases the firing rate of TMN histaminergic neurons in mice [13], increases wakefulness and decreases NREM and REM sleep, and importantly is inactive in Hcrt-R2 KO mice. Phase I clinical trial results, reported in abstract form, indicate that IV TAK-925 promotes wakefulness in sleep-deprived healthy volunteers [122]. The study used a crossover, placebo-controlled, randomized design to compare the wake-promoting effect of a 9-h IV infusion of low (44 mg) and high-dose TAK-925 (112 mg), modafinil (oral tablet, 300 mg) or placebo. The latency to sleep onset during the Maintenance of Wakefulness Test was increased by 8.6 min (placebo), 25.4 min (low-dose TAK-925), 38.8 min (high-dose TAK-925), and 30.9 min (modafinil). TAK-925 demonstrated only minor adverse effects in this study. Additional Phase I trials assessing the effects of TAK-925 in treating narcolepsy, EDS (ClinicalTrials.gov Identifier: NCT03332784 and NCT03748979), obstructive sleep apnea (NCT04091425), and idiopathic hypersomnia (NCT04091438) are in progress.

TAK-994 is related to TAK-925, reported only in abstract form to date. TAK-994 increased wakefulness, decreased sleep/wake fragmentation, and cataplexy-like episodes in two different narcolepsy mouse models [123]. TAK-994 is currently undergoing clinical studies to determine its safety, tolerability and PK/PD in healthy participants (NCT03933488) and narcolepsy patients (NCT04096560).

Preliminary studies with Hcrt-R2 agonists are promising, although the appropriate PK/PD in narcolepsy patients remains a focal point (the effects should last for the duration of a normal day, not too short or too long) when administered both acutely and subchronically (the latter as agonist-induced receptor desensitization is currently unexplored). Since Hcrt receptors are expressed outside of the CNS, peripheral side-effects will also need to be investigated. Based on the powerful effect of Hcrt optogenetic stimulation on the hypothalamic–pituitary–adrenal (HPA) axis [124], cardiovascular adverse effects of HcrtR agonists related to sympathetic activation should be monitored. If Hcrt-R2 agonists prove to be safe and effective and have the appropriate duration of action, this approach is likely the most viable for Hcrt replacement/ supplementation therapy.

Table 1. Hypocretinergic agents investigated in clinical studies

Names	Proprietor	Indication	Stage	Original discovery / key clinical references

Names	Proprietor	Indication	Stage	Original discovery / key clinical references
Dual antagonists				
Almorexant ACT-078573	Actelion	Insomnia	Phase III, discontinued	[26] / [34, 125-127]
ACT-462206	Actelion	Insomnia	Phase I, discontinued in human medicine, currently in development in veterinary medicine	[50] / [33]
Daridorexant (nemorexant) ACT-541468	Idorsia	Insomnia	Phase III / NDA submitted	[31, 51] / [75, 76, 128, 129]
Lemborexant E-2006 (Dayvigo®)	Eisai	Insomnia	Approved 2019 (Japan, USA)	[48] / [69, 70, 72, 130, 131]
Suvorexant MK-4305 (Belsomra®)	Merck	Insomnia	Approved 2014 (Australia, Japan, USA)	[44, 47] / Phase I [132] / Phase II & III [56-60]
Filorexant MK-6096	Merck	Insomnia	Discontinued	[46] / [67]
ORN0829 TS-142	Taisho Pharmaceutical Co.	Insomnia	Phase II	[77]
SB-649868, GSK-649868	GlaxoSmithKline	Insomnia	Phase I/II, Discontinued.	[45] / [52-54]
Hcrt R1 antagonists				

