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Stress and Arousal

The Corticotrophin-Releasing Factor/Hypocretin Circuitry

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Abstract

The hypocretins (also know as orexins) are two neuropeptides now commonly described as critical components for maintaining and regulating the stability of arousal. Several lines of evidence have raised the hypothesis that hypocretin-producing neurons are part of the circuitries that mediate the hypothalamic response to acute stress. New data indicate that the corticotrophin-releasing factor (CRF) peptidergic system directly innervates hypocretin-expressing neurons. CRF depolarizes hypocretin neurons, and this effect is blocked by a CRF-R1 antagonist. Furthermore, activation of hypocretinergic neurons by stress is impaired in CRF-R1 knockout mice. These data suggest that CRF-R1 receptor mediates the stress-induced activation of the hypocretinergic system. A significant amount of evidence also indicates that hypocretin cells connect reciprocally to the CRF system. We propose that upon stressor stimuli, CRF activates the hypocretin system, which relays these signals to brain stem nuclei involved in the modulation of arousal as well as to the extended amygdala, a structure involved in the negative motivational state that drives addiction.

Index Entries: Orexin; lateral hypothalamus; sleep; addiction; relapse; reinstatement.

Introduction

The hypocretins (also known as orexins) are two neuropeptides, hypocretin-1 and -2, derived from the same precursor gene (pre-prohypocretin) produced in a few thousand neurons localized in the perifornical area of the lateral hypothalamus (*1,2;* Fig. 1). Hypocretin-producing neurons project throughout the brain. The distribution of hypocretin terminals is consistent with the partially overlapping, but complementary, distributions of the two hypocretin receptors *(3,4).*

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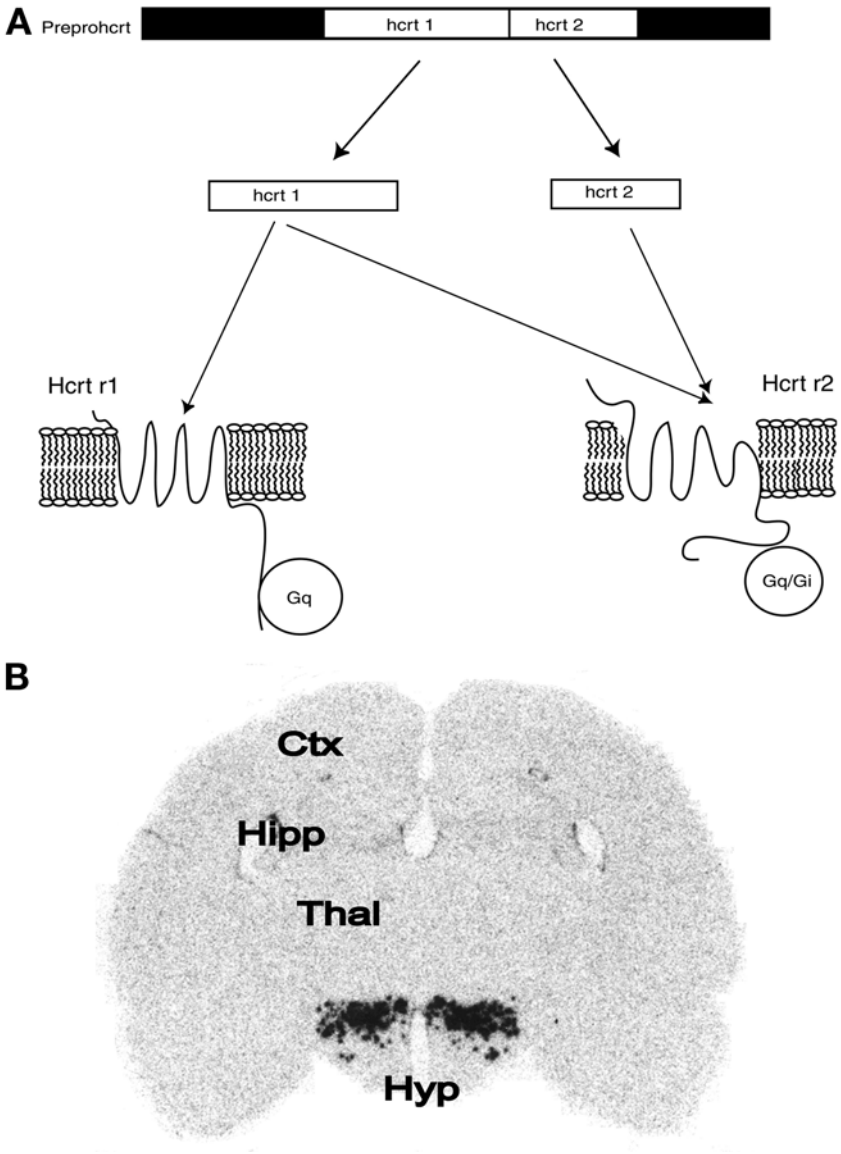

