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The Hypocretin/Orexin System: Implications for Drug Reward and Relapse

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Abstract Hypocretins (also known as orexins) are hypothalamic neuropeptides involved in the regulation of sleep/wake states and feeding behavior. Recent studies have also demonstrated an important role for the hypocretin/orexin system in the addictive properties of drugs of abuse, consistent with the reciprocal innervations between hypocretin neurons and brain areas involved in reward processing. This system participates in the primary reinforcing effects of opioids, nicotine, and alcohol. Hypocretins are also involved in the neurobiological mechanisms underlying relapse to drug-seeking behavior induced by drug-related environmental stimuli and stress, as mainly described in the case of psychostimulants. Based on these preclinical studies, the use of selective ligands targeting hypocretin receptors could represent a new therapeutical strategy for the treatment of substance abuse disorders. In this review, we discuss and update the current knowledge about the participation of the hypocretin system in drug addiction and the possible neurobiological mechanisms involved in these processes regulated by hypocretin transmission.

Keywords Hypocretin · Addiction · Reward · Self-administration · Relapse · Stress

The Hypocretin/Orexin System

Co-discovered in 1998 [1, 2], hypocretins (also known as orexins) have long been described as exclusive central nervous system (CNS) peptides. However, amassing evidence indicates the existence of hypocretins and their receptors in the periphery [3], which suggests the presence of additional sources for these peptides. Multiple aspects of hypocretins functions still remain unknown and this article has been focused on the role of central hypocretin signaling in drug reward and addiction.

Hypocretin-1/orexin-A and hypocretin-2/orexin-B are 33- and 28 amino acid (AA) residue peptides proteolytically cleaved from a common precursor peptide of 130 AA, preprohypocretin/preproorexin [1, 2]. Hypocretins are present from vertebrate fish to mammals [4, 5] and mammalian hypocretin peptides are highly conserved in all the species [6], suggesting a critical role in evolution. Human hypocretin-1 and -2 share 46% sequence homology and hypocretin-1 is identical in a series of mammalian species, while hypocretin-2 differs in 1 or 2 AA from human to other mammalians. The C-terminal sequence of both hypocretin peptides is highly conserved and post-translationally amidated [2]. However, only the N-terminal sequence of hypocretin-1 is cyclized into a pyroglutamyl residue [2]. Hypocretin-1 is also post-translationally stabilized by two intrachain disulfide bonds whereas hypocretin-2 remains a linear peptide [2]. As other neuromodulatory peptides, neuronal hypocretins are stored in secretory vesicles, accumulated at axon terminals, and released in a Ca^{2+}-sensitive manner [1]. Two closely related G protein-coupled receptors that respond to hypocretins have been cloned, hypocretin or orexin receptor-1 (Hcrtr-1/OxR1) and hypocretin or orexin receptor-2 (Hcrtr-2/OxR2) [2]. Human Hcrtr-1 (425 AA) and Hcrtr-2 (444 AA) share 64% sequence identity [2, 6] and Hcrtr-1 binds with 100–1,000 higher affinity to hypocretin-1 than hypocretin-2. In contrast, Hcrtr-2 binds both hypocretins with similar affinity [2, 7, 8]. As the C-terminal region of both peptides is highly conserved, it has

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been proposed that the N-terminal sequence is responsible for the higher preference of Hcrtr-1 for hypocretin-1 [9].

The cellular signals triggered upon hypocretin receptor activation have been largely investigated but remain still to be elucidated. In native receptor-expressing neurons, stimulation of both hypocretin receptors leads to a prominent increase in intracellular Ca^{2+} concentrations through the activation of Gq proteins followed by phospholipase C and subsequent protein kinase C (PKC) stimulation [10–14]. The activation of this kinase phosphorylates and modulates effector ion channels leading to Ca^{2+} entrance, among other effects [6, 15]. Hcrtr-1 is thought to signal only through Gq proteins in neurons, although a recent report showed that Hcrtr-1 stimulation lead to cyclic AMP production in primary cultures of rat astrocytes [16], suggesting the existence of Gs-coupled signal transduction through Hcrtr-1. The Ca^{2+} elevation induced by hypocretin receptor activation explains the commonly reported neuroexcitatory nature of hypocretin peptides on the brain [1]. Thus, the most common response to hypocretins is an increase in action potential frequency achieved by pre- and postsynaptic effects. Hypocretin regulation of presynaptic glutamate- and GABA-releasing neurons has been identified. Thus, PKC at presynaptic terminals phosphorylates and activates presynaptic Ca^{2+} channels facilitating neurotransmitter release [10, 17, 18]. Additionally, postsynaptic-activated PKC phosphorylates and regulates the function of diverse effector ion channels [6, 15], finally leading to depolarization. In contrast, few reports relate hypocretins to synaptic inhibition [19–22]. Although the molecular mechanisms regarding hypocretin-induced synaptic inhibition have not been clarified, Hcrtr-2 has been shown to couple to inhibitory Gi proteins that prevent cyclic AMP formation [23].

In the CNS, hypocretin expression is restricted to a few thousand neurons in some particular regions of the hypothalamus: the perifornical area (PFA), the dorsomedial hypothalamus (DMH), and the dorsal and lateral hypothalamus (LH) [1, 2, 24]. Although hypocretin-containing neurons represent a relatively small number of cells, their projections are widely distributed throughout the brain (Fig. 1) [24]. Hypocretin fibers are spread along the entire hypothalamus, which suggests an important role in energy homeostasis and other autonomic functions. The largest amount of extrahypothalamic projections are found in brain stem nuclei, such as the raphe nuclei, the reticular formation, and especially the locus coeruleus [24], a brain nucleus involved in the regulation of behavioral arousal. Hypocretin neurons also send significant efferent projections to structures related with drug seeking and addiction, such as the paraventricular nucleus of the thalamus, the septal nuclei, the bed nucleus of the stria terminalis, the anterior and central amygdaloid nuclei, and the ventral tegmental area (VTA). Disperse axons are also found throughout the cortex and the medial part of the nucleus accumbens (NAcc) shell [24, 25]. Both hypocretin receptor subtypes are found in similar brain areas than hypocretin fibers and the distribution of both receptors is partially overlapping, although some particular areas essentially express one receptor subtype. Thus, the prefrontal cortex predominantly expresses Hcrtr-1 and the NAcc mainly Hcrtr-2 [26], suggesting that each receptor could regulate distinct functions in the reward circuit.

