9 Hyperarousal and Post-Traumatic Stress Disorder: A Role for the Hypocretin System

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Contents

Abstract

The hypocretins are a pair of neuropeptides produced in a few thousand neurons in the lateral hypothalamus. Extensive evidence suggests that one of the main functions of hypocretin neurons is to stabilize arousal/alertness during periods of wakefulness and to increase arousal-related behaviors, including eating, drinking, grooming, and locomotor activity. The ability of hypocretin neurons to increase arousal-associated behaviors suggests the possibility that these neurons may play a role in the hyperarousal state observed when an animal is exposed to an acute stressor. Consistent with this hypothesis is a variety of observations indicating that centrally administered hypocretins mimic the behavioral and physiological response to stress. In addition, hypocretin neurons receive prominent input and are activated by terminals containing corticotropin-releasing factor (CRF), a neuropeptide that is secreted in response to an acute stressor. There is abundant evidence demonstrating a reciprocal

From: *Post-Traumatic Stress Disorder*: *Basic Science and Clinical Practice*
Edited by: P. J. Shiromani et al., DOI: 10.1007/978-1-60327-329-9_9

connection between hypocretin and CRF neurons, which may be important in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis and the acute stress response. Given that CRF secretion and hyperarousal are both important symptoms of post-traumatic stress disorder (PTSD), hypocretin neurons may play a prominent role in causing many of the other physiological symptoms of the disease. We review the possible role of hypocretin neurons in the allostatic pathophysiology of PTSD, including dysregulation of the stress response, and in the circuitry that regulates sleep and wakefulness.

Key Words: Corticotropin-releasing factor (CRF), HPA axis, hyperarousal, hypocretin, hypothalamus.

INTRODUCTION

One of the major hallmarks of post-traumatic stress disorder (PTSD) is a state of heightened arousal characterized by interrupted sleep, hypervigilance, and an exaggerated startle response *(1)*. This hyperarousal state can also be characterized as changes in physiological indicators of stress, such as higher blood pressure, heart rate, and body temperature *(1,2)*. Much research into the neurobiology of PTSD has focused on the corticotropin-releasing factor (CRF) system and hypothalamus-pituitary-adrenal (HPA) axis, as these systems play an important role in the response to acute and chronic stressors. Indeed, hyperactivity of CRF and the HPA axis has been proposed as contributing to the hyperarousal and physiological symptoms of PTSD *(1–4)*. However, a recently discovered pair of peptides, the hypocretins, has also been shown to play a crucial role in the stability of arousal and alertness *(5)*. Perturbing the hypocretin system can lead to higher indices of arousal and anxiety that mimic the hyperarousal state exhibited in PTSD. In this chapter, we review the data suggesting that hypocretin peptides play a role in arousal and stress and propose a model for hyperactivity of the hypocretin system in PTSD.

THE HYPOCRETINS

The hypocretins (also known as orexins) were discovered independently by two groups in the late 1990s *(6,7)*. They consist of a pair of secreted peptides, hypocretin 1 and hypocretin 2 (Hcrt1 and Hcrt2; also known as orexin A and orexin B, respectively) that are processed from the same precursor, preprohypocretin (ppHcrt) *(6,7)*. The two peptides are produced exclusively in a few thousand neurons in the lateral hypothalamus (LH), distinct from cells in the LH that express melanin-concentrating hormone (MCH) *(6,7)*.

Hypocretin neurons project diffusely throughout the brain to two receptors, Hcrt-r1 and Hcrt-r2 *(6,7)*. Initial work demonstrated that Hcrt-r1 bound Hcrt1 with high affinity and Hcrt2 with 100- to 1,000-fold lower affinity *(7)*. However, Hcrt-r2 was shown to have high affinity for both Hcrt1 and Hcrt2. Hcrt-r1 messenger ribonucleic acid (mRNA) levels are found within the hypothalamus, the locus coeruleus (LC), the cerebral cortex, and several brain stem nuclei. In contrast, Hcrt-r2 mRNA is

expressed in cholinergic nuclei in the brain stem, the ventral tegmental area, and histaminergic neurons in the tuberomammilary nucleus (TMN), as well as overlapping expression with Hcrt-r1 in the hypothalamus *(6–10)*. The in situ hybridization pattern of these receptors is consistent with the map of Hcrt-containing fibers *(8)*. Dense projections to the ventrolateral preoptic area (VLPO), TMN, pedunculopontine tegmental (PPT) area, laterodorsal tegmental (LDT) area, and LC suggest that hypocretins may play a role in regulating arousal as all these nuclei play a role in regulating sleep and wakefulness. Thus, initial study of the physiological function of hypocretins involved the role of Hcrts in arousal and vigilance.

