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Role of Corticotropin-Releasing Factor in Alcohol and Nicotine Addiction

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Abstract

According to the *Diagnostic and Statistical Manual of Mental Disorders*, 5th edition, substance use disorders (SUDs) consist of a problematic pattern of use that results in impairments in daily life and noticeable distress. The two most prevalent SUDs involve alcohol (alcohol use disorder) and nicotine (tobacco use disorder), which are often co-abused. According to the 2018 report by the Substance Abuse and Mental Health Services Administration, 26.7% of individuals 12 years of age and older used tobacco products in the past year, and 65.5% used alcohol. Chronic nicotine and alcohol use results in the dysregulation of brain regions that are involved in stress and emotional systems upon the initiation and cessation of use. Robust preclinical and translational evidence indicates that negative emotional states are key for the development of alcohol and nicotine addiction as subjects continue drug use to obtain relief from the negative emotional states of acute withdrawal and protracted abstinence. The present review explores the role of the neuropeptide corticotropin-releasing factor in the development of negative emotional states.

Corticotropin-Releasing Factor System Overview

Corticotropin-releasing factor (CRF) is a 41-amino-acid neuropeptide that is encoded by the *crh* gene, which was first discovered and characterized as a regulator of stress in 1981 by Vale and colleagues (Vale et al., 1981). CRF_1 and CRF_2 receptors and a family of related signaling peptides (urocortins - Ucn1, Ucn2, and Ucn3) soon emerged as mediators of negative emotional states that are associated with the stress response (Zhao et al., 1998). Corticotropin-releasing factor is expressed throughout the central nervous system, including the paraventricular nucleus of the hypothalamus (PVN) and regions of the extended amygdala, including the central nucleus of the amygdala (CeA), nucleus accumbens (NAc), and bed nucleus of the stria terminalis (BNST) (Figure 1). Corticotropin-releasing factor neurons are also found throughout cerebral cortices, the parasubthalamic nucleus (PSTN), the ventral tegmental area (VTA), the locus coeruleus (LC), the skin, and the gut (Dabrowska et al., 2016; Rodino-Janeiro et al., 2015). Corticotropin-releasing factor is regulated by CRF-binding protein (CRF-BP), a glycoprotein that regulates the extracellular availability of CRF to bind to its receptors (Behan et al., 1995; Ketchesin and Seasholtz, 2015; Seasholtz et al., 2002). Humans express polymorphisms of CRF and CRF-BP that can have significant downstream effects on responses to withdrawal and stress (Barr et al., 2009; Hansson et al., 2007; Ketchesin and Seasholtz, 2015; Ketchesin et al., 2017). We and others have hypothesized that the CRF system plays an important role in substance use disorders (SUDs) by increasing the motivation to use drugs through neuronal activation in the extended amygdala to produce anxiety-like behavior, shifts in reward salience, compulsive-like drug self-administration, and the stress-induced reinstatement of drug seeking (Haass-Koffler and Bartlett, 2012; Koob, 2008; Koob, 2009; Koob, 2010).

CRF_1 and CRF_2 receptors belong to the secretin-receptor (class B1) family subtype of the G protein-coupled receptor family. Each CRF receptor is encoded by distinct genes and has several

splice variants that are expressed in the central nervous system and peripheral tissues. CRF_1 and CRF_2 receptor expression has high overlap, with nearly 70% identical sequence conservation (Dautzenberg and Hauger, 2002). CRF_1 and CRF_2 receptor activation preferentially leads to initiation of the cyclic adenosine monophosphate second messenger pathway through the Gαs protein activation of protein kinase A. In the case of CRF_1, after stressful tasks which elicit high levels of CRF secretion, females have been shown to preferentially signal through Gαs leading to more CRF sensitivity, compared to males which preferentially signal via ß arrestin as shown in figure 2. (Hauger et al., 2006; Miguel et al., 2014; Van Pett et al., 2000). Other signaling pathways include activation of the extracellular signal-related kinase/mitogen-activated protein kinase cascade through β-arrestin (Punn et al., 2006), the Gαq activation of protein kinase C, and the AKT/protein kinase B pathway (Hauger et al., 2006) (Fig. 2). Despite high sequence conservation, CRF peptide has a 10-fold higher affinity for CRF_1 receptors relative to CRF_2 receptors (Perrin et al., 1995). CRF_1 and CRF_2 receptors are widely expressed in the central nervous system, with extensive overlap in expression, but distinct expression profiles are also found between brain regions that are involved in addiction-like behaviors, such as the CeA and BNST (Van Pett et al., 2000) (Fig. 1). These differences play an important role in the recruitment of downstream signaling pathways in SUDs. For a further in-depth review of intracellular signaling pathways, see Hauger et al. (Hauger et al., 2006).