The widespread extension of the hypocretin system in the CNS is in agreement with the variety of physiological functions of hypocretin peptides, that includes energy homeostasis, behavioral arousal, sleep/wake cycles, and reward-seeking and addiction, among others [27]. Recent evidence also points to a role for the hypocretin system in other CNS disorders, such as Alzheimer's disease [28] and panic anxiety disorders [29]. Initially, hypocretins emerged as regulators of feeding behavior and the alternative name "orexin" was devised upon the observation that intraventricular infusion of hypocretins increased food intake in rats [2]. Nevertheless, this response is now considered to be an indirect effect of the wake-promoting effects of hypocretins and not directly related to the regulation of satiety states and energy balance [30, 31]. On the other hand, the role of hypocretin signaling in the promotion of wakefulness/arousal is unquestionable [32] and narcolepsy is the clearest evidence for aberrant hypocretin transmission in pathophysiological conditions. Genetic ablation of preprohypocretin or Hcrtr-2 gene in rodents results in a phenotype similar to human narcolepsy with cataplexy [33, 34]. In agreement, dogs with a mutation in the Hcrtr-2 gene also display the same phenotype [35]. Furthermore, some human narcoleptics have undetectable hypocretin levels in the cerebrospinal fluid [36] and lack hypocretin-producing neurons in the hypothalamus [37]. Thus, Hcrtr-2 agonists might be useful for narcoleptic patients whereas hypocretin receptor antagonists might serve for the treatment of insomnia [38].

Arousal- and wakefulness-promoting stimuli are often associated with stress and anxiety. Indeed, hypocretin-containing neurons are sensitive to corticotrophin-releasing factor (CRF) neurons and can also regulate the activity of these cells [39], suggesting a role for hypocretins in stress-related behaviors [40]. Stress is a key element of the negative emotional states associated to drug withdrawal and represents a crucial factor for drug-seeking behavior [41]. Hypocretin signaling could contribute to drug seeking in part by modulation of stress responses. Thus, intraventricular and intra-VTA infusions of hypocretin-1 decreases the activity of the brain reward system [42, 43], similar to CRF [44] and drug withdrawal [45, 46]. However, in contrast to the previous data, intra-VTA infusion of hypocretin-1 and -2 increased dopamine levels in the NAcc [47, 48] and the prefrontal cortex [49] of rats, indicating a role for hypocretins in the regulation of the dopaminergic mesocorticolimbic

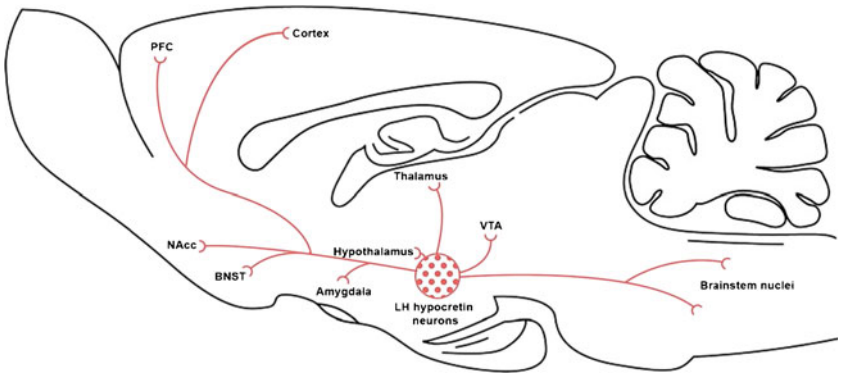

Fig. 1 Schematic representation of the hypocretin system. Neurons expressing hypocretin (*dots*) project widely throughout the brain modulating diverse physiological functions. *PFC* prefrontal cortex, *NAcc* nucleus accumbens, *BNST* bed nucleus of the stria terminalis, *VTA* ventral tegmental area, *LH* lateral hypothalamus

system and reward learning. Moreover, hypocretin-1 is critical for the induction of synaptic plasticity in the VTA [50], which accounts for relapse after long periods of abstinence. In agreement with these findings, several behavioral studies using models of drug reward and relapse support the involvement of hypocretin transmission in the addictive properties of drugs of abuse.

Animal Models of Drug Reward and Relapse

Several predictive animal models are available to study responses related to the rewarding effects produced by the different drugs of abuse. These procedures include the intracranial electric self-stimulation paradigms that evaluate the effects of the drug in the brain reward circuits, the self-administration methods that directly measure the reinforcing properties and the conditioned place preference that assesses conditioned responses related to the rewarding effects. These experimental models have been useful to define the neurobiological substrate involved in the rewarding effects of drugs of abuse that are crucial for the addictive process. However, one of the most important clinical concerns for the treatment of drug addicts is the very high rate of relapse even after long periods of drug abstinence [51]. Several behavioral models have also been validated in experimental animals to mimic some aspects of drug relapse in human addicts. The vast majority of animal studies on drug relapse are based on reinstatement models [52–54]. Reinstatement refers to the recovery of a learned response that occurs when a subject is exposed to some particular stimuli after extinction of such a response [55].

The neurobiological mechanisms underlying the different behavioral responses evaluated in these animal models of reward and reinstatement have been clarified by using several neurochemical and electrophysiological techniques. Electrophysiological techniques that determine the firing rates of neurons have been widely used to investigate the changes in the activity of intrinsic and synaptic currents of neurons involved in the reward circuits [56]. These electrophysiological recordings have been performed in vivo in whole animals as well as ex vivo in isolated brain slice preparations [57]. Ex vivo studies in brain slices are useful to differentiate the intrinsic responses of the neurons from those that are dependent on the activity of afferent neuronal projections. In vivo electrophysiological studies have identified the specific firing responses of neurons involved in reward processes, such as dopaminergic neurons, to several rewarding stimuli including drugs of abuse, and have provided interesting correlates with the responses evaluated in the different behavioral models [56].

Among the different neurochemical procedures, the use of in vivo microdialysis techniques has provided crucial advances in the knowledge of the neurobiological substrate of the reward circuits [58]. In vivo microdialysis techniques in free-moving rodents allow the measurement of the extracellular levels of neurotransmitters in discrete areas of the central nervous system, which represents a direct reflection of the balance between synaptic release and uptake/clearance of these neurotransmitters. The use of this technique in line with behavioral studies has allowed identifying the neurochemical changes that are associated to particular behavioral tasks, such as the enhancement in the extracellular levels of dopamine in the reward pathways after the administration of all the prototypical drugs of abuse [59].

One of the behavioral paradigms widely used to study the effects of drugs of abuse on the activity of the reward circuits is the intracranial electric self-stimulation procedure [60]. In this paradigm, animals are trained to maintain an operant behavior to obtain an electric pulse through an electrode placed in a reward-related brain site; most frequently, the lateral hypothalamic area. The threshold of the minimal current needed to promote intracranial electric self-stimulation is usually estimated. A drug that stimulates the reward circuit will decrease this threshold, which would be related to its rewarding properties, whereas a drug or a state of withdrawal producing aversive effects will enhance the minimal current required to maintain the self-stimulation [61]. Intracranial electric self-stimulation paradigms are useful to investigate the rewarding effects of drugs of abuse, but have not been used as animal models of reinstatement of drug-seeking behavior.