Fig. 1. **(A)** The hypocretins are derived from a single peptide precursor. Hypocretin-1 (also known as orexin A) binds to hypocretin receptor-1 with higher affinity than hypocretin receptor-2, whereas hypocretin-2 (orexin B) binds to both receptors with similar affinities. **(B)** *In situ* hybridization of pre-prohypocretin, showing that hypocretin-producing neurons in the rat brain are restricted to the lateral hypothalamic area.

Evidence from multiple experiments indicates that hypocretin neurons in the lateral hypothalamus receive inputs from diverse sensory and limbic systems to provide a coherent output that results in the stability of the states of vigilance *(5–7).*

The Hypocretins Are Critical for the Maintenance of Arousal

Narcolepsy is a neurological disorder characterized by excessive daytime sleepiness and cataplexy attacks. Patients with narcolepsy

also exhibit sleep-onset rapid eye movement (REM) direct transition from wakefulness to REM sleep *(8),* suggesting the inability to control the boundaries between vigilance states. The link between hypocretins and narcolepsy was revealed when Mignot and colleagues *(9)* reported the mapping of the canine narcolepsy mutation to hypocretin receptor-2. Pre-prohypocretin knockout mice show periods of cataplexy-like attacks and sudden onset of REM sleep *(10,11).* This narcolepsy-like phenotype is also observed in transgenic mice and rats with selective postnatal degeneration of hypocretin-expressing neurons *(12,13),* and the narcolepsy condition can be rescued by either pharmacological or genetic means *(14).* These data unequivocally demonstrate that narcolepsy is a disease of the hypocretinergic system.

Studies in transgenic animals have revealed that in addition to their their key role in the regulation of transitions between vigilance states, the hypocretins may be involved in linking information about nutritional and metabolic state and promotion of arousal. Therefore, whereas most mammals respond to reduced food availability by becoming more wakeful and active, transgenic mice depleted of hypocretin neurons fail to respond to fasting with increased activity and arousal *(15).* Recent data have also indicated that the hypocretinergic system receives input from the brain circuitry that modulates stress.

The Hypocretinergic System May Be a Component of the Stress Response

Behavioral arousal is a key component of the stress response. A well-characterized physiological response to stress affects the hypothalamus–pituitary–adrenal (HPA) axis. Upon stress stimulus, synthesis of the corticotrophin-releasing factor (CRF) is induced in the paraventricular nucleus of the hypothalamus. Stimulation of the pituitary corticotroph cells by CRF stimulates the production of the adrenocorticotropic hormone (ACTH). The primary target of ACTH is the adrenal gland, from which ACTH stimulates the release of glucocorticoids, which, in turn, provide a feedback loop to the pituitary and hypothalamus to stop the response to stressful stimuli *(16).*

As discussed earlier, hypocretin-containing neurons are critical components of the circuitry that modulates and sets the arousal threshold *(7).* Therefore, it is expected that the hypocretinergic system has an important role in the "hyperarousal" state characterizing stress. Indeed, intracerebroventricular injection of hypocretin-1 increases food consumption *(17–20),* locomotor activity *(21–23),* and body temperature *(24,25).* Moreover, central administration of hypocretin-1 stimulates gastric acid secretion and increases arterial blood pressure, heart rate, cerebral blood flow, and sympathetic nerve activity *(26,27);* additionally, mice deficient in pre-prohypocretin display low sympathetic tone *(28).*