Behavioral Effects of CRF and CRF Receptor Manipulations

Numerous studies have investigated the behavioral effects of manipulations of CRF and CRF_1 and CRF_2 receptors (Land et al., 2008; Zorrilla et al., 2013; Zorrilla et al., 2014). Table 1 presents a list of genetic and pharmacological manipulations of the CRF system with regard to stress

and anxiety-like behavior. The central administration of CRF peptide and CRF receptor agonists in rodent models mimics behavioral responses to stress (Britton et al., 1986a; Britton et al., 1986b; Dunn and Berridge, 1990; Sutton et al., 1982). In contrast, CRF_1 receptor knockout mice exhibit less anxiety-like behavior and a decrease in recruitment of the hypothalamic-pituitary-adrenal (HPA) axis despite having equal levels of stress hormones as control animals (Smith et al., 1998; Timpl et al., 1998). Similarly, CRF_1 receptor antagonists exert effects that are opposite to CRF, reducing both stress and anxiety-like behavior (Overstreet et al., 2004b; Zorrilla et al., 2002; Zorrilla and Koob, 2004). These alterations were demonstrated using various behavioral paradigms, including the elevated plus maze (Sahuque et al., 2006), forced swim test (Griebel, 2002), and measures of alcohol withdrawal (Funk et al., 2006; Gehlert et al., 2007) and nicotine withdrawal (Shaham and de Wit, 2016).

CRF_2 receptors are also expressed throughout the brain, including the olfactory bulb, the hippocampus, the amygdala, the septum, dorsal and median raphe nuclei, the cortex, the pituitary, and the spinal cord (Bittencourt and Sawchenko, 2000; Korosi et al., 2006; Van Pett et al., 2000). These regions are important for learning, memory, the stress response, reward, and pain, which is particularly relevant as a negative consequence of withdrawal (Kim et al., 2011). CRF_2 receptor knockout mice exhibit higher sensitivity to stress and an increase in anxiety-like behavior (Neufeld-Cohen et al., 2012). These knockout animals also exhibit the early termination of adrenocorticotropic hormone (ACTH) release rather than the delayed initiation of HPA recruitment, as is the case of CRF_1 receptor knockout animals (Coste et al., 2000). Double knockout of both CRF_1 and CRF_2 receptors significantly blunts the stress response, but such an effect was attributed largely to CRF_1 receptor loss. Priel et al. reported that CRF_2 receptors were unable to compensate for CRF_1 receptor

loss, suggesting that each CRF receptor subtype plays a different role in regulating the stress response (Preil et al., 2001).

Key Brain Regions Related to the CRF System

Corticotropin-releasing factor is widely distributed in the brain, and decreases or increases in CRF signaling in different brain regions can affect a wide range of behaviors. For example, the prefrontal cortex (PFC) is involved in impulse control, goal-directed behavior, and working memory (Devilbiss et al., 2016; Hupalo and Berridge, 2016; Kwako and Koob, 2017). This brain region also exhibits widespread functional and structural abnormalities in individuals with SUDs and sends downstream projections to regions that are involved in motivation, stress, mood, and impulsivity (Klenowski, 2018; Sesack et al., 1989; Zhang et al., 2012). The deletion of CRF_1 receptor signaling in the PFC ameliorates the acute stress-induced impairment of executive function, such as impulse control and decision making (Bryce and Floresco, 2016; George et al., 2012; Hupalo et al., 2019; Uribe-Marino et al., 2016). Another macrostructure that has high CRF expression is the extended amygdala (i.e., CeA, NAc, and BNST) (Alheid and Heimer, 1988; Heimer and Alheid, 1991). The extended amygdala is grouped based on functional connectivity and has been shown to play a prominent role in both fear and anxiety-like behavior, two integral aspects of the stress response (Davis et al., 2010). The CeA is composed predominantly of ɣ-aminobutyric acid (GABA)ergic projection neurons and interneurons (Sun et al., 1994). The lateral CeA sends inhibitory projections to the medial CeA and also projects to the BNST, which is also implicated in stress and addiction-like behaviors (Krettek and Price, 1978; Weller and Smith, 1982). The CeA is a major source of CRF in the BNST, but the BNST also expresses high levels of CRF in the lateral region (Sakanaka et al., 1986). The medial CeA projects to the periaqueductal gray (PAG), which is involved in

withdrawal-induced hyperalgesia (Avegno et al., 2018). The medial CeA also sends inhibitory projections to the hypothalamus, locus coeruleus, nucleus of the solitary tract, and pedunculopontine tegmental nucleus. The network connectivity within this region is positioned to regulate a wide range of behavioral processes, including the stress response and peripheral processes that are involved in somatic signs of withdrawal (Pitklnen A and D.G, 2000).