The most reliable and predictive animal models of reinstatement involve operant self-administration procedures based on operant conditioning paradigms [53]. Self-administration models mimic drug-taking behavior in humans and are widely used to directly evaluate the primary reinforcing properties of drugs. These operant procedures are considered by most researchers to be reliable models of drug consumption in humans with a high predictive value [60]. In these models, animals are trained to respond in order to obtain a drug intravenous infusion or an oral solution in the case of alcohol, typically by pressing a lever or nose poking in a hole. The operant chambers are equipped with these manipulandi that transmit the operant response as well as devices that deliver the drug (reinforcer). The response in the active manipulandum is linked to the delivery of the drug, while the response in the inactive manipulandum results in the delivery of the drug vehicle or lacks any programmed consequence. The fixed ratio and progressive ratio schedule reinforcement programs are commonly used. Under a fixed ratio schedule, the drug is delivered each time a preselected number of responses are completed. Under the progressive ratio schedule, the response requirement to deliver the drug escalates according to an arithmetic progression. The common index of performance evaluated in this schedule is the breaking point defined as the highest number of responses that the animal accomplishes to obtain a single infusion of drug, which provides information about its motivation for the drug. The analysis of this instrumental response provides valuable information about different behavioral aspects of drug consumption. After acquisition of the operant task, the behavioral response can be extinguished by exposing the animals to an additional training where the reward is no longer available. The operant behavior to seek the drug can be then reinstated by using different stimuli.

Several researchers have developed drug reinstatement procedures in rats and mice using the place conditioning paradigm [62]. In the place-conditioned paradigms, the subjective effects of the drug are repeatedly paired to a previously neutral spatial environmental stimulus. Through this repeated association process, the environment acts as a conditioned stimulus. The animal will then prefer (conditioned place preference) or avoid (conditioned place aversion) this conditioned stimulus, depending on the rewarding or aversive effects produced by the administration of the drug. Although a conditioned approach/avoidance towards specific stimuli can also occur in humans as a result of drug consumption [63], the place-conditioning paradigms are not primarily intended to model any particular feature of human behavior. These paradigms mainly represent an indirect assessment of the rewarding or aversive effects of a drug by measuring the response of the animal towards the conditioned stimulus. Two different phases (acquisition and expression) of the place conditioning that have different psychological implications are evaluated in this paradigm. Indeed, acquisition seems to be related to reward and learning whereas expression would be more related to incentive motivation, memory recall or sign tracking. However, some of the effects obtained in the place conditioning paradigms may reflect state-dependent learning due to discriminative stimuli properties of the test drug rather than rewarding effects [64], which represents a limitation for the interpretation of the reinstatement models based on these paradigms.

It has been postulated that the reinstatement of drug-seeking behavior could be related to the appearance of a behavioral sensitization to the motor stimulant responses induced by some drugs of abuse [65]. Thus, behavioral sensitization and reinstatement of drug-seeking behavior involve some overlapping circuitry, and neurotransmitter and receptor systems [65]. However, the relationships between these two distinct behavioral responses still remain controversial.

The rodent reinstatement models based on the operant drug self-administration procedures have been widely validated in rats. Nevertheless, the genetically modified laboratory animals available to date have mainly been inbred mouse strains. Therefore, it is crucial to extend these high predictive drug self-administration reinstatement models in rats to mice. Nowadays, reliable mouse models of relapse based on well-established extinction-reinstatement procedures in rats have been validated. Thus, new mouse models of reinstatement of seeking behavior to cocaine [66], methamphetamine [67], MDMA [68], alcohol [69], nicotine [70], morphine (unpublished results from our laboratory), and even palatable food [71] are now available. These new mouse operant models of reinstatement represent excellent tools for the advancement in the understanding of the neurobiological mechanisms underlying drug relapse.

Stimuli-Triggering Drug Relapse

One of the main strength of the drug self-administration reinstatement models in rodents is their high predictive validity. Predictive validity in an animal behavioral model refers to the extent to which the behavior induced in the experimental paradigm predicts human behavior induced by a similar event, and it is essential for the translational value of the animal model [72]. Indeed, craving and relapse to drug intake are mainly provoked in human addicts by the re-exposure to the drug of abuse [73], drug-associated cues [74], or stressful situations [75] that are exactly the same stimuli used to reinstate drug-seeking behavior in the rodent drug self-administration models.

The re-exposure to the drug is one of the most well-known stimuli triggering the relapse of drug-seeking in

human addicts [73]. In agreement, drug priming administration has been widely used as an effective stimulus to reinstate extinguished responding of drug-seeking in multiple animal models. Drug priming has been the first stimuli used to validate experimental models of renewal of extinguished responding of drug seeking in animals [76], and has been used over three decades for these purposes [64]. In these animal models, the drug is usually administered noncontingently in order to promote the reinstatement of the operant behavior.

The exposure to environmental cues that were previously associated with drug taking is another widely known factor that triggers drug relapse in humans [74]. The reinstatement of drug-seeking behavior induced by conditioned cues associated to drug self-administration has been used to model this situation in animals [77]. Different cues have been employed to reinstate extinguished response of drug seeking. Thus, discrete cues (typically lights or tones) that are associated with each reward delivery during the training period have been widely used to reinstate drug seeking after extinction of the operant behavior [77]. Another procedure consists in the use of discriminative cues. For this purpose, rodents are trained to self-administer a drug in the presence of specific discriminative stimuli and saline in the presence of a different set of stimuli. Operant behavior is then extinguished in the absence of the discriminative stimuli and is reinstated by the re-exposure of these stimuli [78]. Contextual reinstatement stimuli have also been applied. Under these situations, rodents are first trained to self-administer the drug in a context with specific cues that reveals the availability of the reinforcer and the operant behavior is extinguished in a different context that contains other specific cues. The re-exposure to the animal to the drug-paired context reinstates drug-seeking behavior [79].

In humans, negative affective states such as anger, stress, anxiety, or depression can trigger relapse to drug taking [75, 80]. Stress-induced reinstatement of extinguished responding of drug seeking has been widely used to model in animals this human situation [53]. The most successfully employed stressors in these animal reinstatement paradigms are the exposure to intermittent foot shocks [66, 81] and the administration of pharmacological agents inducing stress [82, 83].

The rodent reinstatement models using these three different kinds of stimuli were first validated in rats and are now also available in mice with a similar predictive validity [66–68, 70].