Names	Proprietor	Indication	Stage	Original discovery / key clinical references
JNJ-61393215	Janssen Pharmaceutica (Johnson & Johnson)	Panic, MDD +/- Anxiety	Phase II	[95]
ACT-539313	Idorsia	Probably anxiety disorders, substance abuse, binge eating	Phase I/II discontinued	[93]
Hcrt R2 antagonists				
Seltorexant MIN-202 JNJ-42847922	Janssen Pharmaceutica (Johnson & Johnson)	Insomnia and MDD	Phase III	[100, 101] / [103-105]
MK-1064	Merck	Insomnia	Phase I (discontinued)	[96]/ [97]
Dual agonist				
Intranasal Hcrt	Investigator driven	Narcolepsy	Investigatory	[114, 115].
Hcrt-R2 agonists				
TAK-925 (Danavorexton)	Takeda & Millenium Pharmaceuticals	Narcolepsy, EDS	Phase I	[13]
TAK-944	Takeda	Narcolepsy, NT1, NT2	Phase I	Takeda

4. Circuit-based Indications for Hypocretinergic Agents

Alterations in the hypocretin system have been described for several disorders [reviewed in 158], suggesting replacement therapy or blockade of Hcrt signaling, accordingly, may provide therapeutic benefit. In addition, the Hcrt circuitry and receptor expression profile naturally associate this system with relatively specific clusters of functional roles. These include vigilance states / sleep-wakefulness, aversive learning and memory, reward and motivation, stress resilience and anxious behaviors, cognitive flexibility and feeding and metabolism (see Fig. 2).

---Insert Figure 2 ~ here--- Legend:

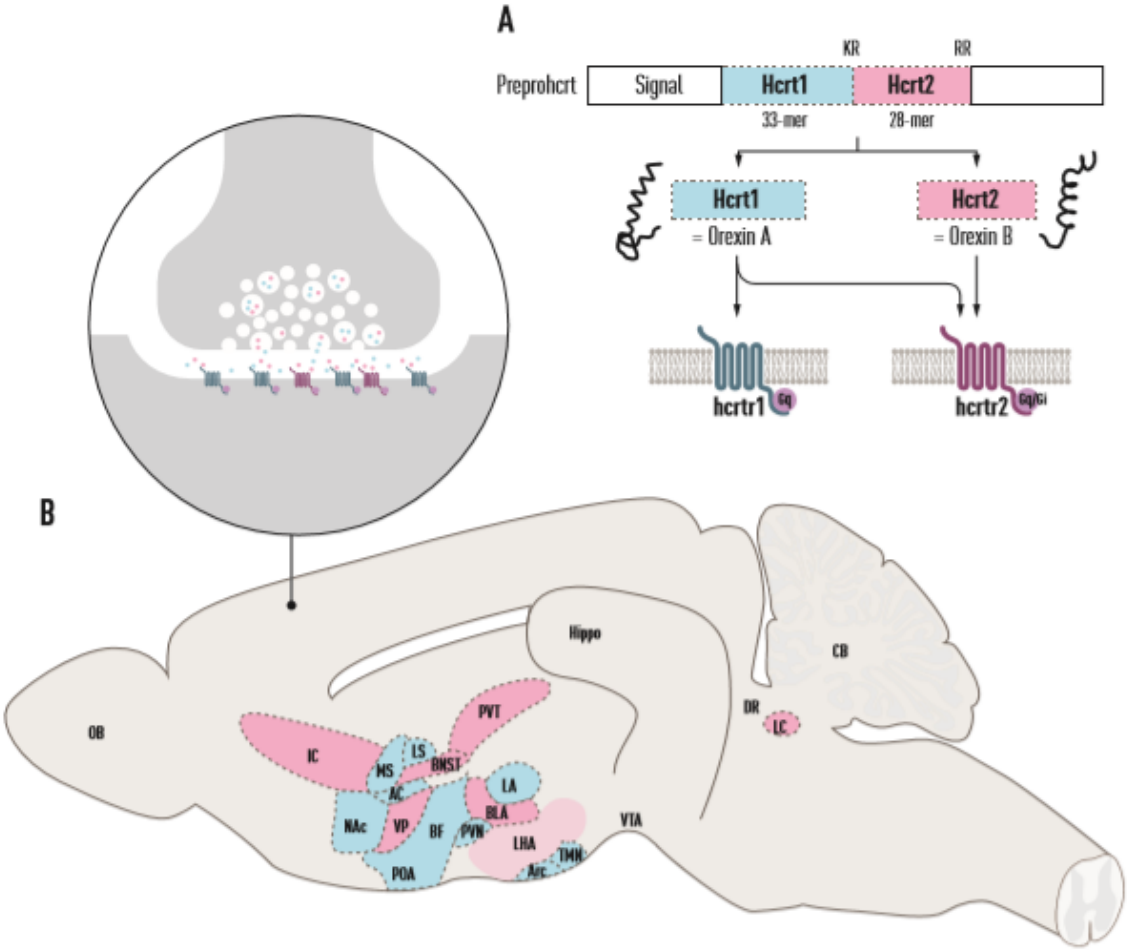

Figure 2. **Hcrt neuron circuitry highlights disease indications potentially targeted with hypocretinergic agents**. Hcrt neurons interconnect with, and integrate input from and output to, structures implicated in disorders associated with vigilance states, reward and motivation, eating and metabolism, mood, stress and cognition. Double-headed arrows denote structures with reciprocal connections with Hcrt neurons; arrows directed to the Hcrt circle represent afferent inputs to, and

arrows outward efferent outputs from, Hcrt neurons. See [159] for a detailed review of Hcrt neurocircuitry. *Abbreviations*: BNST, bed nucleus of the stria terminalis; DR, dorsal raphe; LC, locus coeruleus; LH, lateral hypothalamic area; LS, lateral septum; MDD, major depressive disorder; mPFC, medial prefrontal cortex; MRN, medial raphe nucleus; NAcc, nucleus accumbens; POA, preoptic area; PVN, paraventricular hypothalamic nucleus; PVT, paraventricular thalamic nucleus; TMN, tuberomammillary nucleus; VMH, ventromedial hypothalamus; VTA, ventral tegmental area.

4.1 Brain Hcrt levels in various disorders

Altered levels of Hcrt in cerebrospinal fluid (CSF) and/or plasma or serum have been reported for a number of conditions, particularly neurodegenerative disorders, as reviewed elsewhere [see 158]. Analyses have mostly been conducted with radioimmunoassay (RIA) and enzyme-linked immunosorbent (ELISA) assays for Hcrt-1. Although appropriate for diagnosing NT1, such methods have been criticized for use in fundamental research, as the nature of the actual analytes determined is unclear and may include breakdown products of Hcrt-1 (and perhaps also Hcrt-2 or even prepro-Hcrt) [160]. Determinations of plasma Hcrt in particular need to be interpreted with caution, as RIA and ELISAs have been shown to recognize hydrolyzed peptides that have little in common with active Hcrt receptor ligands [161]. Development of more advanced methods for Hcrt determination, e.g., mass spectrometry, are clearly warranted, as recently described for the assessment of Hcrt-1 levels in a preclinical setting, which is additionally challenged by very low sample volumes [162]. In spite of these limitations, clear alterations of the Hcrt system in several neurodegenerative disorders, generally associate with cognitive and vigilance state symptoms [see 158]. NT1 is the best characterized degenerative disorder associated with alterations in Hcrt levels, since it is characterized by the loss of LH Hcrt neurons [163-166]. Reduced plasma/serum levels of Hcrt-1 have been reported in Anorexia Nervosa [167], attention deficit/hyperactivity disorder [168] and PTSD [169]. The latter study also described substantially decreased Hcrt-1 in CSF of veterans with chronic PTSD. Plasma and CSF Hcrt were positively correlated in patients and controls, and CSF Hcrt strongly negatively correlated with PTSD symptom severity [169]. In MDD, one study showed substantially blunted diurnal variations in CSF Hcrt versus controls which was partially rescued by antidepressant treatment [170]. Brundin and colleagues reported lower CSF Hcrt in MMD with suicide attempt versus other disorders, which negatively correlated with symptoms, and was restored in a subsequent 1 year later follow up in association with improved symptoms [171]. In contrast, elevated CSF Hcrt has been described in panic disorder [172]. More information is clearly required for psychiatric disorders.