The function of CRF in the PVN is essential for activation of the HPA axis. Cells within the PVN secrete CRF, which in turn activates the anterior pituitary to produce ACTH (Smith and Vale, 2006; Vale et al., 1981). Adrenocorticotropic hormone then acts on adrenal cortical cells, resulting in the release of cortisol in humans and corticosterone in rodents (Herman et al., 2016). These hormones (cortisol/corticosterone) then activate glucocorticoid and mineralocorticoid receptors, which initiate intracellular responses to stress (Hauger et al., 2006). This pathway has been extensively characterized. The emergence of novel microcircuitry feedback in the PVN has been explored with regard to local CRF signaling in the PVN. Recent studies reported the presence of fast-responding microcircuitry that involves CRF and CRF_1-positive neurons in the PVN (Jiang et al., 2018b). These CRF_1-positive neurons make local GABAergic synapses on both magnocellular and parvocellular cells in the PVN and long-range glutamatergic synapses on cells in the nucleus of the solitary tract. Jiang et al. ablated CRF_1-positive neurons in the PVN and observed an increase in the quantity of corticosterone that was released in male rodents during stressful situations, and the duration of this elevation was longer than in intact animals (Jiang et al., 2018b). Therefore, the long-range secretion of CRF plays a role in the stress response, but local activation is also important (Herman et al., 2016; Jiang et al., 2018b).

Corticotropin-releasing factor plays a role in various behaviors that are downstream of the stress response. Stressors have been shown to elicit dopamine-dependent behaviors that are

associated with motivation and reward (Wanat et al., 2013). Dopaminergic neurons in the VTA are well known for their contribution to the rewarding effects of drugs of abuse. Downregulation of the mesolimbic dopamine system during withdrawal is common in SUDs and has been well studied in nicotine and cocaine use in both dependent and nondependent animals (Grieder et al., 2014). Intracerebral injections of CRF in the VTA promoted dopamine-dependent behaviors in drug-dependent animals, whereas drug-naive animals did not exhibit the same repression (Bryce and Floresco, 2016; Wanat et al., 2013). CRF function in the VTA and IPN plays an important role in dopamine signaling and motivated behavior. Both rodents and humans express a population of CRF neurons in the VTA that are recruited during withdrawal from nicotine after chronic exposure and contribute to dopaminergic adaptations that mediate negative motivational states that are elicited by nicotine withdrawal (Grieder et al., 2014). Chronic nicotine exposure has been shown to upregulate CRF mRNA expression in dopaminergic neurons in the posterior VTA, which can block CRF_1 receptors and nicotine-induced transient GABAergic inputs to those neurons. Grieder et al. reported that CRF peptide was depleted in the posterior VTA, IPN, and CeA but not in the anterior VTA or PVN during nicotine withdrawal (Grieder et al., 2014). Furthermore, Zhao-Shea et al. demonstrated that local signaling from the VTA to the IPN modulates glutamatergic inputs from the medial habenula, and that the pharmacological blockade of IPN CRF_1 positive neurons using small molecules, or optogenetic silencing of the medial habenular output to the IPN alleviated withdrawal-induced anxiety-like behavior (Zhao-Shea et al., 2015). These findings link the reward system and stress systems based on local circuitry within the VTA and IPN during withdrawal.

Modulation of Alcohol-Related Behaviors by CRF

Alcohol use disorder (AUD) is a chronic disorder that is characterized by the loss of control over alcohol intake, withdrawal symptoms upon the cessation of alcohol use, and relapse after periods of abstinence (Becker, 2008; Weiss, 2005). The abrupt cessation of chronic alcohol intake in humans and animal models is associated with negative mood, irritability, and somatic signs of withdrawal, many of which are at least partially mediated by CRF signaling (Kimbrough et al., 2017; Schuckit, 2009). Chronic alcohol use affects brain stress systems through modulation of the HPA axis and extended amygdala (Koob, 2009). However, chronic activation of the CRF system in the PVN and extended amygdala is known to have opposite effects. Chronic stress and chronic exposure to alcohol are associated with downregulation of the CRF system in the PVN, leading to hyporeactivity of the HPA axis and sensitization of the CRF system in the extended amygdala (Blaine et al., 2016; Breese and Knapp, 2016; Logrip et al., 2013; Piazza et al., 1993; Piazza and Le Moal, 1997; Rivier et al., 1984). Extensive pharmacological and genetic manipulations of the CRF system have been shown to play a fundamental role in alcohol-related behaviors. The section below focuses primarily on preclinical studies that explored the role of CRF in alcohol-related behaviors. Notably, however, small-molecule CRF receptor antagonists have been unsuccessful in the treatment of AUD in clinical trials (Kwako et al., 2015; Shaham and de Wit, 2016). For a further review of these trials, possible caveats, and alternative explanations (e.g., the role of other neuropeptides), see Gilpin, 2012 (Gilpin, 2012).