Role of the Hypocretin/Orexin System in Psychostimulant-Rewarding Properties and Relapse

The involvement of the hypocretin system in the rewarding properties of psychostimulants has not been still fully clarified (Table 1). Amphetamine-sensitized rats showed increased Fos levels in hypocretin neurons of the LH [84, 85]. Additionally, the Hcrtr-1 antagonist SB334867 blocked the effects of amphetamine on extracellular dopamine levels in the NAcc shell and the expression of amphetamine-induced locomotor sensitization [86]. Rats conditioned to cocaine in a place preference paradigm also showed increased Fos expression in LH hypocretin cells [87], even though the administration of SB334867 did not modify the expression of cocaine place preference [88]. Interestingly, mice receiving systemic and intra-VTA injections of the Hcrtr-1 antagonist SB334867 as well as mice lacking the preprohypocretin gene showed attenuated basal and cocaine-enhanced dopamine extracellular levels in the NAcc [48]. Consistent with this, intra-VTA infusion of hypocretin-1 increased basal dopamine extracellular levels in the NAcc, and the effects of cocaine on evoked dopamine release and uptake inhibition in this brain structure [89]. These biochemical results suggest that hypocretin signaling critically modulates cocaine effects on mesolimbic dopamine transmission.

In spite of these findings, contradictory results have been obtained using the operant cocaine self-administration paradigm. So far, self-administration studies have not yet demonstrated the participation of hypocretins in the primary reinforcing properties of cocaine when using a fixed-ratio 1 schedule of reinforcement [42, 48, 89, 90]. Nevertheless, when access to cocaine self-administration is limited or under conditions that require a higher effort to obtain a cocaine infusion, an involvement of the hypocretin system in cocaine reinforcement has been revealed. Thus, SB334867 reduced responding for cocaine when using a 24-h access self-administration procedure in which the number of injections that the animal can receive each hour is limited (discrete trial procedure) [48]. On the contrary, an intraventricular infusion of hypocretin-1 increased lever pressing in the same experimental paradigm [89]. The systemic or intra-VTA administration of SB334867 significantly reduced the breaking point achieved on a progressive ratio schedule of reinforcement in rats self-administering cocaine [48, 91]. Similarly, this Hcrtr-1 antagonist decreased the rate of responding for cocaine by using a self-administration paradigm in which the demand of responding progressively increases to maintain blood levels of cocaine (threshold self-administration paradigm) [48]. Conversely, intra-VTA infusions of hypocretin-1 increased the motivation to self-administer cocaine on a progressive ratio schedule [89], suggesting that the enhancement of hypocretin transmission in this particular brain structure increases the reinforcing efficacy and the motivation to obtain cocaine.

The participation of the hypocretin system in the reinstatement of cocaine seeking induced by the presentation of different stimuli has been extensively investigated. An

Table 1 The hypocretin/orexin system in psychostimulant reward and relapse

Drug	Experimental model	Experimental approach	Effect	Animal species	Reference
Amphetamine	NAcc extracellular DA levels (in vivo microdialysis)	SB334867 (30 mg/kg, sc)	Decrease	Sprague–Dawley rats	[86]
	Behavioral sensitization (expression)	SB334867 (30 mg/kg, sc)	Decrease	Sprague–Dawley rats	[86]
Cocaine	Conditioned place preference (expression)	SB334867 (20 mg/kg, ip)	No effect	C57BL/6J mice	[88]
	NAcc extracellular DA levels (in vivo microdialysis and voltammetry)	SB334867 (30 mg/kg, ip)	Decrease	Sprague–Dawley rats	[48]
		SB334867 (10 nmol, intra-VTA)	Decrease	Sprague–Dawley rats	[48]
		Preprohypocretin KO	Decrease	Sprague–Dawley rats	[48]
		Hypocretin-1 (0.5 nmol, intra-VTA)	Increase	Sprague–Dawley rats	[89]
	Self-administration (FR1 schedule of reinforcement)	Hypocretin-1 (1.5 nmol, icv)	No effect	Wistar rats	[42]
		SB334867 (30 mg/kg, ip)	No effect	Sprague–Dawley rats	[90]
		4PT (30 mg/kg, ip)	No effect	Sprague–Dawley rats	[90]
		SB334867 (30 mg/kg, ip)	No effect	Sprague–Dawley rats	[48]
		Hypocretin-1 (0.5 nmol, icv)	No effect	Sprague–Dawley rats	[89]
	Self-administration (discrete trial procedure)	SB334867 (7.5, 15 and 30 mg/kg, ip)	Decrease	Sprague–Dawley rats	[48]
		Hypocretin-1 (0.5 nmol, icv)	Increase	Sprague–Dawley rats	[89]
	Self-administration (threshold procedure)	SB334867 (15 and 30 mg/kg, ip)	Decrease	Sprague–Dawley rats	[48]
	Self-administration (PR schedule of reinforcement)	SB334867 (10 mg/kg, ip)	Decrease	Sprague–Dawley rats	[91]
		SB334867 (7.5, 15 and 30 mg/kg, ip)	Decrease	Sprague–Dawley rats	[48]
		Hypocretin-1 (0.5 nmol, intra-VTA)	Increase	Sprague–Dawley rats	[89]
	Self-administration (reinstatement)	Hypocretin-1 (0.75 and 1.5 nmol, icv)	Increase	Wistar rats	[42]
		Hypocretin-1 (10 μM, intra-VTA)	Increase	Long–Evans rats	[92]
		Hypocretin-2 (10 μM, intra-VTA)	No effect	Long–Evans rats	[92]
	Self-administration (foot shock stress-induced reinstatement)	SB334867 (30 mg/kg, ip)	Decrease	Wistar rats	[42]
		SB408124 (10 μm, intra-VTA)	No effect	Long–Evans rats	[92]
	Self-administration (cue-induced reinstatement)	SB334867 (20 or 30 mg/kg, ip)	Decrease	Sprague–Dawley rats	[90]
		SB334867 (20 or 30 mg/kg, ip)	Decrease	Sprague–Dawley rats	[90]
		4PT (10 or 30 mg/kg, ip)	No effect	Sprague–Dawley rats	[90]
		SB334867 (30 mg/kg, ip)	Decrease	Sprague–Dawley rats	[94]
		SB334867 (3 μg, intra-VTA)	Blockade	Sprague–Dawley rats	[95]
		SB334867 (3 and 6 μg, intra-PVT)	No effect	Sprague–Dawley rats	[95]
	Self-administration (context-induced reinstatement)	SB334867 (10, 20 or 30 mg/kg, ip)	Decrease	Sprague–Dawley rats	[93]
		SB334867 (30 mg/kg, ip)	Decrease	Sprague–Dawley rats	[94]
	Self-administration (cocaine-induced reinstatement)	SB334867 (10 mg/kg, ip)	No effect	Sprague–Dawley rats	[94]
	Behavioral sensitization (acquisition)	SB334867 (10 mg/kg, ip)	Decrease	Sprague–Dawley rats	[50]
		SB334867 (6 μg, intra-VTA)	Decrease	Sprague–Dawley rats	[50]
	Behavioral sensitization (expression)	SB334867 (10 mg/kg, ip)	No effect	Sprague–Dawley rats	[50]
		SB334867 (6 μg, intra-VTA)	No effect	Sprague–Dawley rats	[50]