Numerous pieces of evidence suggest that the hypocretins are involved in the central component of the HPA axis. Centrally administered hypocretin-1 increases plasma ACTH and corticosterone levels, a response similar to central administration of CRF. Interestingly, hypocretin receptors have been detected at each level of the HPA axis—that is, in the parvocellular part of the hypothalamic paraventricular nucleus, in the pituitary, as well as in the cortex and medulla of the adrenal gland *(29,30).* Additionally, in vitro studies have demonstrated that addition of hypocretins to adrenocortical cultures stimulates norepinephrin release *(31–34)*

Further supporting the role of hypocretins in the HPA axis activation, Ida et al. *(35)* demonstrated that intracerebroventricular administration of the α-helical CRF, a nonselective CRF receptor antagonist, blocked hypocretin-induced grooming and face-washing behavior. More importantly, hypocretin-induced corticosterone increase can also be blocked by pretreatment with the CRF antagonist *(33,36,37).* Furthermore, several acute stress paradigms,

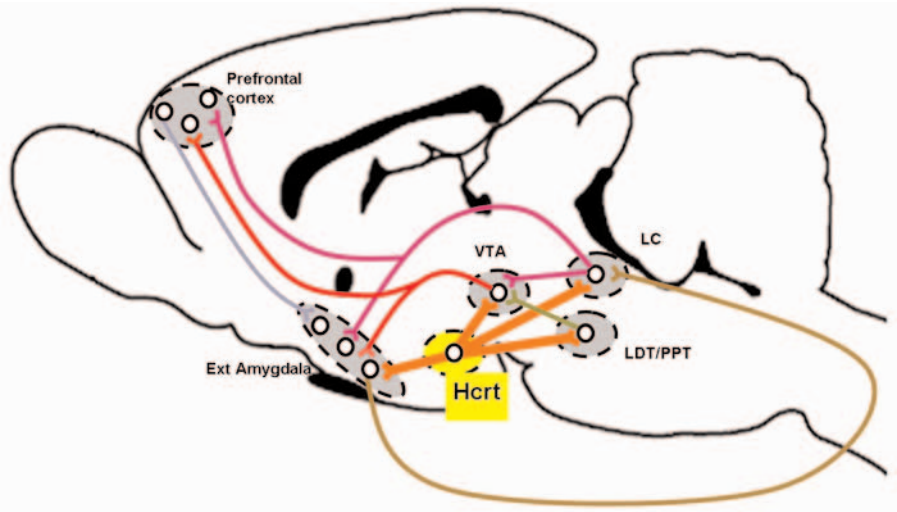

Fig. 2. Interactions between the hypocretins and other transmitters involved in hyperarousal. Corticotrophin-releasing factor neurons from the paraventricular nucleus, or extended amygdala (central nucleus of the amygdala or bed nucleus of the stria terminalis) directly contact and activate hypocretin neurons, which, in turn, activate and provide stability to noradrenergic, histaminergic, and cholinergic nuclei that modulate arousal.

including food deprivation, have been shown to be associated with activation of hypocretin neurons *(38–40)*. Interestingly, pre-prohypocretin knockout mice display diminished behavioral response to emotional stress in the "resident-intruder" test *(28,41)*.

At which level are hypocretins involved in the stress response? Several lines of evidence suggest that the hypocretinergic neurons and CRF-expressing neurons constitute a feedback circuit that regulates arousal in response to stressful stimuli. Recently, anatomical interactions between the CRF and hypocretinergic systems were demonstrated. Synapses occur between CRF-immunoreactive boutons and hypocretin-immunopositive perikarya and dendrites in the lateral hypothalamus *(42)*. Additionally, numerous hypocretin-immunoreactive neurons express CRF-R1/2 receptors. The origin of this innervation remains to be determined, although preliminary evidence suggests strong afferent innervation from the paraventricular nucleus, the central nucleus of the amygdala, and the bed nucleus of the stria terminalis.

