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Role of innate and drug-induced dysregulation of brain stress and arousal systems in addiction: Focus on corticotropin-releasing factor, nociceptin/orphanin FQ, and orexin/hypocretin

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Abstract

Stress-like symptoms are an integral part of acute and protracted drug withdrawal, and several lines of evidence have shown that dysregulation of brain stress systems, including the extrahypothalamic corticotropin-releasing factor (CRF) system, following long-term drug use is of major importance in maintaining drug and alcohol addiction. Recently, two other neuropeptide systems have attracted interest, the nociceptin/orphanin FQ (N/OFQ) and orexin/hypocretin (Orx/Hcrt) systems. N/OFQ participates in a wide range of physiological responses, and the hypothalamic Orx/Hcrt system helps regulate several physiological processes, including feeding, energy metabolism, and arousal. Moreover, these two systems have been suggested to participate in psychiatric disorders, including anxiety and drug addiction. Dysregulation of these systems by chronic drug exposure has been hypothesized to play a role in the maintenance of addiction and dependence. Recent evidence demonstrated that interactions between CRF-N/OFQ and CRF-Orx/Hcrt systems may be functionally relevant for the control of stress-related addictive behavior. The present review discusses recent findings that support the hypotheses of the participation and dysregulation of these systems in drug addiction and evaluates the current understanding of interactions among these stress-regulatory peptides.

Keywords

corticotropin-releasing factor; orexin/hypocretin; nociceptin/orphanin FQ; addiction; reinstatement; relapse; allostasis; stress; anxiety

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1. Introduction

Stress has an established role in the initiation and maintenance of drug abuse and is a major determinant of relapse in abstinent individuals (Brown et al., 1995; Marlatt, 1985; McKay et al., 1995; Sinha, 2001; Wallace, 1989). The significance of stress in drug-seeking behavior and reinforcement has been extensively studied and documented in the animal literature. Stressors can facilitate the acquisition of drug self-administration and increase drug intake in animals (e.g., Sarnyai et al., 2001). Stress also elicits the recovery of extinguished drug- and alcohol-seeking behavior (Le and Shaham, 2002; Shaham et al., 2000; Shaham et al., 2003) in reinstatement models of relapse and enhances the effects of drug cue exposure on reinstatement (Liu and Weiss, 2002). Moreover, the motivating effects of stress are persistent and can precipitate “relapse” despite many weeks of abstinence (Shalev et al., 2001).

In addition to the direct role of stress in drug seeking and consumption, dysregulation of brain stress systems following long-term drug use is important in maintaining drug and alcohol addiction. Chronic drug use produces neuroadaptive changes, both in systems mediating the acute reinforcing effects of drugs of abuse and other neural systems, notably brain systems that regulate behavioral and emotional responses to stress. These latter changes, originally referred to as “between-system” neuroadaptations (Koob and Le Moal, 1997), are thought to represent excitatory adaptive responses to chronic drug or alcohol exposure. These changes are hypothesized to oppose the acute reinforcing and hedonic effects of drug or alcohol exposure, leading to anxiety, mood disturbances, and adverse somatic symptoms that accompany acute withdrawal. Moreover, drug-induced neuroadaptations are hypothesized to be responsible for the persistence of these symptoms, albeit at reduced intensity, during protracted withdrawal (Meyer, 1996). This neuroadaptive activation of brain stress systems has been implicated as a mechanism responsible for the development of an allostatic state underlying compulsive drug taking, loss of control over drug intake, and vulnerability to relapse (e.g., Koob and Le Moal, 2001).

Research aimed at understanding the neural basis of the stress-addiction link has focused predominantly on corticotropin-releasing factor (CRF), a major molecule regulating stress and arousal in the mammalian central nervous system. In addition to the CRF system, two other neuropeptide systems with a role in stress and arousal have received growing attention with respect to their role in drug and alcohol addiction: the nociceptin/orphanin FQ (N/OFQ) opioid-like peptide system and the orexin/hypocretin (Orx/Hcrt) system (e.g., Boutrel et al., 2005; Ciccocioppo et al., 1999b; Ciccocioppo et al., 2000; Harris et al., 2005). These systems have attracted interest because of emerging evidence demonstrating interactions between CRF and N/OFQ and Orx/Hcrt systems that may be functionally relevant for the control of stress-related addictive behavior.

This paper reviews the literature on the respective roles of CRF, N/OFQ, and Orx/Hcrt in drug addiction and evaluates the current understanding of interactions among these stress-regulatory peptides relevant for drug addiction.

2. CRF: Role in Stress and Addiction

CRF is a 41-amino acid peptide that interacts with two G-protein-coupled CRF receptors, CRF₁ and CRF₂, that are positively coupled to adenylate cyclase via the G_s protein (Zorrilla and Koob, 2004; Zorrilla and Koob, 2005). CRF is the primary activator of the hypothalamic-pituitary-adrenal (HPA) “stress” axis via anterior pituitary CRF₁ activation, leading to increased adrenocorticotrophic hormone (ACTH) secretion that ultimately stimulates glucocorticoid production and release from the adrenal gland (Vale et al., 1981). Circulating glucocorticoids exert fine negative feedback control on the HPA axis at several levels, including transcriptional modulation of paraventricular nucleus (PVN) CRF-producing

neurons (Kageyama and Suda, 2009). CRF not only regulates HPA axis activity, but also functions as a neurotransmitter in extrahypothalamic brain regions. CRF in the amygdala, bed nucleus of the stria terminalis (BNST), and septum has been implicated in the integration of emotional responses to stress. CRF neurotransmission has also been identified in the ventral tegmental area (VTA) where it appears to play an important role in the regulation of appetitive and motivational states (Wang et al., 2005; Wang et al., 2007; Ungless et al., 2003; Rodaros et al., 2007). Unlike their effects on the regulation of CRF in the PVN, glucocorticoids appear to upregulate the activity of CRF systems in the extended amygdala, forming a feed-forward circuit (Schulkin et al., 1998). Stress-related addictive behavior is partially related to activation of the HPA axis by CRF (for review, see Goeders, 2002; Sarnyai et al., 2001). However, extrahypothalamic, non-neuroendocrine CRF neurotransmission in brain regions that regulate behavioral and emotional responses to stress plays a significant independent role in addictive behavior (e.g., Sarnyai et al., 2001).

Generally confirming a role for CRF in addictive behavior, manipulations that interfere with CRF transmission reduce the effects of stress on drug seeking and intake. For example, CRF₁ antagonist administration blunts the acquisition of cocaine-induced conditioned place preference (Lu et al., 2003), reduces conditioned reinstatement of cocaine seeking (Gurkovskaya and Goeders, 2001), attenuates footshock-induced reinstatement of cocaine seeking, and reverses stress-induced renewal of cocaine-induced conditioned place preference (for review, see Koob, 2008; Boutrel, 2008; Cleck and Blendy, 2008). Conversely, intracranial administration of CRF reinstates cocaine-seeking behavior (Brown et al., 2009; Erb et al., 2006). Moreover, blockade of central CRF transmission by CRF receptor antagonists or CRF antisera blocks cocaine-induced anxiety-like behavior, locomotor activity, and stress-induced cross-sensitization with psychostimulants (Lu et al., 2003; Przegalinski et al., 2005; Sarnyai et al., 1992; Sarnyai et al., 1995).