SB334867 hypocretin receptor-1 antagonist, *SB408124* hypocretin receptor-1 antagonist, *4PT* hypocretin receptor-2 antagonist, *NAcc* nucleus accumbens, *VTA* Ventral tegmental area, *PVT* paraventricular nucleus of the thalamus, *FR1* fixed ratio 1, *PR* progressive-ratio, *icv* intracerebroventricular, *ip* intraperitoneal, *sc* subcutaneous, *KO* knockout

intraventricular infusion of hypocretin-1 induced reinstatement of a previously extinguished cocaine-seeking behavior [42]. This effect was attenuated by the pretreatment with the α_2 agonist clonidine that decreases noradrenergic activity and the CRF_1/CRF_2 antagonist D-Phe CRF_{12-41}, suggesting a role for noradrenaline and CRF on hypocretin-1-induced reinstatement [42]. In agreement, the systemic injection of a high dose of SB334867 attenuated foot shock stress-induced reinstatement of cocaine seeking [42]. These results suggest that hypocretin and CRF systems interact to regulate cocaine-seeking behavior. Intra-VTA infusion of hypocretin-1, but not hypocretin-2, also induced reinstatement of cocaine seeking [92]. Nevertheless, in contrast to the data described above, this behavioral response was not blocked by the intra-VTA perfusion of the antagonist alpha-helical CRF_{9-41} [92]. Furthermore, foot shock stress-induced reinstatement of cocaine seeking was not attenuated by the intra-VTA administration of the Hcrtr-1 antagonist SB408124 [92]. Therefore, these findings suggest that, at least at the level of the VTA, hypocretin and CRF modulate cocaine-seeking behavior by independent mechanisms.

More recently, Hcrtr-1 signaling was found to be necessary for cue- and context-induced cocaine seeking [90, 93, 94] but not for cocaine-primed reinstatement [94]. Cocaine seeking elicited by drug-paired cues was not blocked by the pretreatment with an Hcrtr-2 antagonist [90], indicating a functional difference between both hypocretin receptors in the regulation of this effect. VTA hypocretin signaling, unlike what occurs in stress-induced reinstatement, has been shown to play a role in cue-elicited cocaine relapse. Thus, the intra-VTA infusion of SB334867 dose-dependently attenuated cue-induced cocaine-seeking behavior [95]. This effect was not observed when infusing the Hcrtr-1 antagonist into the paraventricular thalamus [95].

Remarkably, cocaine-induced glutamatergic synaptic plasticity in VTA dopamine neurons is also dependent on hypocretin inputs [50]. Pretreatment with SB334867 blocked the glutamate-dependent long-term potentiation induced by cocaine in dopamine neurons of the VTA. Moreover, systemic or intra-VTA administration of SB334867 blocked the acquisition of cocaine-induced locomotor sensitization [50]. These data provided the first link between hypocretin signaling and glutamatergic synaptic plasticity in cocaine behavioral responses related to addiction. This hypocretin-dependent cocaine-induced synaptic plasticity in the VTA could be involved in facilitating the transformation of neutral environmental stimuli into salient reward-predictive cues [96], which could explain the role of Hcrtr-1 in cue- and context-induced reinstatement of cocaine seeking.

Role of the Hypocretin/Orexin System in Morphine-Rewarding Properties and Relapse

Several studies suggest that hypocretin transmission plays an important role in the rewarding properties of opioids (Table 2). Rats that exhibited a preference for an environment previously associated to morphine in a place-conditioning paradigm showed an activation of hypocretin neurons, as revealed by Fos immunostaining [87]. Moreover, significant positive correlations were found between the percentages of activated hypocretin neurons in the LH and the morphine preference scores [87]. Bilateral excitotoxic lesions of hypocretin-enriched area of the LH completely blocked the acquisition of morphine-conditioned place preference [97]. Pharmacological studies using the selective Hcrtr-1 antagonist SB334878 confirmed the involvement of this receptor in the rewarding properties of morphine. Thus, systemic [87, 88] or intra-VTA

Table 2 The hypocretin/orexin system in morphine reward and relapse

Experimental model	Experimental approach	Effect	Animal species	Reference
Conditioned place preference (acquisition)	Excitotoxic lesions of LH	Blockade	Sprague–Dawley rats	[97]
Conditioned place preference (expression)	SB334867 (30 mg/kg, ip)	Decrease	Sprague–Dawley rats	[87]
	SB334867 (20 mg/kg, ip)	Decrease	C57BL/6J mice	[88]
	SB334867 (10 nmol, intra-VTA)	Decrease	Sprague–Dawley rats	[47]
	Preprohypocretin KO	Blockade	C57BL/6J background	[47]
	Preprohypocretin KO	No effect	C57BL/6J background	[88]
Conditioned place preference (reinstatement)	SB334867 (30 mg/kg, ip)	Blockade	Sprague–Dawley rats	[87]
	Hypocretin-1 (140 nM, intra-VTA)	Increase	Sprague–Dawley rats	[87]
NAcc extracellular DA levels (in vivo microdyalisis)	Preprohypocretin KO	Decrease	C57BL/6J mice	[47]
Somatic signs of withdrawal	Preprohypocretin KO	Decrease	C57BL/6J background	[98]
	SB334867 (20 mg/kg, ip)	Decrease	C57BL/6J background	[99]
	SB334867 (100 μM, intra-LC)	Decrease	Wistar rats	[100]

LH lateral hypothalamus, *VTA* ventral tegmental area, *NAcc* nucleus accumbens, *LC* locus coeruleus, *SB334867* hypocretin receptor-1 antagonist, *KO* knockout, *DA* dopamine, *ip* intraperitoneal

[47] administration of the Hcrtr-1 antagonist SB334867 reduces morphine-induced place preference in rats. However, conflicting results have been found in mice lacking the prepro-hypocretin gene since a suppression [47] or similar [88] morphine place preference have been reported in these mutant animals. Similar discrepancies were reported in the hyperlocomotor effects of morphine in preprohypocretin knockout mice [47, 88]. In spite of these controversial findings, behavioral studies using operant self-administration procedures to evaluate the role of hypocretins in the reinforcing properties of opioids are still missing. The mechanisms underlying the effects of the hypocretin system on morphine rewarding properties are related to a modulatory response on morphine-induced activation of the mesolimbic dopaminergic pathway. Thus, in vivo microdialysis studies revealed that the enhancement of the extracellular levels of dopamine in the NAcc induced by morphine was significantly decreased by deletion of the preprohypocretin gene [47].