Manipulation of the CRF System and Related Behaviors in Alcohol Use

The role of CRF transmission and stress in alcohol-related behaviors has been extensively examined using neurobiological, pharmacological, and genetic models (Table 2). Preclinical evidence of the anxiogenic properties of CRF has been documented, and humans with AUD often

report that stress and anxiety are motivators for continued drinking (Ludwig and Wikler, 1974). High CRF1 receptor expression was reported to be associated with stress-induced alcohol intake in genetically selected Marchigian Sardinian (msP) alcohol-preferring rats (Hansson et al., 2007) and nongenetically selected animals in a post-dependent state (Sommer et al., 2008). Genetic knockout animals exhibit a wide range of reductions of drinking behavior, and CRF_1 knockouts as well as CRF_1/CRF_2 double knockouts exhibit the greatest behavioral effects (Kaur et al., 2012; Molander et al., 2012; Muglia et al., 1995; Pastor et al., 2008; Pastor et al., 2011; Preil et al., 2001; Smith et al., 1998).

During alcohol withdrawal, CRF release increases in the CeA and BNST in dependent rats (Funk et al., 2006; Olive et al., 2002). For example, Baldwin et al. injected CRF intracerebroventricularly (i.c.v.) and observed a reduction of open arm entries in the elevated plus maze during alcohol withdrawal, indicating an increase in anxiety-like behavior (Baldwin et al., 1991). Injections of CRF and CRF receptor agonists exacerbated alcohol withdrawal-induced stress (Bell et al., 1998; Huang et al., 2010), increased anxiety-like behavior in the elevated plus maze (Zhao et al., 2013), increased withdrawal-induced social anxiety (Overstreet et al., 2004b), and increased the footshock stress-induced reinstatement of alcohol seeking (Le et al., 2002).

Furthermore, the administration of CRF receptor antagonists has been shown to decrease alcohol drinking in alcohol-dependent animals but not in nondependent animals (Funk and Koob, 2007; Gilpin et al., 2008; Richardson et al., 2008). Bruijnzeel et al. showed that CRF receptor blockade with the nonselective CFR_1/CFR_2 receptor antagonist D-Phe CRF_{12-41} prevented elevations of brain reward thresholds that were associated with alcohol withdrawal (Bruijnzeel et al., 2010). Similarly, local intracerebral and i.c.v. injections of small-molecule $CRF1/CRF_2$ receptor antagonists

selectively blocked dependence-induced increases in alcohol self-administration during acute withdrawal (Valdez et al., 2003; Valdez et al., 2004). The CRF_1 receptor antagonists CRA-1000 and MPZP blocked the increase in alcohol intake in response to acute withdrawal-induced stress (Funk et al., 2006; Knapp et al., 2004; Richardson et al., 2008). Injections of the CRF_2 receptor agonist Ucn3 in the CeA similarly reduced the increase in alcohol self-administration that was associated with acute withdrawal (Funk et al., 2006; Funk and Koob, 2007; Sharpe and Phillips, 2009; Valdez et al., 2004). Thus, antagonism of the CRF system reduces alcohol drinking after acute withdrawal and protracted abstinence (Baldwin et al., 1991; Lodge and Lawrence, 2003; O'Callaghan et al., 2005; Rassnick et al., 1993). These results support the hypothesis that the dysregulation of CRF stress systems is only observed in alcohol-dependent individuals and not in nondependent or recreational drinkers (Le et al., 2000; Lowery and Thiele, 2010; Sarnyai et al., 2001; Shaham et al., 2000). For a further review of neurobiological and pharmacological differences between dependent and nondependent animals, see Vendruscolo et al. (Vendruscolo and Roberts, 2014).

Modulation of the CRF System and Anxiety/Stress during Alcohol Use

The blockade of CRF in the CeA has been shown to ameliorate elevations of anxiety-like behavior that is associated with alcohol withdrawal using both small molecules and cellular manipulations to inactivate CeA CRF neurons (de Guglielmo et al., 2016; Rassnick et al., 1993). Systemic (intraperitoneal [i.p.] or subcutaneous [s.c.]) or i.c.v. CRF receptor antagonist administration blocked anxiety-like responses to stressors during protracted abstinence (Albrechet-Souza et al., 2015; Breese et al., 2005; Valdez et al., 2003), whereas CRF receptor agonists increased alcohol self-administration that was associated with protracted abstinence (Funk et al., 2006; Valdez et al., 2003). Huang et al. reported that intracerebral injections of CRF in the CeA and BNST