Most studies in neurodegenerative disorders show evidence of a loss of Hcrt signaling components, suggestive that Hcrt replacement therapy or HcrtR agonists during the daytime may benefit these patients. However, data also suggest that Hcrt tone may be dependent on the state of disease progression, with some disorders (notably AD and PD) showing evidence of elevated Hcrt signaling earlier in the disease course [173-176], suggesting possible early compensatory hyperarousal

mechanisms may be at play that are subsequently overwhelmed as degeneration advances. Under such circumstances, Hcrt agonists may not be ideal, but rather an antagonist administered at night preferable. Hcrt therapeutic approaches for progressive disorders will thus most likely need to take disease status into account, perhaps supported by assessments of Hcrt tone and specific symptom presentation. Critically, future studies which associate disease stage and importantly, circadian time of sampling (ideally assessed over a full 24 hr period), are imperative to guide clinical approaches.

4.2 Vigilance state disorders

Hcrt neurons connect with components of the ascending arousal system, either as afferent (noradrenergic locus coeruleus (LC)) or reciprocal projections [basal forebrain cholinergic system (BF), dorsal and medial raphe nuclei (DRN, MRN), dopaminergic ventral tegmental area (VTA), histaminergic tuberomammillary nucleus (TMN); see 159; Fig. 2]. These structures all express high levels of Hcrt-Rs, the LC predominantly Hcrt-R1, the TMN specifically Hcrt-R2, and others both receptors [177, 178]. Correspondingly, the role of Hcrt in the promotion and maintenance of arousal has been well established [reviewed in 159]. Thus, disorders in which sleep/wakefulness is disrupted, may benefit from hypocretinergic drugs. Therapeutic approaches may include improvement of sleep and sleep architecture using DORAs and HcrtR2 antagonists or stabilizing the wake state in conditions with symptoms of EDS with Hcrt agonists. Hcrt agonists may also provide access to not only increased/stabilized wakefulness during the day, but also improved sleep at night [see 158].

Indications approachable with antagonists to improve sleep evidently include insomnia [see section 3.3, and reviewed in 9, 27, 29, 31, 179]). DORAs and Hcrt-R2 antagonists were initially developed during the Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM IV) era, when primary insomnia was considered as a discrete disorder, a distinction later removed in DSM V [180]. Since then, the clinical focus for hypnotic Hcrt-R antagonists has rapidly expanded to selected populations and secondary insomnias e.g. elderly [65, 127], MDD [105], AD [64], circadian disruptions induced by shift work (NCT02491788), PD (NCT02729714), PTSD (NCT02849548) and PSP (NCT04014387), with strong clinical rationales presented for substance abuse withdrawal [181] and Autism spectrum disorders [182].

Indications that may be approached with Hcrt-R2 agonists or Hcrt replacement therapy (see section 3.2) include narcolepsy (type 1 and type 2), chronic fatigue syndrome, EDS in neurodegenerative disorders, PTSD, attention deficit, age-related cognitive decline, attention deficit/hyperactivity disorder and EDS caused by sleep deprivation. Compelling rationale for the use of Hcrt activation strategies in such disorders are comprehensively reviewed in Seigneur & de Lecea [158]. In addition to ameliorating EDS by promoting wakefulness and attention, enhancing Hcrt tone during wake period may also improve night-time sleep, as demonstrated in an elegant preclinical study defining the effects of Hcrt-modulated theta and gamma power during wakefulness on subsequent sleep *via* sleep homeostatic mechanisms [185].