2.1. Drug-induced neuroadaptive dysregulation of the HPA axis

Stress-related addictive behavior is partially related to changes in HPA axis activity (e.g., Goeders, 2002; Sarnyai et al., 2001). For example, plasma corticosterone levels are increased in animals self-administering cocaine (Mantsch et al., 2000). More importantly, plasma corticosterone levels before acquisition of low-dose cocaine self-administration correlate with subsequent drug-reinforced responding (Mantsch et al., 2001). Thus, basal HPA axis activity may predict individual vulnerability to initial drug use, and individual differences in HPA axis responses to drug intake may represent a moderating vulnerability factor, when high doses of the drug are self-administered (Koob and Kreek, 2007). Furthermore, in rats with extended access to cocaine self-administration, both downregulation of HPA axis activity and impaired glucocorticoid receptor function have been observed (Mantsch et al., 2007). Specifically, dysregulation of glucocorticoid receptor-mediated negative feedback on HPA axis activity has been reported, which may contribute to drug-induced stress system alterations (Mantsch et al., 2007). Similar observations have been reported following chronic alcohol exposure, after which decreased activity of hypothalamic CRF neurons (Lee et al., 2000) and reduced anterior pituitary responsiveness to CRF (Dave et al., 1986) are evident. These preclinical findings are consistent with the clinical literature demonstrating that alcoholics and drug addicts show reduced HPA axis activity or a blunted HPA axis response to acute stress (Wand and Dobs, 1991).

2.2. Drug-induced neuroadaptive dysregulation of extrahypothalamic CRF

Repeated or long-term exposure to drugs of abuse leads to adaptations in the extrahypothalamic CRF circuitry with functional and behavioral consequences relevant for the development of drug addiction. This has been shown at several levels of analysis for psychostimulants, opiates, and ethanol. The focus here will be on the effects of chronic cocaine and ethanol. Following

chronic cocaine treatment, neuroplasticity in the central nucleus of the amygdala (CeA) occurs via a CRF-dependent mechanism (Fu et al., 2007; Pollandt et al., 2006). Moreover, repeated cocaine administration cross-sensitizes locomotor activity and *c-fos* responses in the CeA to intracerebroventricular CRF administration (Erb et al., 2003; Erb et al., 2005; Erb and Brown, 2006). Behavioral evidence of CRF system neuroadaptation has been obtained in rats voluntarily self-administering cocaine, specifically in rats with a history of escalated cocaine intake associated with daily extended access (6 h/day) to intravenous cocaine (Mantsch et al., 2008). Rats showing escalated cocaine intake exhibit behavioral signs consistent with cocaine dependence. These signs include (i) increased motivation to obtain cocaine, measured by progressive-ratio responding, (ii) vertical shifts in the cocaine dose-response function, suggesting an increased hedonic set point (Ahmed and Koob, 1998; Ahmed and Koob, 1999; Wee et al., 2007), (iii) anhedonia-like symptoms upon cocaine withdrawal, measured by elevations in brain stimulation reward thresholds (Ahmed et al., 2002), (iv) resistance to extinction, and (v) facilitated reinstatement of cocaine seeking (Ahmed and Cador, 2006). In these animals, stressors and intracerebroventricular CRF administration more effectively reinstate cocaine-seeking behavior than in rats without a history of escalation (Mantsch et al., 2008). Additionally, CRF₁ antagonists reduce cocaine intake in rats with a history of extended daily cocaine access compared with non-escalated rats, suggesting increased activity of the CRF receptor system (Specio et al., 2008).

A second line of evidence illustrating dysregulation of extrahypothalamic CRF function by chronic drug use derives from intracranial microdialysis studies in which withdrawal from chronic ethanol, cocaine, cannabinoid, and morphine treatments was shown to result in substantial elevations in extracellular CRF levels in the amygdala (Merlo-Pich et al., 1995; Richter and Weiss, 1999; Rodriguez de Fonseca et al., 1997; Weiss et al., 2001) and BNST (Olive et al., 2002). This hypersecretion of CRF in the amygdala is an effect common to withdrawal from all major drugs of abuse studied to date (Weiss et al., 2001) and is associated with aversive and anxiety-like behavioral manifestations that are reversible by functional antagonism of CRF transmission (Basso et al., 1999; Sarnyai, 1998).

The alcohol literature also indicates the involvement of CRF neurotransmission in the medial raphe nucleus (MRN) in addictive behavior, particularly stress-induced ethanol seeking and relapse. CRF microinjection into the MRN elicits marked reinstatement of extinguished alcohol seeking behavior (Le et al., 2002). Conversely, intra-MRN administration of the nonselective CRF receptor antagonist D-Phe CRF₁₂₋₄₁ prevents footshock stress-induced reinstatement of ethanol seeking and reduces stress-induced *c-fos* expression in the CeA (Funk et al., 2003), confirming a significant role for MRN CRF systems in alcohol seeking induced by stress.

More recently, CRF neurotransmission in the VTA has been identified as an important participant in the regulation of appetitive and motivational states. Specifically, footshock stress increases extracellular CRF levels in the VTA, and, similar to stress, intra-VTA infusion of CRF elicits reinstatement of cocaine-seeking behavior (Wang et al., 2005). The behavioral actions of CRF were associated with increased glutamate and dopamine release in the VTA, but only in cocaine-experienced rats, suggesting that cocaine self-administration leads to neuroadaptive changes in CRF neurotransmission within the VTA (Wang et al., 2005). Consistent with this hypothesis are findings showing that application of CRF to VTA slice preparations facilitates excitatory glutamatergic responses of dopamine neurons (Ungless et al., 2003) and that the magnitude and duration of the CRF-induced potentiation of *N*-methyl-D-aspartate (NMDA) receptor-mediated neurotransmission is significantly enhanced by repeated cocaine exposure (Hahn et al., 2009). Interestingly, in the VTA, in contrast to other brain regions where CRF₁ receptors play a primary role, the effects of CRF are blocked by selective CRF₂, but not CRF₁, receptor antagonists (Ungless et al., 2003; Wang et al., 2007). The actions of CRF on dopamine transmission in the VTA may also involve the CRF binding

protein (a protein that binds free CRF or urocortin-1 with high affinity; Behan et al., 1995). CRF₆₋₃₃, a fragment without known receptor agonist activity that competitively displaces CRF from the CRF binding protein, prevented CRF from stimulating VTA dopamine transmission and eliciting cocaine seeking in rats (Ungless et al., 2003; Wang et al., 2007). These findings suggest a role not only for CRF₂ receptors in the VTA, but also the CRF binding protein in the regulation of addictive behavior.

2.3. Drug-induced neuroadaptation and genetic factors in the dysregulation of extrahypothalamic CRF: implications for addiction

Perhaps the most extensive and compelling evidence of a link between extrahypothalamic CRF dysregulation, heightened stress sensitivity, and exacerbated drug seeking and intake can be found in the ethanol literature.