increased the stress response during alcohol withdrawal (Huang et al., 2010). The lateral BNST contains CRF neurons (Cummings et al., 1983; Dabrowska et al., 2016; Morin et al., 1999; Sakanaka et al., 1986) and receives CRF-containing projections from the CeA (Sakanaka et al., 1986). Projections from the CeA to the BNST have also been shown to mediate anxiety-like behavior through the CRF activation of BNST CRF neurons (Pomrenze et al., 2019). The CRF system in the BNST has been shown to mediate the stress-induced reinstatement of drug-seeking behavior through both CRF_1 and CRF_2 receptors (Erb and Stewart, 1999). Functional studies found that the selective optogenetic stimulation of CRF_2-expressing neurons in the BNST ameliorated the adaptive stress response (Henckens et al., 2017). de Guglielmo et al. reported that the optogenetic inactivation of CRF-dependent amygdalofugal pathways in the BNST reversed addiction-like behaviors, including alcohol drinking and withdrawal signs, in alcohol-dependent rats (de Guglielmo et al., 2019). Corticotropin-releasing factor release increased in the CeA and BNST in alcohol-dependent rats (Funk et al., 2006; Olive et al., 2002), and CRF levels decreased after animals resumed drinking (Olive et al., 2002), likely through a negative reinforcement mechanism (i.e., the relief of withdrawal-induced stress). Direct pathways from the extended amygdala contribute to the stress response during alcohol use. The BNST gives rise to extensive projections to the PVN and hypothalamic nuclei (Alheid and Heimer, 1988; Herman et al., 1994), suggesting that increases in extracellular CRF levels at the level of the BNST may also contribute to HPA axis activation that is commonly observed during alcohol withdrawal (Tabakoff et al., 1978). Therefore, targeting regions throughout the CRF system beyond the CeA may be an alternative strategy for the treatment of AUD. For a list of manipulations of the CRF system in alcohol use, see Table 2.

Modulation of Nicotine-Related Behaviors by CRF

Tobacco use is highly addictive because of the presence of nicotine. Cigarette smoking remains the leading preventable cause of death in the United States (Samet, 2013). Stress has been shown to promote the use of cigarettes and other tobacco products. The rewarding and cognitive-enhancing effects of nicotine play an important role in the initiation of smoking (Bruijnzeel, 2012b; Rezvani and Levin, 2001). However, after the development of nicotine dependence, individuals continue to smoke to prevent dysphoria and anxiety that are associated with smoking cessation (Bruijnzeel and Gold, 2005). This is supported by the observation that most smokers relapse during the first week of abstinence when affective withdrawal signs are most severe. Relapse rates have been shown to be higher in individuals with depression (Hughes et al., 2004; Jarvis, 2004), and smokers report that stress is the primary reason for relapse during protracted abstinence (Breese et al., 2005; Heilig et al., 2010). Similar to stressors and alcohol, acute nicotine activates the HPA axis, but its impact on behavior depends on the dose and time of administration. For example, low doses of nicotine promote antidepressant effects and anxiolytic states (Andreasen et al., 2008; Balerio et al., 2005; Plaza-Zabala et al., 2010; Varani et al., 2012), whereas chronic exposure and high doses of nicotine have been shown to increase anxiety-like behavior, produce conditioned place aversion, and recruit CRF PVN neurons (File et al., 1998; Yu et al., 2008). Below is a discussion of the role of CRF in nicotine-related behaviors. For a comprehensive list of modulations of the CRF system in nicotine-related behaviors, see Table 3.

Nicotine, CRF, and Anxiety

Anxiety-like behaviors are commonly seen during withdrawal and protracted abstinence from nicotine. The role of CRF in mediating anxiety-like behavior has been shown to be specific to conditioned rather than unconditioned anxiety, indicating that CRF may be involved in negative

reinforcement processes that lead to excessive use rather than the simple expression of withdrawal symptoms (Tucci et al., 2003). Manipulations of the CRF system in animal models of nicotine addiction have elucidated roles of both the peptide and its receptors in anxiety- and depression-like behaviors (Bruijnzeel, 2012a; Molas et al., 2017). Grieder et al. reported that nicotine dependence increased CRF mRNA expression in the posterior VTA, measured by *in situ* hybridization (Cohen et al., 2015; Grieder et al., 2014; Slawecki et al., 2005). They found that CRF_1 receptor blockade in the VTA prevented anxiety-like behavior during nicotine withdrawal (Grieder et al., 2014), and the upregulation of CRF in the VTA–interpeduncular nucleus–medial habenula circuit was involved in the increase in anxiety-like behavior during withdrawal (Zhao-Shea et al., 2015). George et al. found that abstinence from nicotine dependence increased anxiety-like behavior and led to greater nicotine intake after abstinence, and CRF_1 receptor antagonism prevented this behavior (George et al., 2007). Interestingly, CRF_1 and CRF_2 receptors have opposing effects on anxiety-like behavior. Selective CRF_1 receptor antagonism reduced these behaviors (Grieder et al., 2014; Tunstall and Carmack, 2016; Zhao-Shea et al., 2015), whereas selective CRF_2 receptor agonism ameliorated negative affective states during chronic nicotine exposure and withdrawal symptoms in mice (Bagosi et al., 2016). Brain regions that have been shown to be involved in mediating this behavior include the VTA, NAc, amygdala, and hypothalamus (Grieder et al., 2014; Torres et al., 2015; Yu et al., 2008). Torres et al. also reported higher CRF levels in the NAc, amygdala, and hypothalamus in female rats than in male rats during nicotine withdrawal, although males had higher levels of corticosterone (Torres et al., 2013; Torres et al., 2015). Increases in CRF levels in the hippocampus have also been shown to mediate anxiety-like behavior following nicotine dependence (Slawecki et al., 2005). Altogether, CRF-mediated anxiety is a large contributor to nicotine dependence and perpetuates

nicotine use through negative emotional states. Further dissection of the circuitry and signaling may contribute to the development of novel therapies for tobacco use disorder.