4.3 Manipulating sleep architecture as a precision medicine approach.

One of the clinically most interesting features of DORAs is their ability to enhance REM sleep, whereas most traditional hypnotics suppress REM [35]. Preclinical studies comparing individually and co-dosed Hcrt-R1 and Hcrt-R2 antagonists, and dosing selective antagonists into the opposing receptor KO mouse, suggests this is due to the combined effects of blocking both Hcrt-Rs simultaneously [84, 86, 133]. This effect is present to such a degree that REM sleep disinhibition in HcrtR-R2 KO mice has become routinely used for *in vivo* validation of Hcrt-R1 antagonist target engagement (e.g. with JNJ-54717793 [88] and JNJ-61393215 [95]). Although it can be debated as to whether REM sleep enhancement may be beneficial or detrimental, REM sleep is a normal component of sleep architecture, and no other hypnotic principle replicates this effect. All other hypnotics, antipsychotics and antidepressants typically have either no effect on REM, increase REM sleep latency, or directly suppress REM sleep (and in the case of antidepressants, sometimes for decades, begging the question as to the role of REM sleep suppression in antidepressant efficacy). Given that many neurological and neuropsychiatric disorders show specific REM sleep impairment, the ability of DORAs to enhance REM sleep provides an opportunity to tailor hypnotic treatments to suit specific sleep symptomatology in a precision medicine manner. At present, clinical evidence for this theorem is unproven, but testing is underway through the evaluation of DORAs and Hcrt-R2 antagonists in subjects with PTSD and MDD [NCT02669030; NCT04080752; NCT02849548; 104, 105].

4.4 Reward-related disorders

Hcrt neurons connect strongly with a number of reward-related centers, including reciprocal projections to the dopaminergic VTA, nucleus accumbens, serotonergic DR and the amygdala, afferent input from PVN neurons and project to the ventral pallidum and insular cortex, with Hcrt-Rs expressed in all of these regions (reviewed in [159]. Thus, the Hcrt system is intimately implicated in reward and addiction [see 186]. Accordingly, there is a wealth of preclinical literature on, and strong clinical rationales for, exploring in particular Hcrt-R1 antagonists, but also hcrt-R2 antagonists (studied to a lesser degree), on the various components of addictive behaviors, such as self-administration, craving/drug seeking, withdrawal and reinstatement/ relapse in substance use disorders such as alcohol, cocaine and opioids [see 6, 187, 188-190]. Sleep and anxiety are co-morbid in substance-use disorders and indeed may contribute to their maintenance. Hcrt-R antagonists could be expected to provide benefit *via* these domains as well [187]. Current clinical trials explore Hcrt-R antagonists in substance use disorders and associated symptoms (e.g., opioids: NCT04262193, NCT03789214, NCT04287062; cocaine: NCT02785406, NCT03937986; alcohol: NCT03897062, NCT04229095). In addition, neuronal activity in the paraventricular thalamus, projecting to the VTA and nucleus accumbens, is important for the establishment of opioid-associated memories, and Hcrt neurons projecting to PVT may be key to modulating this circuit [191, 192].

4.5 Major Depressive Disorder

A number of structures that connect with Hcrt neurons and express Hcrt-Rs have been associated with MDD, such as the dorsal raphe nucleus, ventral pallidum, hippocampus, accumbens, amygdala, VTA, locus coeruleus, BNST, arcuate nucleus, PVN, SON, insula, prelimbic and infralimbic cortices. Furthermore, Hcrt-R1 gene variants rs10914456 and rs2271933 are associated with MDD [195]. A number of the aforementioned structures also subserve reward functions. Therefore, reduced Hcrt signaling through these structures could be implicated in anhedonia, a core symptom of MDD [196]. This may suggest Hrct replacement strategies/ agonists as prospective antidepressants for MDD. This is supported preclinically by enhanced stress resilience observed with a Hcrt-R2 agonist and opposing effects of Hcrt-R2 antagonists [134], Hcrt-1-induced restoration of BDNF levels in preclinical models of PTSD and PD [197, 198] and neurogenesis enhancement by ICV Hrct-1 [199]. The latter two effects are central theses of antidepressant mechanisms of action [200]. However, recent Hcrt-2R antagonist studies show antidepressant effects in clinical studies when used as hypnotics [103, 105]. Together these findings suggest that daytime enhancement of Hcrt-R signaling and/or reduced Hcrt-R2 tone during sleep, perhaps viewable as a restoration of circadian amplitude in Hcrt signaling, may provide access to antidepressant activity *via* the Hcrt system.