THE ROLE OF HYPOCRETINS IN AROUSAL

Extensive evidence now suggests that a main function of hypocretins is to enhance arousal and wakefulness. Shortly after their discovery, a mutation in Hcrt-r2 was demonstrated to be the cause of the sleep disorder narcolepsy in a canine model of the disease *(11)*. Hcrt knockout animals also display a striking narcolepsy phenotype, including a dysregulation of REM (rapid eye movement) sleep and "cataplexy-like" attacks, a hallmark of human narcolepsy *(12)*. Genetic ablation of Hcrt neurons also results in a narcolepsy phenotype *(13)*. Indeed, human narcoleptic patients were shown to have decreased hypocretin levels in their cerebrospinal fluid, and postmortem analysis of narcoleptic brains revealed a loss of hypocretin neurons in the hypothalamus *(14–16)*. Abnormal states of arousal due to mutations in the hypocretin system have even been documented in zebrafish *(17)*. Thus, the impairment of the Hcrts or their receptors demonstrates the necessity of this system to sustain normal states of arousal and alertness.

In addition to these loss-of-function studies, several gain-of-function studies also demonstrated that these peptides are sufficient to cause a state of hyperarousal. Intracerebroventricular injection of Hcrt1 or Hcrt2 increases the time spent awake and decreases the time spent in slow-wave and REM sleep *(18–20)*. Hcrt-induced increases in time spent awake are correlated with a relative increase in arousal-related behaviors, including eating, drinking, grooming, and locomotor activity *(18–21)*. In addition to intracerebroventricular injection of Hcrt1 and Hcrt2 peptides, artificial stimulation of Hcrt neurons using a light-activated cation channel, channelrhodopsin 2, increases the probability of transitions from sleep to wakefulness during both slow-wave and REM sleep *(22)*.

Taken together, these studies demonstrate that the Hcrt system mediates an arousal continuum from sleep to hypervigilance. Not only is hypocretin necessary for normal states of arousal and alertness, but overactivity of the hypocretin system leads to a hyperarousal phenotype in which animals not only spend more time in an awake state but also display an increase in arousal-associated behaviors. Because arousal is a prominent component in the biological stress response, it has been hypothesized that Hcrts can play a role in stress and anxiety-like states. This hypothesis is supported by data suggesting that overactivity of hypocretins may induce behavioral and physiological indicators of stress *(5)*.

THE HYPOCRETINS AND STRESS

A variety of observations indicate that centrally administered Hcrts mimic the behavioral and physiological responses to stress *(5,23)*. For example, intracerebral ventricular injection of Hcrt1 in rodents elicits a majority of stress-related behaviors, including grooming, chewing of inedible material, increased locomotor activity, and food consumption *(18,19,24–27)*. Furthermore, hyperactivity of the Hcrt system is also correlated with a variety of autonomic processes associated with high levels of arousal or stress, such as elevation of heart rate, body temperature, mean arterial blood pressure, and oxygen consumption *(28–32)*. Interestingly, all of these behavioral and physiological responses are observed in animal models of stress and anxiety *(33–37)*. These physiological responses and states of hyperarousal are also observed in human anxiety disorders, including PTSD *(1–4,38)*.

Hypocretin cells also seem to be activated by environmental stressors. Many studies showed an increase in c-Fos, an immediate early gene and marker of neural activity, in Hcrt cells in response to acute stressful stimuli, including a brightly lit, novel environment, food deprivation, and cold exposure, in addition to more chronic stressors such as foot shock and immobilization stress *(5,23,25,39–41)*.

How do Hcrts affect arousal and the stress response? As mentioned, Hcrts probably affect arousal by projecting to different arousal-promoting nuclei in the brain, including the LC, TMN, raphé nuclei, and cholinergic nuclei of the brain stem, as well as multiple regions of the cortex *(6–10)*. The Hcrt system has also been shown to directly interact with circuitry associated with the stress response, including the HPA axis and amygdala.

THE HYPOCRETINS INTERACT WITH THE NEURAL CIRCUITRY ASSOCIATED WITH STRESS/ANXIETY

One of the best-studied systems into the neurobiology of stress, anxiety, and PTSD disorders is that of CRF and the HPA axis. In response to environmental stressors, CRF is secreted by the paraventricular hypothalamic nucleus (PVN) *(42–47)*. Stimulation of pituitary corticotroph cells by CRF results in the production of adrenocorticotropic hormone (ACTH), which elicits the release of glucocorticoids, such as cortisol, from the adrenal gland *(48)*. Cortisol then acts on peripheral and visceral physiological responses to support "fight-or-flight" reactions, such as increasing heart rate, blood pressure, body temperature, and oxygen consumption, all hallmarks of the hyperarousal state of PTSD *(1–4,38,47,48)*. Indeed, patients with PTSD exhibit higher levels of CRF compared to healthy controls *(1–3)*. However, these patients also exhibit a *decreased* level of baseline plasma cortisol, suggesting hyperactivity in the central release of CRF and a hyperfeedback of ACTH levels secreted by the pituitary *(1–3)*. The exact dysregulation of this negative feedback is unknown.