Similar to the effects of other drugs of abuse, a factor in the dysregulation of extrahypothalamic CRF dysfunction is chronic ethanol use itself. Chronic ethanol exposure produces functional adaptation of the CRF system in the CeA (Weiss et al., 2001) and BNST (Olive et al., 2002). Withdrawal from chronic ethanol exposure profoundly elevates extracellular CRF levels (Merlo-Pich et al., 1995; Richter and Weiss, 1999; Rodriguez de Fonseca et al., 1997; Weiss et al., 2001). This hypersecretion of CRF results in aversive and anxiety-like behavioral effects. Microinjection of CRF receptor antagonists into the CeA reverses increased anxiety-like reactions and ethanol self-administration observed in ethanol-dependent rats during acute withdrawal but does not alter these behaviors in ethanol nondependent rats (Funk et al., 2006; Rassnick et al., 1993; Valdez et al., 2002). Moreover, stress-induced reinstatement of ethanol seeking is exacerbated in a CRF antagonist-reversible manner (D-Phe CRF₁₂₋₄₁) in rats with a history of ethanol dependence (Liu and Weiss, 2002). These findings indicate that adaptations occur not only in the regulation of CRF release, but also CRF receptors. Systemic administration of selective CRF₁ antagonists or CRF₁ deletion effectively reduces anxiety-like behavior during acute ethanol withdrawal, revealing a role for the CRF₁ receptor in these adaptations (Breese et al., 2004; Knapp et al., 2004; Overstreet et al., 2004; Timpl et al., 1998). Similarly, nonpeptide CRF₁ antagonists reduce increased ethanol self-administration typically associated with acute withdrawal from repeated, intermittent passive ethanol exposure in rats at doses that do not modify ethanol intake in nondependent rats (Funk et al., 2007; Sabino et al., 2006).

A second line of evidence supporting the link between dysregulation of extrahypothalamic CRF function and high ethanol intake is innate dysfunction of the CRF system identified in rats genetically selected for high ethanol preference. In early studies, ethanol-naïve Sardinian alcohol-preferring (sP) rats exhibited an innate upregulation of CRF function in the CeA, reflected by elevated basal CRF output. Additionally, these animals showed increased behavioral sensitivity to stress and anxiogenic stimuli, measured on the elevated plus maze, compared with their alcohol-nonpreferring (sNP) counterparts (Richter et al., 2000). Thus, these alcohol-preferring rats show abnormalities in stress sensitivity and CRF function in the CeA that resemble those in chronic ethanol-exposed genetically heterogeneous rats (i.e., Wistar rats not selected for ethanol preference). Notably, however, despite these abnormalities in stress responsiveness in sP rats, the CRF₁ antagonist LWH-63 was shown to suppress only withdrawal-induced but not spontaneous ethanol intake in this line of rats (Sabino et al., 2006). Nonetheless, LWH-63 and other CRF₁-selective antagonists produced clear anti-stress-like actions in nondependent sP rats, such as attenuation of stress-induced suppression of ethanol intake (Sabino et al., 2006). Therefore, innate upregulation of the CRF system in sP rats may convey vulnerability to high alcohol consumption, but further dysregulation of the CRF system by chronic alcohol exposure may exacerbate CRF₁ receptor-dependent high alcohol drinking in these animals. Similarly, increased alcohol intake may be related to

additional CRF₁ gene single nucleotide polymorphisms (SNPs), such as those found in the sP-derived Marchigian Sardinian alcohol-preferring (msP) rat line (Fonareva et al., 2009; Hansson et al., 2006).

Perhaps the most direct evidence for similarities in addiction-relevant consequences of both genetically determined and chronic ethanol-induced abnormalities in extrahypothalamic CRF function (and other brain stress-systems; see below) comes from studies with msP alcohol-preferring rats in which high alcohol preference co-segregates with increased sensitivity to stress, creating a phenocopy of the postdependent phenotype in nonselected Wistar rats (Ciccocioppo et al., 1999a; Hansson et al., 2006). A screen for differential gene expression identified an innate upregulation of the *Crhr1* transcript encoding the CRF₁ receptor in several limbic brain areas of msP rats, including the CeA, medial nucleus of the amygdala (MeA), basolateral nucleus of the amygdala (BLA), and hippocampal regions, was associated with a genetic polymorphism of the *Crhr1* promoter and accompanied by increased CRF₁ receptor density. Interestingly, in this line of rats, voluntary ethanol consumption led to a reduction of anxiety-like and depression-like behaviors (Ciccocioppo et al., 1999a; Ciccocioppo et al., 2006) associated with significant downregulation of the *Crhr1* transcript in the CeA, MeA, and nucleus accumbens (Hansson et al., 2007). These findings suggest that msP rats may consume large amounts of ethanol to self-medicate a “negative affective state” resulting from excess extrahypothalamic CRF₁ receptor activity. Consistent with this hypothesis, the selective CRF₁ antagonist antalarmin was devoid of effects on ethanol self-administration in nonselected Wistar rats but significantly suppressed this behavior in msP rats. Similarly, antalarmin did not significantly attenuate footshock stress-induced reinstatement of ethanol seeking in Wistar rats but fully blocked the effects of footshock on reinstatement in msP rats (Hansson et al., 2006). Overall, these findings suggest a causal role of CRF₁ receptor upregulation in the behavioral phenotype of msP rats.

The findings in msP rats described above demonstrate that the *Crhr1* genotype and *Crhr1* expression regulate alcohol-taking behavior and interact with environmental stress to reinstate alcohol seeking. Considering the similarities between genetically determined and chronic ethanol-induced abnormalities in the function of extrahypothalamic CRF stress systems discussed earlier, chronic ethanol intoxication may upregulate the *Crhr1* transcript, CRF₁ receptors, or CRF peptide levels in genetically nonselected Wistar rats, resulting in an “ethanol-dependent” behavioral phenotype similar to msP rats, characterized by high alcohol intake and stress sensitivity.

Confirming this hypothesis, alcohol intake and stress sensitivity increased in Wistar rats with a history of ethanol dependence. These behavioral changes were accompanied by upregulation of *Crhr1* transcript expression in the BLA and MeA, upregulation of CRF messenger RNA (mRNA) in the CeA, and downregulation of *Crhr2* expression in the BLA (Sommer et al., 2008). These findings suggest that neuroadaptations encompassing CRF function in the amygdala contribute to the high ethanol drinking and high stress responsivity of postdependent Wistar rats. Somewhat consistent with these findings, postdependent Wistar rats, tested 8 h following withdrawal from a 4 week ethanol vapor dependence induction procedure, exhibited significantly decreased CRF₁ binding in the CeA, MeA, and BLA (Fig. 1), an effect that remained unaltered for at least 3 weeks (data not shown). Thus, increased *Crhr1* gene expression may represent a compensatory response to the decrease in membrane CRF₁ binding elicited by chronic ethanol exposure. Given the well-documented increase of CRF peptide availability in the amygdala postdependence (Zorrilla et al., 2001), the decrease in membrane CRF₁ binding may be attributable to agonist-induced receptor activation and internalization, which is profound and occurs rapidly (within 5 min of activation; Reyes et al., 2006, 2008; Perry et al., 2005). Considering these findings, the decrease in CRF₁ receptor membrane levels in the presence of increased CRF peptide tissue content and CRF₁ synthesis may reflect the