4.6 Anxiety and stress-related disorders

Consistent with the predominant expression of Hcrt-R1 (*versus* Hcrt-R2) in key anxiety-related structures (such as the BNST, LC, BLA and central amygdala, cingulate cortex, insular cortex and ventral pallidum), a critical role for this receptor is emerging in complex, anxiety-related emotional behaviors. This is supported by a plethora of preclinical evidence in models of fear, stress, PTSD and panic ([88, 201-205]; [see also 90, 206-208]) and the association of Hcrt-R1 polymorphisms such as HCRTR1 C/T Ile408Val with panic and agoraphobia, including limited responses to cognitive behavior therapy [209], although the mechanism of altered signaling for this variant has not yet been deciphered [210]. Hcrt-R1 antagonists are being investigated in clinical studies for panic [JNJ-61393215, 95]. A study investigating suvorexant in panic disorder is also listed on clinicaltrials.gov (NCT02593682).

Insomnia is common in PTSD, but so too is dysregulated REM sleep, including REM sleep fractionation, and nightmares [211, 212]. REM sleep is associated with emotional memory consolidation [213, 214]. Consolidation of fractionated REM sleep in PTSD with a REM-enhancing DORA may therefore aid consolidation of cognitive behavior therapy (extinction learning), as currently investigated with suvorexant (NCT02849548). Restoration of normal sleep architecture may be key in PTSD, via reduction of nocturnal arousals and normalization of REM sleep architecture, as shown recently when comparing suvorexant and zolpidem in a model of rodent fear conditioning / extinction [215]. Indeed, it is interesting to speculate whether REM suppressing agents such as SSRIs,

which are commonly prescribed in PTSD, may disrupt REM sleep extinction learning and exacerbate this aspect of the disorder. Furthermore, the PK of most DORAs is relatively long (see [27, 216]), carrying over into the following day period. Hcrt-R1 antagonists reduce conditioned fear and facilitate its extinction in animal models, while Hcrt-1 or optogenetic activation of the Hcrt-LC circuit increased fear and fear generalization [217-219]. Residual Hcrt-R1 antagonism provided by DORAs the following day may therefore also be beneficial for PTSD daytime symptoms.

Another school of thought, supported by preclinical data, suggests that low Hcrt tone during the stressful event may be associated with an increased risk of developing PTSD, *via* disruption of the adaptive stress response [see 158]. This thesis would suggest implementation of Hcrt replacement/Hcrt-R agonist approaches at or near the time of triggering events may be of benefit in suppressing development of PTSD and indicates that the course of the disorder will likely need to be taken into to account to ensure successful translation of Hcrt agents into a clinical setting for PTSD.

Related to their role in reward, anxiety and mood, a recent study has indicated that Hcrt signaling indirectly mediates aggressive behavior in mice, through GABAergic interneurons in the lateral habenula [220]. Furthermore, the HCRTR1 rs2271933 G > A gene variant is associated with enhanced aggression [221]. This may open the possibility of modulating aggression, a common symptom of PTSD [222], in this and other disorders with Hcrt-R antagonists.