Dysphoria

Dysphoria and other depressive-like symptoms are characterized in rodents primarily by measuring brain reward thresholds using intracranial self-stimulation. Animal studies that have sought to elucidate the role of CRF in nicotine-induced dysphoria have reported a wide range of results. Bruijnzeel et al. found that the dysphoric state that was associated with nicotine withdrawal was at least partially mediated by high CRF levels in the CeA. Selective CRF_1 receptor antagonism in the CeA attenuated dysphoria-like behaviors during nicotine withdrawal (Bruijnzeel et al., 2009). Furthermore, a recent study reported that CRF receptor blockade in the CeA but not BNST prevented elevations of brain reward thresholds that were associated with nicotine withdrawal (Marcinkiewcz et al., 2009). However, Qi et al. found that CRF overexpression in the CeA diminished the dysphoric-like state that was associated with nicotine withdrawal (Qi et al., 2014). These conflicting results may be explained by differential neuroadaptive changes in CRF_1 and CRF_2 receptor gene expression. In both studies, the authors found that the ratio of CRF_1 and CRF_2 expression was altered, in which CRF overexpression decreased CRF_1 receptor expression and increased CRF_2 receptor expression. Another possibility is that other mechanisms may exert compensatory effects, especially in the overexpression study by Qi et al., in which the alterations were longer lasting. Overall, these data support other findings that CRF_2 receptor agonism or overexpression reduces anxiety-like behavior during withdrawal. Nonetheless, throughout the extended amygdala, CRF overexpression has a wide array of effects. For example, another study by

Qi et al. showed that CRF overexpression in the BNST reduced dysphoric-like symptoms in rats after mecamylamine-precipitated nicotine withdrawal (Qi et al., 2016). Further research needs to be conducted to more conclusively elucidate the role of CRF in regulating nicotine-associated dysphoria.

Stress-Induced Reinstatement

Stress-induced reinstatement is usually tested in rodent models following a specific period of abstinence from nicotine. Stress is usually induced by footshock or the pharmacological stressor yohimbine. Corticotropin-releasing factor has been shown to mediate footshock-induced reinstatement in nicotine-dependent animals (Plaza-Zabala et al., 2010). Zislis et al. showed that the footshock-induced reinstatement of nicotine seeking decreased after i.c.v. administration of the nonselective CRF receptor antagonist D-Phe CRF_{12-41} (Zislis et al., 2007). Another study by Bruijnzeel et al. reported similar results with regard to the reinstatement of nicotine seeking using a selective CRF_1 receptor antagonist, but these effects were not seen with a selective CRF_2 receptor antagonist (Bruijnzeel et al., 2009), suggesting that CRF_1 receptors mediate the stress-related reinstatement of nicotine use. Brain regions that are involved in the regulation of stress-induced reinstatement primarily include the extended amygdala (CeA, NAc, and BNST). The BNST is involved in the CRF-mediated stress-induced reinstatement of cocaine seeking (Erb and Stewart, 1999; Vranjkovic et al., 2014). More specifically, a CeA-to-BNST pathway contains CRF that mediates the stress-induced reinstatement of cocaine seeking (Erb et al., 2001). However, unclear is whether such a pathway also mediates the reinstatement of nicotine seeking. The basolateral amygdala has been shown to project to the BNST and regulate CRF-mediated fear- and stress-related behaviors (Walker and Davis, 2008). One possibility is that the basolateral amygdala also plays a

role in the stress-induced reinstatement of nicotine seeking. Overall, there is notable consistency in findings that CRF_1 receptor antagonism reduces behaviors that are related to negative affect, including anxiety-like behavior, dysphoria-like behavior, and stress-induced relapse, especially during nicotine withdrawal.

Sex Differences in the Role of CRF in Alcohol and Nicotine Addiction

Nicotine and alcohol are regularly co-abused. Chronic exposure to both substances activates extrahypothalamic CRF systems. Strong evidence indicates that individuals with AUD are more likely to smoke and *vice versa* (Britt and Bonci, 2013; DiFranza and Guerrera, 1990; Doyon et al., 2013). Furthermore, Leao et al. found that nicotine use facilitated the transition to alcohol dependence (Leao et al., 2015), further establishing the existence of cross-talk between the mechanisms that underlie nicotine- and alcohol-related behaviors. Interestingly, AUD and tobacco use disorder appear to be differentially regulated in males and females (Agabio et al., 2017; Ceylan-Isik et al., 2010; Pogun et al., 2017). Overall, men have a higher rate of substance abuse (Administration, 2017), but women are more likely to use drugs in the context of stress-related drug use and have more difficulty quitting (Pogun and Yararbas, 2009; Schulte et al., 2009).