consequences of sustained CRF₁ receptor stimulation driven by high CRF peptide availability. The consistent observation of higher efficacy of CRF₁ receptor antagonists in postdependent rats is accounted for by the prevention of sustained CRF₁ stimulation in these animals. A second, independent explanation for the increased behavioral efficacy of CRF₁ antagonists in postdependent animals, tentatively supported by electrophysiological data, is that CRF₁ signaling efficiency may be enhanced, possibly because of adaptation of signal transduction mechanisms. A final alternative explanation is that the increased CRF antagonist efficacy in ethanol-dependent and postdependent animals results from a more favorable receptor occupancy profile achieved by CRF₁ receptor antagonists in the presence of fewer membrane receptors. The latter interpretation is perhaps less likely because it predicts that CRF₁ antagonists reduce ethanol self-administration in nondependent animals given a sufficiently high dose (and degree of receptor occupancy). However, as discussed above, “anti-drinking” effects have typically not been observed even at the highest CRF antagonist doses in nondependent animals. Indeed, even doses of the CRF₁ antagonist R121919 that almost fully (~85%) occupy brain CRF₁ receptors in ethanol-naïve rats (Gutman et al., 2003) did not alter ethanol self-administration in nondependent animals (Sabino et al., 2006; Funk et al., 2007.)

Thus, substantial evidence implicates increased sensitivity to stress associated with dysregulation of extrahypothalamic CRF function as a major determinant of vulnerability to and maintenance of high alcohol intake. Moreover, dysregulation of extrahypothalamic CRF function leading to enhanced stress sensitivity can preexist as a genetically determined condition or develop as a result of chronic high-dose alcohol exposure.

3. Nociceptin/Orphanin FQ

N/OFQ is a 17-amino acid peptide that shows structural homology with opioid peptides, particularly dynorphin A (Meunier et al., 1995; Reinscheid et al., 1995). N/OFQ binds with high affinity at opioid receptor-like 1 (ORL-1), now included in the opioid receptor family and renamed the NOP receptor (nociceptin/orphanin peptide receptor), but does not activate μ , κ , or δ opioid receptors. N/OFQ and its receptor are widely distributed in the central nervous system, with the highest density in the CeA, BNST, medial prefrontal cortex (mPFC), VTA, lateral hypothalamus (LH), nucleus accumbens, and brain stem areas, including the locus coeruleus (LC) and dorsal raphe (DR; Darland et al., 1998; Neal et al., 1999). Interestingly, N/OFQ has been found to act in the brain as a functional antiopioid peptide by blocking opioid-induced supraspinal analgesia (Mogil et al., 1996a; Mogil et al., 1996b; Morgan et al., 1997), morphine-induced conditioned place preference (Ciccocioppo et al., 2000; Murphy et al., 1999), and morphine-induced increases in extracellular dopamine levels in the nucleus accumbens (Di Giannuario and Pieretti, 2000). Moreover, N/OFQ inhibits stress-induced ethanol seeking (Martin-Fardon et al., 2000) and exerts general anti-stress-like effects by acting as a functional antagonist of extrahypothalamic CRF transmission (Ciccocioppo et al., 2003a; Ciccocioppo et al., 2003b).

3.1. Role in drug and alcohol addiction

Manipulation of the N/OFQ system by pharmacological agents or gene deletion modifies the reinforcing effects of psychostimulants (Kotlinska et al., 2002; Kotlinska et al., 2003; Sakoori and Murphy, 2004; Sakoori and Murphy, 2008; Zhao et al., 2003), morphine (Ciccocioppo et al., 2000; Murphy et al., 1999; Sakoori and Murphy, 2004), ethanol (Ciccocioppo et al., 1999b; Kuzmin et al., 2003; Sakoori and Murphy, 2008), and nicotine (Sakoori and Murphy, 2009) measured by the acquisition of conditioned place preference and consumption (bottle choice drinking paradigm) tests. A role of N/OFQ in the regulation of psychostimulant-induced behavioral sensitization has also been described. Specifically, intracerebroventricular or intra-VTA administration of N/OFQ before repeated cocaine or amphetamine treatments prevents the development of locomotor sensitization, suggesting that the VTA may be a substrate of N/

OFQ actions (Kotlinska et al., 2003; Lutfy et al., 2002). Moreover, intra-VTA administration of N/OFQ reduces dopamine levels in the nucleus accumbens (Murphy and Maidment, 1999). Altogether these findings suggest that N/OFQ participate in regulating mesolimbic dopamine neurotransmission and possibly the rewarding effects of several drugs of abuse.

The role of N/OFQ in drug reward has been studied most extensively with ethanol. Activation of the N/OFQ system (i) blunts the reinforcing and motivating effects of ethanol across a range of behavioral measures, including ethanol self-administration (Ciccocioppo et al., 1999b), conditioned place preference (Kuzmin et al., 2003), and conditioned reinstatement (Ciccocioppo et al., 2004), (ii) reverses increased anxiety-like behavior associated with neuroadaptive changes in N/OFQ function associated with a history of ethanol dependence (Aujla et al., 2007), (iii) attenuates alcohol withdrawal symptoms (Stopponi et al., 2009), and (iv) produces substantial anti-anxiety-like and anti-stress-like effects in animal models of anxiety (Rodi et al., 2008), CRF-induced anorexia (Ciccocioppo et al., 2001; Ciccocioppo et al., 2003b), and stress-induced reinstatement of ethanol seeking (Martin-Fardon et al., 2000). Importantly, activation of the N/OFQ system failed to block stress-induced reinstatement of cocaine seeking (Martin-Fardon et al., 2000). The reasons for the lack of effect of N/OFQ on stress-induced reinstatement of cocaine seeking are presently not clear. However, emerging evidence suggests that the N/OFQ system may be differentially affected by alcohol and psychostimulants. For example, acute ethanol administration increases NOP receptor density in cortical areas, whereas acute administration of cocaine does not (Rosin et al., 2003). Furthermore, under chronic treatment conditions, alcohol administration decreases N/OFQ brain tissue levels (Lindholm et al., 2002), whereas repeated cocaine administration increases N/OFQ immunoreactivity in the brain (Lutfy et al., 2008).