Interestingly, the hypocretins have been shown to functionally interact with the HPA axis. Hypocretin receptors, especially Hcrt-r2, are abundantly present in the PVN *(49)*. Bath application of Hcrt1 and Hcrt2 depolarizes a majority

of magnocellular and parvocellular PVN neurons in vitro *(50)*. The effect of hypocretins on magnocellular PVN neurons is likely indirect, as tetrodotoxin (TTX) treatment blocks Hcrt1-elicited depolarizations *(51)*. Furthermore, these effects are also blocked by kynurenic acid, demonstrating the role of glutaminergic interneurons in the action of Hcrt1 *(51)*. In contrast, Hcrt1 depolarizes parvocellular neurons, and this effect is not blocked by TTX *(51)*. While the exact biochemical identity of neurons in the PVN that respond to Hcrt administration is unknown, it is clear that Hcrt can stimulate the release of CRF into the portal vessels *(52)*. Intracerebroventricular Hcrt1 administration increases plasma levels of both glucocorticoids and ACTH release *(5,25,52–54)*.

Not only can the hypocretins depolarize CRF-containing cells in the PVN, but CRF can also affect hypocretin cells in the LH. CRF-immunoreactive boutons are found adjacent to Hcrt cells, and Hcrt cells express CRF receptors *(55)*. Application of CRF to hypothalamic slices depolarizes the membrane potential and increases firing rate in a subpopulation of hypocretinergic cells *(55)*. These CRF-induced depolarizations are dose dependent and blocked by astressin, a CRF receptor 1 antagonist *(55)*. Furthermore, behavioral stressors such as intermittent foot shock and restraint can induce the activation of Hcrt cells in mice but not in CRF receptor 1-deficient animals. Taken together, these studies demonstrate that CRF neurons and Hcrt neurons form a circuit that may mediate the neuroendocrine and hyperarousal responses to stress. These studies also demonstrate that an increase in CRF release, as is documented in PTSD, could lead to an increase in Hcrt-cell activity and consequently a state of hyperarousal.

Retrograde tracing studies suggest that hypocretin cells receive not only CRF projections from the PVN but also strong projections from CRF-containing cells in the central nucleus of the amygdala (CE) and bed nucleus of the stria terminalis (BNST) *(56)*. These two structures serve as output structures of the amygdala and play a role in regulating anxiety *(1–4)*. It has been suggested that altered plasticity within the amygdaloid complex is responsible for the chronic anxiety experienced in PTSD *(4)*.

Another subregion within the amygdala, the basolateral nucleus (BLA), seems to serve as an integrator and relay center for incoming sensory, memory, and limbic information necessary for anxiety responses *(57)*. Much research has demonstrated that the BLA is a crucial site of synaptic plasticity that contributes to changes in behavior following a stressful or traumatic event *(58–64)*. The BLA sends direct, excitatory projections to the CE and BNST, suggesting that altered plasticity in anxiety disorders causes hyperactivity of the amygdala and its efferent subregions *(1)*.

Consistent with this hypothesis are the consistent findings in functional magnetic resonance imaging (fMRI) studies that increased amygdala activation is associated with several anxiety and mood states *(65–67)*. In rodent models, CRF mRNA is increased in the amygdala following stressful paradigms *(68)*. Restraint, drug withdrawal, and neonatal stressors cause an increase of CRF in the amygdala *(69–70)*. These studies suggest that altered plasticity causes hyperactivity in the amygdala, with Hcrt cells in the LH receiving direct efferent projections from CRF-containing cells in the CE and BNST.

A ROLE FOR HYPOCRETINS IN PTSD

Taken together, there is much evidence suggesting that hyperactivity of the Hcrt system may contribute to the hyperarousal state experienced in PTSD:

- PTSD is a hyperarousal disorder, and hyperactivity of the Hcrt system causes a state of hyperarousal in all animals tested, including mice, rats, fish, and birds.
- Hyperactivity of the Hcrt system also causes autonomic and neuroendocrine changes that are symptoms of PTSD in human patients.
- PTSD patients have an overabundance of CRF resulting from stressful stimuli, and CRF directly excites Hcrt neurons.
- The CE and BNST, structures of the amygdaloid complex that are hyperactive in PTSD, send direct projections to Hcrt cells.