Men have a greater rate of alcohol intake than women. According to the United States Centers for Disease Control and Prevention, men are twice as likely to binge drink than women, and approximately double the percentage of men *vs*. women met the criteria for AUD in 2016 (Naimi et al., 2003; Nolen-Hoeksema, 2004). However, the number of women with AUD is increasing quickly compared with men (Grant et al., 2017; Slade et al., 2016). Therefore, understanding possible sex differences in the CRF system that contribute to AUD is important. Downstream of the HPA axis, corticosterone levels are higher in female rodents than in males, and more CRF-positive cells are

recruited to the PVN in females than in males (Figueiredo et al., 2007; Kudielka and Kirschbaum, 2005; Schreiber and Gilpin, 2018; Silva and Madeira, 2012). Female rodents also exhibit more rewarding effects of alcohol, a phenomenon that has been shown to be hormone-dependent. Ovariectomized females do not exhibit such higher rewarding effects of alcohol. Furthermore, estradiol administration has been shown to increase alcohol consumption in female rodents (Ford et al., 2002; Quirarte et al., 2007). Female rats have also been shown to have higher corticosterone levels and exhibit greater sensitivity to the yohimbine-induced reinstatement of alcohol seeking (Bertholomey et al., 2016). These findings corroborate data that show that women are more likely than men to have comorbid AUD and anxiety disorders (Kessler et al., 1997).

With regard to nicotine, the gender gap is closing, in which female rates of smoking, health effects, cessation, and relapse are nearing historical rates that are seen in men. Clinical studies found that the degree to which stress promotes tobacco use is greater in women than in men. Women are more likely to smoke to alleviate negative affective states and regulate mood compared with men (Mykletun et al., 2008; Perkins et al., 1999; Perkins et al., 2013). Female rodents also present greater symptoms and behaviors that are associated with nicotine addiction, including higher nicotine preference, higher anxiety-like behavior, and higher levels of plasma corticosterone and stress-related gene expression. Torres et al. reported sex differences in the role of CRF in nicotine intake, in which CRF enhanced the reinforcing effects of nicotine in females but not in males or ovariectomized females (Uribe et al., 2019). Intact females also exhibited increases in measures of anxiety compared with controls and decreases in CRF_1 receptor, CRF_2 receptor, dopamine D_3 receptor, and estrogen receptor 2 transcripts and an increase in CRF-BP transcripts in the NAc compared with ovariectomized females during nicotine exposure (Torres et al., 2015). Interestingly, estradiol has been shown to increase the rewarding effects of nicotine, which could explain why

females have greater nicotine intake compared with males (Flores et al., 2016). These findings illustrate the importance of ovarian hormones in sex differences in the stress response and nicotine use. However, different phases of the estrous cycle, which causes fluctuations of female hormone levels, did not influence nicotine self-administration in females, in which females still exhibited higher intake than males across the estrous cycle (Perkins et al., 1999). Such sex differences may be related to organizational changes in the brain that occur during adolescence (Vigil et al., 2016). This is relevant to human studies because women are more likely to relapse and maintain nicotine use (Perkins et al., 1999; Smith et al., 2016; Torres and O'Dell, 2016).

Conclusions

The present review discussed the role of the CRF system in behaviors that are associated with AUD and tobacco use disorder in preclinical models. The majority of brain CRF system-modulated behaviors occur during drug abstinence/withdrawal, including anxiety-like behavior, dysphoria-like behavior, cognitive impairments, and stress-induced relapse. These behavioral effects have been attributed to actions of CRF on CRF_1 and CRF_2 receptors within a distributed network of brain regions, including the PFC, PVN, CeA, BNST, VTA, and IPN. These studies indicate that the recruitment of a distributed brain CRF stress system may provide redundant circuits that promote negative affective states during alcohol and nicotine withdrawal. Further studies are needed to identify specific manipulations of these circuits than can decrease alcohol and nicotine use while maintaining a focus on sexually dimorphic stress-related systems in SUDs.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Table 1. Small-molecule and genetic manipulations of the CRF system in stress, anxiety, dysphoria.