3.2. Genetic predisposition to alcohol abuse

Data from both animals and humans implicate innate dysregulation of the N/OFQ system as a possible factor in alcoholism. msP rats exhibit traits frequently associated with alcoholism, including high sensitivity to stress, a high anxiety phenotype, and depression-like symptoms. These behavioral characteristics of msP rats are ameliorated by alcohol consumption (Ciccocioppo et al., 1999a). Ethanol self-administration in msP rats is highly sensitive to suppression by N/OFQ and N/OFQ analogs (Ciccocioppo et al., 1999b; Ciccocioppo et al., 2000; Ciccocioppo et al., 2004; Economidou et al., 2006), suggesting that hypofunction of the N/OFQ system may be a factor in high alcohol intake by msP rats. Apparently contradicting this hypothesis, msP rats exhibit elevated expression of N/OFQ and NOP receptor mRNA, with particularly high levels in the BNST and CeA (Economidou et al., 2008), and significantly increased NOP receptor binding in the CeA, BNST, VTA, and several cortical structures, indicating an innate overall upregulation of the N/OFQ-NOP system (Economidou et al., 2008). However, msP rats show a distinct pattern of N/OFQ functional abnormalities in the CeA where N/OFQ-stimulated [³⁵S]GTPγS binding was significantly lower in msP than Wistar rats, despite elevated NOP receptor expression and binding in the msP line (Economidou et al., 2008). Thus, “uncoupling” of the NOP receptor from G-protein-mediated signal transduction in the CeA may lead to regionally selective hypofunction of the N/OFQ system which facilitates alcohol drinking. This hypothesis is supported by data showing that stimulation of NOP receptors in the CeA by site-specific microinjection of N/OFQ reduces alcohol self-administration in msP rats (Economidou et al., 2008).

At the clinical level, recent association studies suggest a possible link between alcoholism and polymorphisms in the gene encoding the NOP receptor. One study revealed a link between alcoholism and two SNPs in the N/OFQ gene (Xuei et al., 2008). This observation was corroborated by findings showing that the gene encoding the NOP receptor (SNP rs6010718) is significantly associated with both Type I and Type II alcoholism in a Scandinavian sample

of alcoholics (Huang et al., 2008). Thus, genetic variants of the N/OFQ and NOP genes may be linked to vulnerability for alcohol dependence.

3.3. Dysregulation of the N/OFQ system following ethanol dependence

The hypothesis that N/OFQ system dysregulation is a factor in high alcohol intake is consistent with recent findings showing that Wistar rats with a history of ethanol dependence exhibit neuroadaptive changes in the N/OFQ system that are associated with increased stress sensitivity and alcohol intake. Wistar rats were trained to operantly self-administer 10% ethanol and then subjected to induction of ethanol dependence using a 3 week ethanol vapor inhalation procedure. In subsequent tests of ethanol self-administration conducted 1 week following withdrawal, the ethanol intake-reducing effects of N/OFQ emerged in postdependent rats, whereas nondependent controls rats remained less sensitive to the effects of N/OFQ (Fig. 2).

Further evidence of N/OFQ system dysregulation following chronic ethanol exposure has been obtained in tests of the effects of N/OFQ on anxiety-like behavior measured by the shock-probe defensive burying and elevated plus maze tests. In rats made dependent by repeated intragastric ethanol intubation and tested 1 week following ethanol withdrawal (Braconi et al., in press), N/OFQ dose-dependently modified the behavior of Wistar rats in a manner consistent with anxiolytic actions (Fig. 3A and B). These effects, however, emerged at lower doses in postdependent compared with nondependent animals (Fig. 3A and B), consistent with increased sensitivity to the anxiolytic action of N/OFQ in postdependent Wistar rats (Aujla et al., 2007). Notably, the anxiolytic effects of N/OFQ in these tests were more prominent in postdependent rats, whereas N/OFQ produced only small reductions in anxiety-like behavior in nondependent animals (see Fig. 3A and B for comparison). This scenario is similar to the anxiolytic effects of CRF antagonists (Valdez et al., 2002) and suggests that ethanol-induced neuroadaptations convey increased sensitivity to the actions of these neuropeptides. Indeed, in these studies, neuroadaptive changes in the N/OFQ system were evident in rats with a history of ethanol dependence, demonstrated by enhanced NOP gene expression in the BNST (Fig. 3C). A second line of evidence for ethanol-induced neuroadaptive changes in N/OFQ function comes from electrophysiological findings in CeA slice preparations in which N/OFQ decreased the facilitation of GABA_A neurotransmission by ethanol, and this effect was significantly increased in the CeA of ethanol-dependent rats (Roberto and Siggins, 2006). The upregulation of N/OFQ function in major stress-regulatory sites revealed by these studies is likely to be relevant for the increased sensitivity to the effects of N/OFQ on ethanol intake and anxiety-like behavior in postdependent rats, as well as for the reported reversal of ethanol withdrawal symptoms by N/OFQ (Stopponi et al., 2009), although this hypothesis remains to be confirmed.

4. Orexin/Hypocretin

Orexin A (hypocretin-1) and orexin B (hypocretin-2) are recently discovered hypothalamic neuropeptides that regulate a range of physiological processes, including feeding, energy metabolism (Willie et al., 2001), and arousal (Sutcliffe and de Lecea, 2002; Taheri et al., 2002). Two Orx/Hcrt receptors have been identified: Hcrt-r1 and Hcrt-r2. Major Orx/Hcrt neuron populations are found in the LH, a brain region long associated with reward and motivation (for review, see DiLeone et al., 2003), perifornical hypothalamus (PFA), and dorsomedial hypothalamus (DMH). Orx/Hcrt neurons in the DMH and PFA project to brain stem nuclei and have a major role in the regulation of arousal and modulation of stress responses (Baldo et al., 2003; Winsky-Sommerer et al., 2004).

Rapidly growing evidence indicates a possible central role of Orx/Hcrt neurons in the LH in drug addiction. These neurons project to the paraventricular thalamus (PVT), nucleus accumbens shell, ventral pallidum (VP), VTA, CeA, and BNST (Baldo et al., 2003; Peyron et al., 1998) and were originally implicated in the regulation of feeding behavior (Edwards et al.,

1999; Haynes et al., 2000; Haynes et al., 2002; Sakurai et al., 1998). Recent evidence, however, shows that Orx/Hcrt neurons in the LH play a significant role in the modulation of reward function and, particularly, drug-directed behavior (Harris et al., 2005).

4.1. Recruitment of the Orx/Hcrt system by drugs of abuse

Orx/Hcrt neurons in the LH become activated by stimuli associated not only with food but also morphine and cocaine reward (Harris et al., 2005). Similarly, the expression of conditioned place preference induced not only by food, but also morphine and cocaine, is associated with activation of Orx/Hcrt neurons in the LH (Harris et al., 2005). Consistent with these observations, intra-VTA microinjection of orexin-A produces renewal of morphine-induced conditioned place preference, whereas administration of the Hcrt-r1 antagonist SB334867 decreases the expression of morphine-induced conditioned place preference (Harris et al., 2005). SB334867 also blocks the acquisition of cocaine-induced behavioral sensitization and blocks the potentiation of excitatory currents by cocaine in VTA dopamine neurons (Borgland et al., 2006). Blockade of Hcrt-r1 decreases ethanol (Lawrence et al., 2006) and nicotine self-administration (Hollander et al., 2008), inhibits cue-induced reinstatement of ethanol (Lawrence et al., 2006) and cocaine seeking (Smith et al., 2009), and attenuates stress-induced reinstatement of cocaine (Boutrel et al., 2005) and ethanol seeking (Richards et al., 2008). Thus, behavioral and functional evidence indicates a role for Orx/Hcrt signaling in the neurobehavioral and motivational effects of cocaine and other drugs of abuse (Borgland et al., 2006; for review, see Bonci and Borgland, 2009).