4.7 Cognitive disorders

Hcrt projections and receptor expression in cortical regions, the lateral septum, hippocampus, cholinergic and noradrenergic structures suggest a role for Hcrt in cognitive processes. This has been demonstrated with preclinical studies, determining a role for Hcrt signaling in hippocampal-dependent social [223] and spatial memory [e.g., 224, 225-227] independently of sleep. Hrct increases ACh release in the prefrontal cortex [228-230] and the Hcrt-R1 antagonist SB-334867 impaired performance in an acetylcholine-mediated sustained attention task [231]. Cognitive flexibility is impaired in Hcrt-KO mice [232] and improved in aged rats by intranasal Hcrt [109]. Lower plasma Hcrt-1 is associated with reduced cognitive flexibility in subjects with anorexia nervosa [167], while NT1 patients perform sub-optimally in the decision-making Iowa Gambling Task [233]. Motivational effects of Hcrt agonism on cognitive performance may also be at play in some tasks [234]. The Hcrt system plays an important role in the cellular correlate of learning and memory, long term potentiation, in the hippocampus and VTA [235-237] dependent on functional Hcrt-R1 receptors and plasticity-associated kinases [238-240].

Sleep is disrupted in most neurodegenerative proteinopathies characterized by cognitive dysfunction, including AD, PD, HD, LBD, FTD and MND, often early in the course of disease, and has thus been proposed as an early biomarker for some of these disorders [see 241, 242-246]. Hypnotic Hcrt-R

antagonists may provide benefits in these disorders relative to benzodiazepines, z-drugs or sedative antipsychotics and antidepressants, since Hcrt-R antagonist-induced sleep is not accompanied by memory disruption, a finding reported many times in animals [e.g., 247, 248-251]. This is a particular point of opportunity given the cognitive decline that occurs as a major symptom in most of these disorders. It is also likely that the arousal-ready state that hypocretinergic hypnotics engender in both humans and animals [41, 42, 252] may help to reduce the risk of falls at night, which is a notable risk with sedatives and classic hypnotics in these patient populations [253, 254]. A further potential bonus of enhancing sleep with Hcrt-R antagonists in proteinopathies, includes removal of neurotoxic metabolites *via* glymphatic clearance – an astrocyte-mediated component of the brain's lymphatic drainage system (see [255]). Of particular relevance to AD and tauopathies, this has been demonstrated preclinically for tau and amyloid-beta [Aβ; 256, 257] and in models of Aβ amyloidogenesis when enhancing sleep using DORAs [258-260].

In addition to neurodegenerative disorders characterized by cognitive decline such as AD and FTD, these data suggest Hcrt agonists may theoretically benefit subjects with age-related cognitive decline, attention disruption caused by sleep deprivation or circadian dysregulations (e.g., jet lag) and Attention Deficit/Hyperactivity Disorder [158, 261, 262].

4.8 Eating disorders

In line with Hcrt projections and expression of Hcrt-R in feeding and metabolic centers such as the arcuate nucleus, ventral pallidum, PVN, insular cortex and hypothalamus, early research determined a role for Hcrt in feeding behavior [15]. Subsequent studies have established that Hcrts stimulate food intake and motivation for food rewards in an energy status-dependent fashion, and can promote binge eating [reviewed in 263]. Hcrt-R antagonists have thus been investigated in preclinical models of binge eating. Piccoli and colleagues [264] investigated the DORA SB-649868, Hcrt-R1 antagonist GSK1059865 and Hcrt-R2 antagonist JNJ-10397049 in a binge eating model in female rats. The DORA and Hcrt-R1 antagonists, but not Hcrt-R2 antagonist, selectively reduced binge eating of a highly palatable food without affecting normal food intake, at doses that did not induce sleep. These data are promising for the advancement of Hcrt-R1 antagonists into clinical investigations for binge eating and possibly bulimia nervosa. Idorsia was developing ACT-539313, an Hcrt-R1 antagonist for various indications including binge eating and first in-humans data have been published [93, 94, 265]. On the other hand, very extensive phase III studies with the DORAs suvorexant, lemborexant and daridorexant, which lasted up to 12-14 months, have not resulted in weight reduction in these insomnia patients whether lean or obese (or for that matter in "healthy" controls) [see 56, 60, 61, 131, 266].