The hypocretin system also participates in the behavioral and biochemical manifestations of the morphine-withdrawal syndrome. Somatic signs of morphine withdrawal were attenuated in preprohypocretin knockout mice [98] and in mice pretreated with SB334867 before naloxone-precipitated morphine withdrawal [99]. Recently, a specific participation of Hcrtr-1 located within the locus coeruleus in morphine physical dependence has been reported [100]. Consistent with these behavioral data, naltrexone- or naloxone-precipitated morphine withdrawal leads to the induction of Fos expression in hypocretin cells [98, 99], and spontaneous morphine withdrawal increases hypocretin mRNA levels in the LH [101].

Few studies have examined the implication of the hypocretinergic system in animal models of opioid relapse. Chemical activation of LH hypocretin neurons reinstated an extinguished morphine place preference [87], which was completely blocked by prior systemic administration of the Hcrtr-1 antagonist SB334867 [87]. In addition, the injection of hypocretin-1 in the VTA, but not in areas surrounding this brain structure, caused a significant reinstatement of the previously extinguished morphine place preference [87], suggesting a role for hypocretins acting on the VTA in the mechanisms driving to relapse of morphine seeking. Behavioral studies using operant self-administration paradigms would be useful to confirm the involvement of hypocretins in this behavioral response related to morphine addictive properties.

Role of the Hypocretin/Orexin System in Nicotine-Rewarding Properties and Relapse

Nicotine addiction is a complex neurochemical process in which many neurotransmitters are involved [58, 102] and growing evidence suggests that hypocretins play also a crucial role in nicotine addictive effects [103] (Table 3). Acute nicotine injections as well as mecamylamine-precipitated nicotine withdrawal increased Fos expression in hypocretin neurons of the LH [104–106]. Additionally, acute nicotine-induced activation of the paraventricular nucleus of the hypothalamus was dependent on hypocretin transmission [105]. On the other hand, intravenous nicotine self-administration modified Hcrtr-1 mRNA levels in the arcuate nucleus and the rostral lateral areas of the hypothalamus in rats [107]. In agreement, noncontingent chronic nicotine administration also regulated preprohypocretin and hypocretin receptor mRNA levels in the rat hypothalamus [108]. At the behavioral level, pretreatment with the Hcrtr-1 antagonist SB334867 or the Hcrtr-1/-2 antagonist almorexant decreased intravenous nicotine self-administration in rats under a fixed-ratio 5 schedule of reinforcement [107, 109]. In addition, SB334867 decreased the number of nicotine rewards earned under a progressive ratio schedule [109] suggesting that hypocretins acting on Hcrtr-1 regulate nicotine reinforcement and the motivation to seek the drug. Interestingly, stroke-associated damage to the insular cortex in human smokers resulted in spontaneous cessation of the smoking habit and a low urge to smoke [110]. Based on these findings, it was hypothesized that hypocretin transmission in the insular cortex might have a role in nicotine reinforcement. Notably, intra-insular infusion of the Hcrtr-1 antagonist SB334867 decreased nicotine intake in rats [109]. The mechanism by which hypocretins modulate nicotine rewarding and motivational properties could involve the regulation of the stimulatory effects of nicotine in brain reward systems. Indeed, pretreatment with SB334867 blocked the nicotine-induced lowering of the intracranial self-stimulation thresholds in rats [109]. Consistent with rodent studies, recent evidence in human smokers also points to a role of hypocretin transmission in tobacco addiction. Thus, a negative correlation between hypocretin plasma concentration and nicotine craving has been shown [111].

The hypocretin system also seems to participate in nicotine-seeking behaviors. Indeed, hypocretin-1, through Hcrtr-1 activation, reinstated a previously extinguished nicotine-seeking behavior in mice [105]. The CRF_1 receptor antagonist antalarmin did not block the effects of hypocretin-1 on reinstatement, whereas the Hcrtr-1 antagonist SB334867 did not modify the CRF-dependent stress-induced reinstatement of nicotine seeking [105]. These results suggest that the mechanism by which hypocretin-1 induces reinstatement of nicotine seeking is independent of the CRF system. Additional research will be needed to elucidate the potential participation of hypocretins in the other two modalities to reinstate nicotine-seeking behavior (nicotine-associated cues and re-exposure to nicotine).

Table 3 The hypocretin/orexin system in nicotine reward and relapse

Experimental model	Experimental approach	Effect	Animal species	Reference
Intracranial self-stimulation (nicotine-induced lowering of threshold)	SB334867 (4 and 6 mg/kg, ip)	Increase	Wistar rats	[109]
Self-administration (FR5 schedule of reinforcement)	SB334867 (4 mg/kg, ip)	Decrease	Wistar rats	[109]
	SB334867 (0.2, 1 or 5 μg, intra-insula)	Decrease	Wistar rats	[109]
	SB334867 (30 mg/kg, ip)	Decrease	Long–Evans rats	[107]
	Almorexant (300 mg/kg, po)	Decrease	Decrease Long–Evans rats	[107]
Self-administration (PR schedule of reinstatement)	SB334867 (1–4 mg/kg, ip)	Decrease	Wistar rats	[109]
Self-administration (reinstatement)	Hypocretin-1 (0.75 nmol, icv)	Increase	C57BL/6J mice	[105]
Self-administration (footshock stress-induced reinstatement)	SB334867 (5 and 10 mg/kg, ip)	No effect	C57BL/6J mice	[105]
Somatic signs of withdrawal	SB334867 (5 and 10 mg/kg, ip)	Decrease	C57BL/6J	[106]
	SB334867 (10 nmol, intra-PVN)	Decrease	C57BL/6J	[106]
	TCSOX229 (5 and 10 mg/kg, ip)	No effect	C57BL/6J	[106]
	Preprohypocretin KO	Decrease	C57BL/6J background	[106]

ip intraperitoneal, *icv* intracerebroventricular, *po* per os (oral administration), *SB334867* Hypocretin receptor-1 antagonist, *TCSOX229* hypocretin receptor-2 antagonist, *KO* knockout, *PVN* paraventricular nucleus of the hypothalamus, *FR5* fixed-ratio 5, *PR* progressive ratio

Impaired attention is an established cognitive symptom of nicotine withdrawal that could contribute to smoking relapse. Interestingly, hypocretins could participate in the attention-enhancing effects of nicotine [112, 113], which suggests that the hypocretin system may contribute to nicotine addiction not only by the modulation of the rewarding effects but also through the modification of nicotine-cognitive effects.

On the other hand, a selective involvement of Hcrtr-1 in the expression of nicotine withdrawal has been recently reported [106]. Pretreatment with SB334867, but not with the specific Hcrtr-2 antagonist TCSOX229, attenuated the somatic signs of nicotine withdrawal in mice. In addition, a crucial role for the hypothalamic paraventricular nucleus in the modulation of this effect was revealed. Thus, the increase in Fos expression that occurred in the paraventricular nucleus of the hypothalamus during nicotine withdrawal was dependent on hypocretin transmission and local infusion of the Hcrtr-1 antagonist into this brain area attenuated the somatic manifestations of withdrawal [106].