Intracellular recordings of hypocretin neurons, identified by enhanced green fluorescent protein staining in hypothalamic slices from orexin/epithelial growth factor protein, indicate that CRF directly depolarizes hypocretinergic cells *(42)*. This effect likely is mediated through CRF-R1 because astressin (a CRF-R1-selective antagonist) blocked the CRF-induced depolarization of hypocretin neurons. The functional significance of the CRF–hypocretins interaction was tested during acute stress, such as restraint or footshock stress. Restraint stress dramatically increases pre-prohypocretin messenger RNA steady-state concentration *(40)*. Both acute stress paradigms induce c-Fos immunoreactivity in hypocretin-producing neurons of wild-type mice. However, activation of c-Fos in hypocretinergic neurons after footshock and restraint stress was decreased in mice that were deficient in CRF-R1 *(42)*. These results suggest that the stress-induced activation of hypocretinergic neurons occurs through the CRF-R1 receptor. The hypocretinergic system may be a component of the central response to acute stress activated by CRF (Fig. 2).

Interestingly, the effect of hypocretins on the stress response appears to be specific and finely regulated: in vitro, hypocretin-1 inhibits CRF-induced ACTH release via a pertussis-toxin-sensitive mechanism but does not affect baseline levels of ACTH from the pituitary *(43).*

Hypocretins can exert some electrophysiological effects on paraventricular nucleus (PVN) neurons. Hypocretin receptors are present in the PVN; hypocretin-2 is the most abundant *(44).* Bath application of hypocretin-1 or hypocretin-2 results in depolarization of the majority of PVN neurons *(45).* These effects, observed in whole-cell patch clamp recording, were not blocked by tetrodotoxin, which suggests postsynaptic effects *(33,46).* Additionally, magnocellular neurons in PVN slice preparations are depolarized by hypocretin-1, and these effects are abolished by tetrodotoxin treatment *(46).* Therefore, the effect of the hypocretins on the PVN neurons is, in all likelihood, indirect. Supporting this view, data from Follwell et al. *(46)* showed that the depolarizing effect of hypocretin-1 on the PVN magnocellular neurons was abolished by kynurenic acid, thus demonstrating the role of glutaminergic interneurons in the action of hypocretin-1.

Hypocretin neurons are reciprocally connected with neuropeptide Y (NPY)-containing neurons *(47),* another peptidergic system involved in the multiple responses to acute stress. *(48).* NPY and hypocretin share many target areas, including the components of the extended amygdala that have a prominent role in the responses to acute stress *(49,50).* Interestingly, intracerebroventricular administration of NPY increases sedation *(51)* and has anxiolytic activity in response to some stimuli *(52–54).* NPY potently hyperpolarizes hypocretin neurons in vitro *(55).* Therefore, it is possible that some of the behavioral effects of NPY are mediated by inhibition of the hypocretinergic system.

This circuitry between CRF, hypocretin, and NPY may have significant relevance in multiple physiological and pathological situations—particularly in hyperaroused states associated with motivation and addiction.

Relevance of the CRF–Hypocretin Interaction in Drug Addiction

The relationship between stress and addiction is well-established, and the extended amygdala has been demonstrated to play a key role in mediating both positive and negative reinforcement associated with drug addiction *(56–58).* The extended amygdala is comprised of the medial subregion of the nucleus accumbens (shell of the nucleus accumbens), the bed nucleus of the stria terminalis, and the central nucleus of the amygdala. This structure receives numerous afferents from limbic regions, such as the basolateral amygdala and hippocampus, and sends efferents not only to the medial part of the ventral pallidum, but also to the lateral hypothalamus, further defining the specific brain areas that interface classical limbic structures with the extrapyramidal motor system. Therefore, the extended amygdala provides a connection for the basal forebrain to the classical reward systems of the lateral hypothalamus via the medial forebrain bundle reward system *(59,60).*

Interestingly, the hypocretinergic system projects to all the major components of the extended amygdala—namely, the central amygdala, the shell of the nucleus accumbens and the bed nucleus of the stria terminalis (Fig. 2; refs. *4* and *50*). Because hypocretins have been shown to be involved in the γ-aminobutyric acid (GABA)ergic modulation of the mesolimbic dopamine system, *(61–63),* this peptidergic system fulfills all the neuro-anatomical and functional criteria to modulate critical connections regulating both positive- and negative-reinforcing properties of drugs of abuse.