4.2. Differential recruitment of the Orx/Hcrt system by drugs and natural rewards

Pharmacological manipulation of the Orx/Hcrt system is particularly effective in modifying the conditioned effects of drug cues in studies of conditioned place preference and reinstatement (Harris et al., 2005; Lawrence et al., 2006; Smith et al., 2009). Understanding whether Orx/Hcrt has a selective role in drug-seeking behavior or whether this role extends to motivated behavior in general is interesting. In a series of studies addressing this question, blockade of Hcrt-r1 receptors by SB334867 selectively reversed conditioned reinstatement induced by a stimulus conditioned to cocaine, without interfering with the effects of the same stimulus when conditioned to a highly palatable conventional reinforcer (Fig. 4). Moreover, although the stimuli conditioned to cocaine and the conventional reinforcer were equally effective in eliciting reinstatement (Fig. 5, *inset*), only the cue conditioned to cocaine recruited a larger number of Fos-positive Orx/Hcrt cells in the LH (Fig. 5). Thus, Orx/Hcrt neurons in the LH appear to preferentially regulate conditioned drug seeking compared with behavior motivated by a natural reward.

The pharmacological and neural mapping data above are somewhat difficult to reconcile with the role of the Orx/Hcrt system in behavior motivated by food (i.e., a natural reward) in addition to its more recently discovered role in drug reward. One hypothesis concerning the control of drug-seeking behavior is that neural circuits mediating the effects of drug cues are not specific to addiction-related events, but rather are “normal” circuits activated to a greater degree, thereby creating new motivational states or tilt processes that normally govern responding for natural rewards toward drug-directed behavior (Kelley and Berridge, 2002). Drugs of abuse may produce this effect by neuroadaptively altering neural systems that regulate “normal” motivation. Evidence of drug-induced dysregulation of the Orx/Hcrt system exists with alcohol. Prepro-orexin mRNA is upregulated in the LH of inbred alcohol-preferring (iP) rats following chronic ethanol consumption (Lawrence et al., 2006).

A possibility derived from this hypothesis is that the Orx/Hcrt system may, over the course of repeated drug use, acquire a preferential role in mediating the effects of stimuli conditioned to drugs of abuse *vs.* natural rewards. A major Orx/Hcrt projection exists from the LH/PFA to

the PVT (Parsons et al., 2006), and the PVT has been proposed to be a key relay gating Orx/Hcrt-coded reward-related communication between the LH/PFA and both the ventral and dorsal striatum (Kelley et al., 2005). Moreover, this “hypothalamic-thalamic-striatal axis” has been suggested to have evolved to prolong the central motivational state in the case of feeding beyond the fulfillment of immediate energy needs, thereby promoting the development of energy reserves for potential future food shortages (Kelley et al., 2005). Maladaptive recruitment of this system by drugs of abuse may “tilt” its function toward drug-directed behavior, which may explain the increased sensitivity of the Orx/Hcrt system to antagonist interference with drug-seeking behavior as opposed to behavior directed toward natural rewards.

A role for Orx/Hcrt projections from the LH to PVT in drug seeking is, in fact, strongly suggested by the finding that drug-related associated contextual cues activate these neurons (Dayas et al., 2008). Significantly larger numbers of Fos-positive hypothalamic Orx/Hcrt neurons were seen in rats exposed to contextual stimuli previously associated with ethanol availability than in rats exposed to the same stimulus previously paired with non-reward (Fig. 6). Moreover, presentation of the ethanol-related stimuli also increased the number of Fos-positive PVT neurons (Fig. 7), and these neurons were closely associated with Orx/Hcrt fibers (for additional details, see Dayas et al., 2008).

Further supporting a role for this projection in regulating drug-seeking behavior, recent data confirm that context-induced reinstatement of alcoholic beer seeking is associated with recruitment of a PVT-ventral striatum pathway (Hamlin et al., 2009). Furthermore, Orx/Hcrt injection into the PVT exerted a priming-like effect, reinstating extinguished cocaine-seeking behavior (Fig. 8).

Maladaptive recruitment of the Orx/Hcrt system by drugs of abuse is also suggested by findings describing neuroadaptive changes within the VTA. For example, voluntary cocaine and natural reward self-administration induces common, short-lasting, neuroadaptation in VTA dopaminergic neurons (i.e., increased glutamatergic function; Chen et al., 2008). This enhanced synaptic strength, however, is persistent and resistant to extinction only in rats self-administering cocaine and not in rats self-administering a non-drug reinforcer (Chen et al., 2008). Interestingly, several lines of evidence suggest that participation of the VTA in cocaine-induced neuronal and behavioral changes requires Orx/Hcrt inputs. For example, activation of Hcrt-r1 in the VTA is necessary for the development of cocaine-induced locomotor sensitization (Borgland et al., 2006), and Orx-A/Hcrt-1-mediated NMDA receptor plasticity in the VTA is increased in rats self-administering cocaine (Borgland et al., 2009). Additionally, short-lasting neuroadaptations in VTA dopaminergic neurons induced by high-fat chocolate food pellets have been described (Borgland et al., 2009), suggesting that the Hcrt/Orx-VTA system initially participates in the regulation of the motivation to obtain potent reinforcers in general (drug or highly palatable food). In contrast, drug-induced neuroadaptation of the Hcrt/Orx-VTA system is long-lasting, an effect that may be linked to the possible “tilting” of this system toward promoting and controlling drug-directed behavior.

5. CRF Interactions with N/OFQ and Orx/Hcrt Relevant for Drug Seeking

5.1. N/OFQ

Major interactions exist between CRF and N/OFQ in the CeA and BNST, brain regions with a pivotal role in the regulation of behavioral responses to stress. Using CRF-induced anorexia as a model, the anorectic effects of intracerebroventricular or intra-BNST CRF administration are reversed by microinjection of N/OFQ into the BNST but not the CeA or other CRF-rich brain regions, including the LC, ventromedial hypothalamus (VMH), paraventricular nucleus of the hypothalamus (PVN), or DR (Ciccocioppo et al., 2003b). Confirming this effect,

anorexia induced by intra-BNST administration of CRF in food-deprived rats was reversed by microinjection of N/OFQ into the BNST but not other CRF-rich sites (Ciccocioppo et al., 2003b). These findings suggest that N/OFQ acts as a functional CRF antagonist in the BNST, although the mechanism underlying this effect remains to be determined.