Role of the Hypocretin/Orexin System in Alcohol-Rewarding Properties and Relapse

Hypocretin transmission seems to play an important role in the addictive properties of alcohol [114], although conflicting results are reported in the literature (Table 4). The pretreatment with SB334867 decreased operant responding for alcohol under a fixed ratio 3 schedule in alcohol preferring rats [115], while no effect of this Hcrtr-1 antagonist was observed on water responding. Subsequent studies confirmed the involvement of Hcrtr-1 in the reinforcing effects of alcohol. Thus, SB334867 also reduced alcohol, but not sucrose, self-administration in Long–Evans rats [116]. The same Hcrtr-1 antagonist reduced ethanol preference on a two-bottle free-choice paradigm in rats [117]. In addition, a recent report has revealed a role for Hcrtr-1 in the motivation to self-administer alcohol since SB334867 pretreatment reduced the breaking point for alcohol, but not for sucrose, on a progressive ratio schedule of reinforcement [118]. Consistent with these behavioral data, the infusion of hypocretin-1 in the hypothalamic paraventricular nucleus or the LH, but not into the NAcc, stimulated voluntary ethanol intake without significantly altering food and water intake [119]. Chronic ethanol consumption in alcohol preferring rats increased the area of expression of mRNA encoding preprohypocretin within the LH [115], although a reduction of hypocretin expression in the perifornical lateral hypothalamus has also been observed following chronic ethanol intake in rats [120]. Interestingly, a recent report has revealed a novel role for Hcrtr-2 in the reinforcing effects of ethanol. Thus, the specific Hcrtr-2 antagonist JNJ-10397049 dose-dependently reduced ethanol self-administration without changing saccharin self-administration in rats [121]. In addition, the same antagonist attenuated the acquisition and expression of ethanol-induced place preference. Surprisingly, the Hcrtr-1 antagonist SB408124 was ineffective in reducing the reinforcing effects of ethanol in this study [121]. In agreement, blockade of Hcrtr-1 by SB334867 did not affect the acquisition and

Table 4 The hypocretin/orexin system in alcohol reward and relapse

Experimental model	Experimental approach	Effect	Animal species	Reference
Oral ethanol intake	Hypocretin-1 (0.9 nmol, intra-PVN)	Increase	Sprague–Dawley rats	[119]
	Hypocretin-1 (0.9 nmol, intra-LH)	Increase	Sprague–Dawley rats	[119]
	Hypocretin-1 (0.9 nmol, intra-NAcc)	No effect	Sprague–Dawley rats	[119]
Two-bottle free-choice	SB334867 (30 mg/kg, ip)	Decrease	Sprague–Dawley rats (high-ethanol preferring)	[117]
Conditioned place preference (acquisition and expression)	SB408124 (30 mg/kg, ip)	No effect	DBA/2J mice	[121]
	JNJ10397049 (10 mg/kg, ip)	Decrease	DBA/2J mice	[121]
	SB334867 (15 and 30 mg/kg, ip)	No effect	DBA/2J mice	[122]
Self-administration (FR3 schedule of reinforcement)	SB334867 (20 mg/kg, ip)	Decrease	Alcohol preferring (iP) rats	[115]
	SB334867 (10, 15, and 20 mg/kg, ip)	Decrease	Long–Evans rats	[116]
	SB408124 (3–30 mg/kg, ip)	No effect	Wistar rats	[121]
	JNJ10397049 (3 and 10 mg/kg, ip)	Decrease	Wistar rats	[121]
Self-administration (PR schedule of reinforcement)	SB334867 (5 mg/kg, ip)	Decrease	Alcohol preferring (iP) rats	[118]
Self-administration (cue-induced reinstatement)	SB334867 (20 mg/kg, ip)	Blockade	Alcohol preferring (iP) rats	[115]
	SB334867 (20 mg/kg, ip)	Blockade	Alcohol preferring (iP) rats	[123]
Self-administration (yohimbine stress-induced reinstatement)	SB334867 (5 and 10 mg/kg, ip)	Blockade	Long–Evans rats	[116]

PVN paraventricular nucleus of the hypothalamus, *LH* lateral hypothalamus, *NAcc* nucleus accumbens, *SB334867* hypocretin receptor-1 antagonist, *SB408124* hypocretin receptor-1 antagonist, *JNJ10397049* hypocretin receptor-2 antagonist, *FR3* fixed ratio 3, *PR* progressive ratio, *ip* intraperitoneal

expression of ethanol-induced conditioned place preference in mice [122], suggesting that Hcrtr-1 does not influence ethanol's primary or conditioned rewarding effects. Differences in experimental procedures and animal species could explain the discrepancies about the role of Hcrtr-1 in the ethanol rewarding responses, but additional research will be necessary to fully characterize and clarify the role of the hypocretin system in these motivational properties of ethanol.

Alcohol relapse induced by associated environmental stimuli is also modulated by the hypocretin system. Thus, SB334867 prevented cue-induced reinstatement of alcohol-seeking in alcohol preferring rats [115]. An attenuation of alcohol relapse by this Hcrtr-1 antagonist was also observed in the same animal species after protracted alcohol abstinence [123]. Moreover, context- [124] and cue-induced alcohol seeking [125] was found to activate hypocretin-containing neurons in the hypothalamus as revealed by Fos protein expression studies. Neuropeptide S, an endogenous brain peptide which promotes arousal and anxiolytic-like responses [126], seems to be involved in the modulation that hypocretins exert on ethanol-seeking behavior. Thus, the activation of neuropeptide S receptors in the LH intensified relapse to ethanol-seeking elicited by environmental-conditioning factors [127]. This increase of alcohol seeking induced by neuropeptide S required also the activation of the hypocretin system [127]. Moreover, Hcrtr-1 signaling is involved in stress-induced reinstatement of alcohol-seeking behavior. Accordingly, SB334867 blocked the relapse to ethanol-seeking induced by yohimbine, an α_2 noradrenergic antagonist that provokes a stress-like response in both humans and laboratory animals [116]. Consistent with the preclinical data, recent studies in humans suggest an involvement of hypocretins in the affective dysregulation that appears in alcohol-dependent patients during alcohol withdrawal and craving [128, 129].