Several lines of evidence suggest that hypocretins are involved in the modulation of the brain reward function. First, both lesions experiments and the intracranial self-stimulation (ICSS) paradigm have suggested an important role of the lateral hypothalamus in reward *(64,65).* Compared to other brain regions, ICSS in the lateral hypothalamus (also called LHSS) is the most potent by far *(66).* Second, maintenance

of energy homeostasis requires the coordination of systems that regulate feeding, body temperature, and autonomic and endocrine functions with those that modulate an appropriate state of arousal and motivation. Strikingly, hypocretin-producing cells are in the lateral hypothalamus and project throughout the brain. Although the role of CRF in the regulation of both emotional and sensory stimuli is well-documented *(57,67)*, the involvement of the hypocretins in the regulation of motivated behaviors remains unclear. However, the close interaction between the CRF and the hypocretin peptidergic systems *(35,36, 42,68)* places hypocretin neurons as a key system in the integration of emotional stimuli as well as in the integration of sensory inputs and suggests a role for this system in the stabilization of motivated behaviors. Indeed, a single infusion of hypocretin-1 elevates ICSS thresholds *(69)*, which is a similar effect to that found following CRF administration *(70)*. Furthermore, hypocretin-deficient mice exhibit a dramatic attenuation of morphine withdrawal symptoms *(71)*. These observations suggest a role for hypocretins in drug withdrawal.

Therefore, the hypocretinergic system is an interesting candidate as a component of the circuitries that underlie vulnerability to relapse during a prolonged abstinence from drugs of abuse *(72,73)*. Hence, the hypocretin system (receiving sensori stimuli and relaying them to brain stem nuclei and the HPA axis as well as to arousal- and stress-related forebrain regions) could be activated by drug intoxication. At cessation of drug presentation, the hypocretin system may act as an alarm signal that prepares the organism for withdrawal and its consequences on energy homoeostasis. Interestingly, leptin, which hyperpolarizes hypocretin neurons, also attenuates fasting-induced heroin-seeking behavior *(74)*. Therefore, leptin may block activation of the hypocretin system *(15,44,75,76)*, preventing relapse for food or drug seeking *(15,77–79)*.

Recent data have raised an important role of the NPY system in alcohol dependence *(80–82)*. Notably, acute withdrawal from ethanol is associated with decreases levels of NPY *(83)*, and NPY knockout mice self-administer significantly higher amounts of alcohol compared to wild-type controls *(84)*. It has been hypothesized that brain CRF systems may become hyperactive, and the NPY antistress system may be compromised with the development of alcohol dependence. Recent findings suggest a role for NPY projections in attenuating hypocretin neurons activity in vitro *(55)*. Further studies are needed to delineate respective roles of CRF, NPY, and hypocretin systems in the modulation of arousal and negative motivational state driving addiction. Understanding the alterations in such fundamental homeostatic systems within the brain is key to preventing various pathophysiological dysfunctions, including affective and addictive disorders *(85)*.

In conclusion, we propose that the hypocretinergic system could play a relevant role in the regulation of both homeostatic (upon stress stimuli as well as during drug withdrawal) and allostatic (after a prolonged period of drug abstinence) functions related to arousal, stress, and motivation. In addition to being key regulators of the transitions between vigilance states, the hypocretin peptides influence diverse physiological functions and consummatory behaviors. Thus, hypocretins may be important molecules involved in changing the set point associated with allostasis, and its priming by stress may facilitate the resumption of drug-seeking behavior.

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