Evidence also supports interactions between N/OFQ and CRF in the CeA. In the alcohol-preferring msP rat line, administration of N/OFQ into the CeA, a key component of the extrahypothalamic CRF system, effectively reduces ethanol intake (Economidou et al., 2008). More direct evidence for N/OFQ-CRF interactions in the CeA comes from electrophysiological studies showing that CRF in the CeA, similar to ethanol, facilitates GABA release and increases GABA_A receptor-mediated inhibitory postsynaptic potentials (IPSPs), an effect blocked by N/OFQ (Roberto and Siggins, 2006; Cruz et al., 2009a; Cruz et al., 2009b). Furthermore, basal GABA release in CeA neurons of ethanol-dependent rats is elevated and N/OFQ-induced inhibition of IPSPs is increased, indicating enhanced sensitivity to N/OFQ. Additionally, the ability of CRF to increase IPSPs is augmented in CeA neurons of ethanol-dependent rats, suggesting that the interactive effects of N/OFQ and CRF on GABAergic transmission in the CeA undergo significant neuroadaptation during the development of ethanol dependence (Cruz et al., 2009a; Cruz et al., 2009b). Altogether, these findings suggest that N/OFQ reduces ethanol drinking (see section 3.3), partially via a functional CRF antagonist action observed in the extended amygdala. Although direct evidence for this possibility is still lacking, alcohol-preferring msP rats show substantial N/OFQ gene overexpression in the CeA and BNST and overexpression of the NOP receptor gene in the CeA and BLA. These findings tentatively confirm the hypothesis that msP rats exhibit an upregulation of the N/OFQ system in the CeA, BNST, and BLA. The upregulation of the N/OFQ system may represent a compensatory response to a concurrent innate upregulation of the extrahypothalamic CRF system (Hansson et al., 2006) that contributes to increased spontaneous ethanol intake in this line of rats (Economidou et al., 2008).

5.2. Orx/Hcrt

Emerging evidence suggests that the Orx/Hcrt system participates in regulating stress responses and may be an integral part of the brain stress system (Paneda et al., 2005). For example, Orx/Hcrt peptides have been shown to induce activation of the HPA axis, an effect that is prevented by α -helical CRF₉₋₄₁, a nonselective CRF antagonist (Jaszberenyi et al., 2000). CRF-immunoreactive terminals make direct synaptic contacts with hypocretin-expressing neurons in the LH, and numerous Orx/Hcrt neurons express CRF₁ and CRF₂ receptors, providing an anatomical basis for potential modulation of these neurons by CRF (Winsky-Sommerer et al., 2004). Furthermore, CRF and Orx/Hcrt appear to interact in regulating the effects of behavioral responses to stress, considering the evidence that acute restraint stress results in upregulation of orexin/hypocretin mRNA in the LH (Reyes et al., 2003) and both restraint and footshock stress induce *c-fos* expression in the perifornical region of the LH, an effect that is reduced in CRF₁ knockout mice (Winsky-Sommerer et al., 2004). Finally, the LH Orx/Hcrt system projects to the BNST, a CRF-rich brain region with a critical role in the control of stress responses and stress-induced drug seeking (Cummings et al., 1983; Erb et al., 2001), suggesting that CRF and Orx/Hcrt may interactively regulate behavioral responses to stress in the BNST. Consistent with this hypothesis are recent findings showing that SB334867 attenuates stress-induced reinstatement of cocaine-seeking (Boutrel et al., 2005) and that Orx-A/Hcrt-1-induced reinstatement of cocaine-seeking is reversed by intracerebroventricular administration of the CRF₁/CRF₂ antagonist *D*-Phe CRF₁₂₋₄₁ (Boutrel et al., 2005). Although unclear is whether *D*-Phe CRF₁₂₋₄₁ produced this effect in the BNST, this finding provides direct evidence for interactions between CRF and Orx/Hcrt relevant for drug-seeking behavior associated with stress. However, in a recent study (Wang et al., 2009), intra-VTA administration of the Hcrt-r1 antagonist SB408124 was not effective in reducing stress-induced reinstatement of cocaine

seeking, and intra-VTA injection of α -helical CRH₉₋₄₁ had no effect on either glutamate release, dopamine release, or cocaine seeking induced by intra-VTA infusion of Orx-A/Hcrt-1. These findings suggest that although CRF and Orx/Hcrt may interact to regulate drug-seeking behavior in other brain areas, such interactions do not occur in the VTA.

6. Conclusion/Perspectives

Neuroadaptive changes in the extrahypothalamic CRF system induced by chronic drug use represent a major factor in the maintenance of drug and alcohol dependence. Growing evidence suggests that the N/OFQ and Orx/Hcrt neuropeptide systems functionally interact with and regulate the extrahypothalamic CRF system. Understanding the precise functional role of these interactions will be an important area of future research and ultimately may offer a possible avenue for restoring “normal” CRF function following chronic drug abuse. Finally, recent findings have identified interactions between the Orx/Hcrt and N/OFQ systems (Xie et al., 2008). N/OFQ potently inhibits the activity of Orx/Hcrt neurons (Xie et al., 2008), leading to the hypothesis that N/OFQ also modulates Orx/Hcrt-modulated functions, including behavioral response to stress, anxiety, reward, and addiction. Investigation of these interactions will be an important focus of future research on stress-regulatory neuropeptidergic systems.

Abbreviations

CRF	corticotropin-releasing factor
Orx/Hcrt	orexin/hypocretin
N/OFQ	nociceptin/orphanin FQ
NOP	nociceptin/orphanin receptor

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Figure 1.

Reduced amygdala CRF₁, but not CRF₂, receptor binding in rats during acute ethanol withdrawal following chronic, intermittent ethanol vapor exposure. Panels show mean ($\pm$ SEM) specific CRF₁ and CRF₂ binding in the central nucleus of the amygdala (CeA), medial nucleus of the amygdala (MeA), and basolateral amygdala (BLA) determined by quantitative autoradiography. Total specific binding of the CRF₁/CRF₂ receptor radioligand $[^{125}\text{I}]\text{-Tyr}^0\text{-sauvagine}$ was defined using a saturating concentration of the subtype-nonselective peptide antagonist D-Phe CRF₁₂₋₄₁ (10 mM). CRF₁ vs. CRF₂ receptor binding was defined using the highly selective nonpeptide CRF₁ antagonist R121919, in which residual specific binding of $[^{125}\text{I}]\text{-Tyr}^0\text{-sauvagine}$ that was not displaced by R121919 (1 mM) was defined as CRF₂ binding, and R121919-sensitive binding was defined as CRF₁ binding. Autoradiography was performed on coronal brain sections (20 μm) obtained from male Wistar rats during acute withdrawal (8 h) from a 28 day intermittent ethanol vapor inhalation procedure ($n = 5$; 14 h on; 10 h off; target blood alcohol levels, 200–225 mg%) and from air-exposed control rats ($n = 5$). Optical densitometry was performed using ImageJ (NIH), and values were normalized to prefabricated autoradiography microscans (Amersham).

Figure 2.

Effects of N/OFQ on ethanol-reinforced responses in ethanol nondependent ($n = 7$) and postdependent (right panel: Dependent) Wistar rats ($n = 9$). Rats self-administered ethanol on a fixed-ratio schedule in 30 min sessions in which each reinforced response resulted in the delivery of 0.1 ml of 10% (w/v) ethanol. A mixed-factorial ANOVA did not reveal significant main effects of ethanol dependence history ($F_{1,14} = 0.6$, not significant) or an ethanol dependence history $\times$ dose interaction ($F_{3,42} = 0.2$, not significant). However, a main effect of dose was detected ($F_{3,42} = 4.0$, $p < 0.01$). Separate one-way ANOVAs revealed a significant effect of N/OFQ in postdependent ($F_{3,24} = 4.2$, $p < 0.01$) but not nondependent rats ($F_{3,18} = 0.9$, $p = 0.4$). For postdependent rats, Newman-Keuls *post hoc* tests revealed significant differences between vehicle- (0 µg) and N/OFQ-treated rats at all doses tested (* $p < 0.05$).