On the other side of the spectrum, the role of Hcrt signaling in anorexia nervosa (AN) is unclear, with some studies showing altered Hcrt levels in e.g., plasma, and other not [see 158, 263]. However, the findings that centrally administered Hcrt-1 induces feeding in satiated animals [121, 267, 268], the

complexity of treating AN and comorbid psychiatric conditions, and the limited efficacy of current therapies [269], together indicate that should dual or Hcrt-R1 agonists become available, this is a concept very much worth testing in AN.

4.9 Cancer and immune modulation

Hcrt plays a role in both the neuroimmune axis and in immune-independent tumor-induced alterations in sleep and metabolism. In the later, mammary tumor growth was associated with enhanced Hcrt neuron activity, likely mediated *via* aberrant satiety signaling systems, disrupting metabolism through Hcrt-induced hyperarousal/reduced sleep and sympathetic activation [270]. Enhancing sleep with a DORA (almorexant), rescued both sleep and metabolic alterations in tumor bearing mice. These findings illustrate a role for Hcrt signaling in tumor-induced alterations in sleep and metabolism, and point to the potential for hypnotic Hcrt-R antagonists to improve these symptoms in cancer patients [270]. More broadly, these data define a mechanism by which cancer induces changes in brain functions that promote cancer proliferation and cements the bidirectional relationship between altered sleep-wakefulness (a common symptom across different types of cancers), metabolism and the manifestation and progression of cancer [reviewed in 271]. Although immune signaling *via* IL-6 was separated from sleep-wakefulness disruption/metabolic axis in the study by Borniger and colleagues [270], this does not preclude additional effects of Hcrt on other immune signaling pathways associated with cancer, or with peripheral immune function in general. Indeed, efferent connections of the Hcrt system with the HPA axis and associated glucocorticoid secretion and/or sympathetic tone, point to a mechanism by which the Hcrt system may influence peripheral immune responses. Stress-mediating corticotrophin-releasing hormone positive neurons in the PVN (CRH^{PVN} neurons) project to Hcrt neurons, and optogenetic stimulation of this projection promotes wakefulness, as does restraint stress. Hyperarousal in both conditions was abolished by CRISP-Cas9 knockdown of the *crh* gene, indicating that the CRH^{PVN}/Hcrt circuit mediates stress-induced hyperarousal. Optogenetic stimulation of CRH^{PVN} neurons also drove changes in circulating peripheral immune cell populations, as assessed by single-cell mass cytometry by time of flight (CyTOF) profiling, mimicking stress-induced immunosuppression [272]. These data suggest a common mechanism underlying insomnia and stress-induced systemic immune suppression. Indeed, links between immune function, sleep and Hcrt were demonstrated by McAlpine and colleagues in the context of atherosclerosis, whereby sleep disruption increased the production of monocytes and neutrophils from bone marrow, which is also observed in Hcrt KO mice, and was reversable by Hcrt supplementation [273]. These data demonstrate robust interactions between sleep, stress and peripheral immune responses, and highlight a central role for Hcrt in this relationship. How these novel and exciting findings may play out in the scope of drug discovery and development for immune/metabolic-related disorders, or indeed as Hcrt-agent associated adverse effects, remains to be determined in future lines of research.

5 Conclusion

In summary, the hypocretin/orexin system sits at a neuronal crossroad of integrated pathways that modulate the broad construct of arousal. As a result, interventions of the Hcrt system simultaneously

modulate neurotransmitters canonically associated with wakefulness and alertness namely dopamine, norepinephrine, acetylcholine and histamine. Hypnotic HcrtR antagonists show specific advantages and reduced adverse effects compared to conventional hypnotics, such as Z-drugs and benzodiazepines. The duality of Hcrt functions in arousal and reward opens additional therapeutic opportunities in substance abuse, eating and panic disorders, cognitive disruption and emotion-related disorders including anxiety, MDD and PTSD. These features are likely to encourage drug discovery and development initiatives to continue to deliver a solid pipeline of compounds targeting the Hcrt system for an ever-growing list of indications.

Conflict of Interest

The authors declare that there is no conflict of interest related to this manuscript.

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GRAPHICAL ABSTRACT