Potential Mechanisms Involved in the Modulation of the Addictive Properties of Drugs of Abuse by Hypocretins

The data described above suggest that the hypocretin system plays a crucial role in the addictive properties of several major drugs of abuse, including psychostimulants, opioids, nicotine and alcohol. However, hypocretin transmission regulates the primary reinforcing effects of opioids, nicotine, and alcohol, but not those of psychostimulants. The differential participation of the hypocretin system in the reinforcing properties of psychostimulants could be explained by the different action upon the mesolimbic system. Although all drugs of abuse increase dopamine extracellular levels in the NAcc, opioids, nicotine, and alcohol increase dopaminergic cell firing in the VTA whereas psychostimulants directly inhibit dopamine uptake in the NAcc. These differences suggest that the VTA might be a crucial

site of action for hypocretins to mediate the rewarding effects of different drugs of abuse [130]. Therefore, behavioral effects that depend on increased VTA dopaminergic activation (opioid/alcohol/nicotine reinforcement) would be attenuated by Hcrtr-1 antagonists because they may require hypocretin transmission. However, behaviors which are independent of the activation of VTA dopamine cells (primary cocaine reinforcement) could avoid this critical site of action of hypocretins. On the other hand, different hypocretin neuron populations of the hypothalamus seem to be involved in the modulation of the rewarding processes [131]. Thus, LH hypocretin neurons that project to the VTA could be mainly activated by reward-related stimuli [87] while those located in the PFA/DMH may have a more important role in the regulation of arousal and response to stressful situations [99]. Further studies are required to confirm this different physiological role of the diverse hypocretin cell populations.

Hypocretins also regulate the reinstatement of drug-seeking behaviors induced by drug-associated cues as mainly described for cocaine and alcohol. The VTA seems to be also a critical brain structure of hypocretin action for these effects. Thus, blockade of VTA Hcrtr-1 signaling attenuates cue-induced reinstatement of cocaine seeking [95]. Notably, cocaine-induced glutamatergic synaptic plasticity in VTA dopamine neurons, which is involved in relapse [132], depends on hypocretin inputs [50]. Consistent with the implication of VTA as a major site of action for hypocretins and its importance shaping the association between drugs and environmental stimuli, electrophysiological studies have shown an increase of the excitability of VTA dopaminergic neurons by the application of these neuropeptides to midbrain slices [133]. Moreover, this brain structure expresses both hypocretin receptor subtypes [26, 47] and receives substantial projections from LH hypocretin neurons [134], although a small proportion makes synaptic contacts within the VTA [135]. The possible participation of VTA hypocretin transmission in cue-induced reinstatement of drug-seeking behavior of other substances of abuse such as alcohol, nicotine and opioids has still not been elucidated.

Hypocretin projections are found throughout the brain and hypocretin receptors are present in multiple brain regions. Accordingly, hypocretin signaling within brain areas distinct from VTA have also been involved in the regulation of the addictive effects of different drugs of abuse. Thus, Hcrtr-1 into the insular cortex is critically involved in the reinforcing properties of nicotine [109]. Moreover, Hcrtr-1 activation in the hypothalamic paraventricular nucleus [106] and the locus coeruleus [100] regulates the somatic signs of nicotine and morphine withdrawal syndromes, respectively. In addition to the VTA, hypocretin transmission in other brain structures has also been suggested to participate in the processes driving to relapse to drug-seeking behavior. Accordingly, the presentation of cues previously associated to ethanol availability activates neurons of the thalamic paraventricular nucleus and it has been suggested that this effect is mediated by hypocretin neurons [125]. In addition, although different brain regions could participate in the regulation of stress-induced reinstatement of cocaine seeking, VTA is not involved in this behavioral response [92].

The distribution of hypocretin receptors in the brain is partially overlapping and sometimes complementary, suggesting that these receptors could regulate different physiological functions. Indeed, the current knowledge of the pharmacology of the hypocretin system points to a role for Hcrtr-1 in drug-taking behaviors while Hcrtr-2 would be mainly involved in the regulation of sleep–wake cycle [27]. However, so far most of the hypocretin addiction research has focused on elucidating the function of Hcrtr-1. In contrast, very little is known about the possible role of Hcrtr-2 in these processes due to the lack of available selective Hcrtr-2 antagonists. Thus, research in the hypocretin field would largely benefit on the development of new selective antagonists for the different hypocretin receptors. Moreover, the use of very high doses of the Hcrtr-1 antagonist SB334867 in some in vivo studies could have induced disruptive behavioral effects and even block both hypocretin receptor subtypes [136]. Therefore, future research using genetically engineered mice for one or both hypocretin

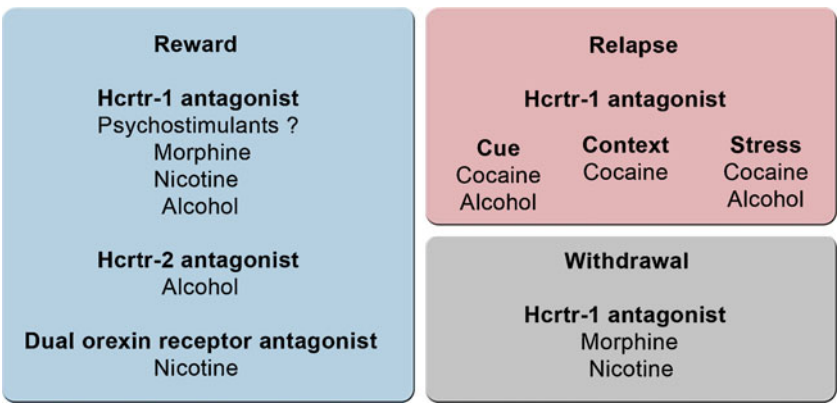

Fig. 2 Diagram showing the potential therapeutic utility of hypocretin receptor antagonists on different stages of drug addiction. *Hcrtr-1* hypocretin receptor-1, *Hcrtr-2* hypocretin receptor-2

receptors will be crucial to clarify the specific contribution of each receptor to the different stages of drug addiction. Additionally, experiments using viral vectors to knockdown or re-express hypocretin receptors in time and space-controlled conditions will also be important to determine the neural circuits underlying hypocretin signaling in drug addiction.

Conclusions

The findings reviewed in this article reveal strong evidence for a critical role of hypocretin signaling in drug addiction. The available data suggest that hypocretin transmission not only participates in the primary reinforcing and motivational properties of drugs of abuse but is also involved in the processes that drive relapse to drug seeking. Hence, hypocretin receptor antagonists might represent a new generation of compounds to treat a wide variety of addictive processes (Fig. 2). However, important questions regarding the functional role of the hypocretin system remain to be elucidated. Although the importance of VTA as a critical site of hypocretin action is well documented, future studies will be required to identify other specific brain areas underlying hypocretin activity. The precise contribution of the different hypocretin receptors in the regulation of behaviors associated with addiction needs to be clarified. Several studies have evaluated the interaction between cannabinoids and hypocretins in some physiological responses such as the regulation of feeding [137, 138] and nociception [139]. However, the possible role of the hypocretin system in the addictive properties of *Cannabis*, which is one of the most commonly used illicit drugs, remains to be demonstrated. Finally, the improvement of our understanding of the signaling cascades activated by the stimulation of hypocretin receptors will be crucial for the development of more specific drugs with different efficacy profiles.

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Conflict of Interest The authors declare that they have no conflict of interest.

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