Figure 3.

Effects of N/OFQ on anxiety-like behavior in nondependent (**A**) and postdependent (**B**) Wistar rats measured in the elevated plus maze (% Open Arm Time, left y-axis) and defensive burying test (Burying Duration, right y-axis). Postdependent animals exhibited increased anxiety-like behavior measured by both the elevated plus maze and defensive burying test (N/OFQ, 0 μg). N/OFQ dose-dependently decreased anxiety-like behavior in both models of anxiety, with anxiolytic-like effects appearing at a lower dose (1 μg) in postdependent compared with nondependent rats. This observation is consistent with increased sensitivity to the anxiolytic action of N/OFQ in postdependent Wistar rats. For both behavioral tests, significant differences were found between the two groups (two-way ANOVA; elevated plus maze, $F_{1,68} = 9.9$, $p <$

0.01; defensive burying, $F_{1,68} = 7.4, p < 0.05$), with significant effects of N/OFQ dose (elevated plus maze, $F_{3,68} = 6.3, p < 0.001$; defensive burying, $F_{3,68} = 12.08, p < 0.001$). A group (dependent, postdependent) $\times$ dose interaction was also confirmed in the defensive burying test ($F_{3,68} = 3.1, p < 0.05$). $*p < 0.05$, $**p < 0.01$, compared with N/OFQ (0 μ g); $^+p < 0.05$, $^{+++}p < 0.001$, compared with nondependent ($n = 9$ –10 animals/group; Newman-Keuls *post hoc* test). (C) NOP receptor mRNA levels in the BNST of postdependent (Withdrawal) and nondependent (Vehicle) Wistar rats. Data were obtained using real-time reverse transcription polymerase chain reaction and compared with mRNA expression in experimentally naive age-matched controls (100%). $**p < 0.01$, compared with Vehicle; $^{++}p < 0.01$, compared with naive ($n = 6$ animals/group).

Figure 4.

Effects of the specific Hcrtr1 antagonist SB334867 (0–3 mg/kg, IP, 30 min before testing) on conditioned reinstatement induced by a cocaine (COC)-related stimulus (S⁺, left) or a stimulus conditioned to sweetened condensed milk (SCM; S⁺, right). These data provide preliminary evidence that blockade of Hcrtr1 reverses the motivational effects of cocaine-related environmental stimuli but not stimuli conditioned to a highly palatable conventional reinforcer. ++*p* < 0.01, compared with non-reward-related stimulus (S⁻); ***p* < 0.01, compared with 0 mg/kg (*n* = 6 animals/group).

Figure 5.

Recruitment of Orx/Hcrt neurons by a stimulus (S⁺) conditioned to cocaine (COC) but not sweetened condensed milk (SCM). Male Wistar rats were subjected to reinforcement contingencies in which responses at the active lever were differentially reinforced in the presence of a distinct discriminative stimuli S^D associated with reinforcers (cocaine or SCM) availability vs. non-availability. A constant 70 dB white noise served as a discriminative stimulus (S⁺) for availability of the reinforcer (cocaine or SCM), while illumination of a 2.8 W house light located at the top of the chamber's front panel served as a discriminative stimulus (S⁻) signaling non-availability of the reinforcer (i.e., saline solution instead of cocaine or no consequence instead of SCM). Sessions were initiated by extension of the levers into the chambers and concurrent onset of the respective S^D which remained present until termination of the session by retraction of the levers. In the presence of the S⁺, responses at the right, active lever were reinforced by cocaine or SCM on a fixed-ratio 1 schedule and, similar to training, were followed by a 20 s timeout period signaled by illumination of a cue light above the lever. In the presence of the S⁻, depression of the right active lever was followed by an intermittent

tone during which the lever remained inactive for 20 s. Three daily sessions (each lasting 1 h for the cocaine group and 20 min for the SCM group) separated by 30 min intervals were conducted, with two “reward” sessions and one “non-reward” session sequenced in random order. After 8 training days (i.e., a total of 16 “reward” and 8 “non-reward” sessions), both the cocaine and SCM groups were placed on extinction conditions in daily 30 min sessions during which the reinforcers and S^D were withheld until a criterion of ≤ 4 responses/session for 3 consecutive days was reached. Fos-positive (Fos⁺) Orx/Hcrt neurons in the lateral hypothalamus (LH) were then counted following COC or SCM S^+ presentation (reinstatement tests in which both the COC and SCM S^+ elicited robust recovery of responding; see inset) and compared with Fos-positive counts obtained following the final extinction (EXT) session. Fos-positive orexin neurons: $**p < 0.05$, compared with EXT; $+p < 0.05$, COC S^+ vs. SCM S^+ . **Inset:** Mean ($\pm$ SEM) number of responses during reinstatement tests in the presence of the COC S^+ or SCM S^+ . Both stimuli were equally effective in eliciting responding. $**p < 0.01$, compared with EXT ($n = 8$ animals/group).

Figure 6.

Mean ($\pm$ SEM) number of Fos-positive Orx/Hcrt cells in the perifornical/lateral hypothalamus (PF/LHA) (**D**) or dorsomedial hypothalamus (DMH) (**E**) in rats exposed to either the ethanol- (S^+) or non-reward-related stimulus (S^-). A significantly greater number of Fos-positive Orx/Hcrt cells was observed in animals exposed to the S^+ (vs. S^-) in both the PF/LHA and DMH. (**B** & **C**) Photomicrographs illustrating the effects of S^- or S^+ exposure on the number of Fos-positive Orx/Hcrt cells in the PF/LHA. (**A**) Schematic illustrating the rostro-caudal level from which photomicrographs (**B** & **C**) of PF/LHA tissue, immunolabeled for Fos-protein and Orx/Hcrt, were made. * $p < 0.05$, *** $p < 0.001$, compared with S^- . Scale bar = 20 μ m. Taken with permission from Dayas et al. (2008).

Figure 7.

(A) Schematic illustrating the rostro-caudal level from which photomicrographs of paraventricular thalamus (PVT) tissue were made. (B) Counts (mean ± SEM) of Fos-positive PVT cells were significantly increased in animals exposed to the ethanol S⁺ vs. S⁻ cue. ** $p < 0.001$, compared with S⁻. (C & D) Photomicrographs illustrating the effects of S⁻ or S⁺ exposure on the number of Fos-positive PVT neurons. Scale bar = 50 μm. Taken with permission from Dayas et al. (2008).

Figure 8.

Intra-PVT Orexin-A (ORX) injection reinstates extinguished cocaine-seeking behavior. Rats were first trained to intravenously self-administer cocaine (0.25 mg/infusion, fixed-ratio 1, timeout 20 s, signaled by the illumination of a cue light above the active lever) for 2 weeks. Following self-administration training (SA), the animals were placed on extinction conditions for 2 weeks. Extinction sessions were identical to self-administration sessions, including the signaled 20 s TO period, but cocaine was no longer available. Under extinction conditions, microinjection of orexin A (ORX, 150 pmole) into the PVT immediately before the test reinstated extinguished cocaine-seeking behavior. * $p < 0.05$, compared with Vehicle (Veh; $n = 6$ animals/group).