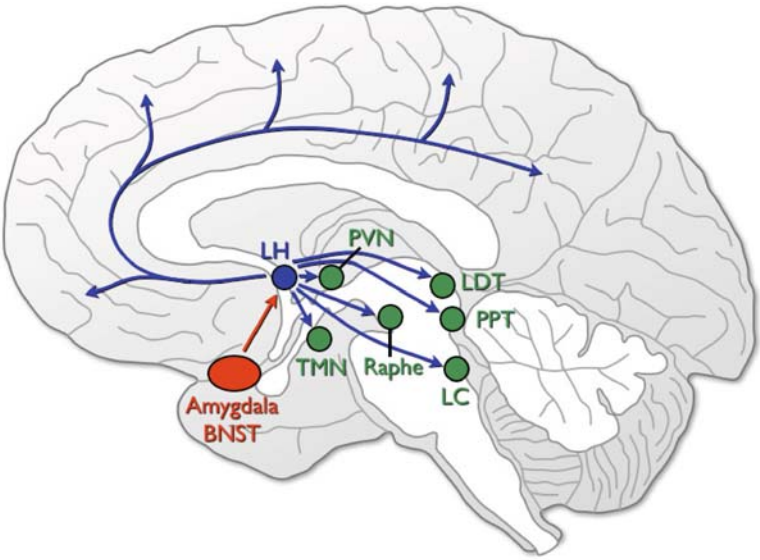

Fig. 1. A model for the role of hypocretins in post-traumatic stress disorder (PTSD). Altered plasticity in the amygdala causes an increase in activity in corticotrophin-releasing factor (CRF)-containing neurons of the central nucleus of the amygdala and bed nucleus of the stria terminalis (BNST). These nuclei send robust projections to hypocretin neurons in the lateral hypothalamus (LH). The hypocretin neurons are well positioned to cause a heightened state of arousal, projecting to multiple brain systems that promote arousal and the response to stress, such as CRF-containing neurons in the paraventricular hypothalamic nucleus (PVN), histaminergic neurons in the tuberomammilary nucleus (TMN), serotoninergic neurons in the raphé nuclei, cholinergic neurons in the lateral dorsal tegmental nucleus (LDT) and pedunculopontine tegmental nucleus (PPT), and noradrenergic neurons in the locus coeruleus (LC). Thus, hyperexcitation of hypocretin neurons could orchestrate the hyperarousal state of PTSD (*See Color Plates*)

Because the CE and BNST send robust projections to Hcrt cells in the LH, we propose a simple model in which hyperexcitability of CRF cells in the amygdala, as has been shown in PTSD, causes an increase in activity in Hcrt cells (Fig. 1). This increase would in turn result in a hyperarousal state such as seen with stimulation of the Hcrt system. The Hcrt cells are perfectly situated to cause an increase in arousal and a stress-like state as they project to multiple nuclei that promote arousal (such as the LC, TMN, and raphé nuclei), as well as the PVN and HPA axis.

The hypocretin cells are also perfectly situated to contribute to the sleep disturbances found in PTSD. It is estimated that 70–91% of patients with PTSD have difficulty falling asleep or staying asleep *(71)*. Individuals with PTSD are more likely to report waking up restless with excessive body movements during sleep compared to individuals with insomnia and no PTSD *(72)*. Furthermore, patients with PTSD exhibit inappropriate awakenings from normal REM sleep *(73)*. As mentioned, these are the same effects seen in animal models in which artificial stimulation of hypocretin neurons causes an increase in the probability of a transition from sleep to wakefulness *(22)*. Intracerebroventricular injection of hypocretins increases the time spent awake and decreases the time spent in slow-wave and REM sleep *(18–20)*. Some have hypothesized that overactivity of the LC/noradrenergic system is responsible for the sleep disturbances observed in PTSD; indeed, some reports indicated that PTSD patients exhibit an increase in noradrenergic activity *(74,75)*. The LC receives robust projections from hypocretin cells, yet so far no study has examined an increase in hypocretin tone as causal or correlational with the sleep disturbances experienced in PTSD.

Future studies should directly examine a role for hypocretin cells in PTSD. It will be interesting to find out if PTSD patients have hyperactivity in the LH and an increase in cerebrospinal fluid hypocretin concentration as compared with healthy controls, especially following stressful stimuli. In animal models, it will be necessary to test whether stimulation of the CE or BNST causes an increase in activity of hypocretin neurons. Finally, a growing number of pharmacological agents target the hypocretin system in humans *(76)*. It will be clinically important to examine the effects of a hypocretin antagonist in patients suffering from PTSD and other anxiety disorders to see if a decrease in hypocretin activity helps to moderate an abnormal hyperarousal state.

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