Target	Manipulation	Basal	Stress	Anxiety	Dysphoria	Other Behaviors	References
CRF	Agonist (i.c.v.)	↑	↑	↑	↑	Learning/memory Feeding Fear Reproduction Shock-induced freezing	(Cullen et al., 2001) (Land et al., 2008) (Sherman and Kalin, 1988)
CRF_1 receptor	Antagonist (i.c.v.)	↓	↓	↓	↓	Depression Feeding	(Swerdlow et al., 1989) (Heinrichs et al., 1992) (Arborelius et al., 1999) (Zorrilla et al., 2002)
CRF_2 receptor	Antagonist (i.c.v.)	↑/↓	↑/↓	↑/↓	↓	Feeding	(Cullen et al., 2001) (Pelleymounter et al., 2000) (Takahashi, 2001) (Takahashi et al., 2001) (Land et al., 2008)
CRF_1 receptor	Genetic KO	↓	↓	↓	↓	Learning/memory Alcohol addiction Feeding	(Smith et al., 1998) (Timpl et al., 1998) (Sillaber et al., 2002) (Bradbury et al., 2000)
CRF_2 receptor	Genetic KO	—	↑	↑	—	Depression Feeding	(Bale et al., 2000) (Coste et al., 2000) (Kishimoto et al., 2000)
CRF_1/CRF_2	Genetic KO	↓	↓	↓♀ ↑♂	—	Grooming Sexually dysmorphic anxiety	(Bale et al., 2002) (Preil et al., 2001)
CRF	Genetic KO	↓	↓	—	—	Grooming, feeding	(Muglia et al., 1995)
CRF	Genetic overexpression	↑	↓	↑	—	Reproduction	(Stenzel-Poore et al., 1992) (Groenink et al., 2002)
CRF-BP	Genetic KO	—	—	↑	—	Weight gain Maternal aggression	(Karolyi et al., 1999) (Gammie et al., 2008)
CRF-BP	Genetic overexpression	—	↓	↑	—	Feeding Locomotor activity	(Lovejoy et al., 1998) (Burrows et al., 1998) (Stinnett et al., 2015)

CRF_2 receptor	Optogenetic activation	—	↓	↓	↓	Impaired fear memory	(Anthony et al., 2014) (Henckens et al., 2017)
CRF_1 receptor	Genetic KO	↑	↑	↑	—	Increased cortisol Slower recovery from stress	(Jiang et al., 2018a)
CRF	Viral overexpression	↑	↑	↑	↑	Startle Depression	(Keen-Rhinehart et al., 2009)

KO, knockout; i.c.v., intracerebroventricular; i.p., intraperitoneal. Adapted and updated from Vale and Bale (Bale and Vale, 2004).

Table 2. Small-molecule and genetic manipulations of the CRF system in alcohol use disorder.

Target	Manipulation	Result	Reference
CRF	Genetic KO	KO mice consumed more alcohol than WT	(Olive et al., 2002)
CRF_1 receptor	Genetic KO	KO mice did not exhibit increased alcohol self-administration during withdrawal	(Chu et al., 2007)
		KO mice exhibited decreased HPA recruitment, measured by corticosterone	(Pastor et al., 2008)
		KO mice exhibited increased alcohol intake, increased withdrawal-included alcohol self-administration	(Molander et al., 2012)
CRF_2 receptor	Genetic KO	Alcohol intake was slightly reduced	(Sharpe et al., 2005)
		When stressed, KO animals consumed more alcohol than WT	(Sillaber et al., 2002)
		Alcohol intake was slightly reduced, but reduction was not maintained throughout the study	(Kaur et al., 2012)
CRF_1/CRF_2 receptor	Genetic KO	KO mice did not exhibit alcohol-induced locomotor sensitization but exhibited decreased HPA axis recruitment, measured by corticosterone in response to alcohol intake	(Pastor et al., 2008)
		Repeated forced swim test increased alcohol intake	(Pastor et al., 2011)
CRF-BP	Genetic KO	No change in alcohol intake in mouse DID paradigm	(Ketchesin and Seasholtz, 2015)
CRF	Genetic overexpression	KO mice consumed less alcohol than WT	(Palmer et al., 2004)
CRF_1/CRF_2 receptor	Agonist	Decreased alcohol intake during two-bottle choice (i.c.v.)	(Bell et al., 1998)
		Reduced alcohol intake in dependent vapor-exposed rats (i.c.v.)	(Thorsell et al., 2005)
		CRF exacerbated withdrawal-induced stress, measured by social interaction (i.c.v., intra-CeA, intra-BNST)	(Huang et al., 2010)

		Alcohol-withdrawn animals spent less time on the open arms of the elevated plus maze, indicating increased stress response (i.c.v.)	(Zhao et al., 2013)
		Reduced social interaction as a measure of withdrawal-induced anxiety (i.c.v.)	(Overstreet et al., 2004b)
		Induced alcohol reinstatement (i.c.v., intra-MRN)	(Le et al., 2002)
CRF_1/CRF_2 receptor	Antagonist (D-Phe $CRF_{12/41}$)	Anxiety reduced (i.c.v.)	(Valdez et al., 2003)
		Decreased footshock-induced reinstatement (i.c.v.)	(Liu and Weiss, 2003)
		Decreased alcohol-seeking behavior in dependent rats (intra-CeA)	(Funk et al., 2006)
		Decreased alcohol withdrawal-induced alcohol self-administration (intra-CeA)	(Finn et al., 2007)
		Lower elevation of brain reward thresholds during alcohol withdrawal (i.c.v.)	(Bruijnzeel et al., 2010)
CRF_1 receptor	Antagonist (MTIP)	Decreased anxiety-like behavior in elevated plus maze during acute alcohol withdrawal (i.p.)	(Gehlert et al., 2007)
CRF_2 receptor	Agonist (Ucn3)	Decreased alcohol intake after escalation (i.c.v.)	(Sharpe and Phillips, 2009) (Valdez et al., 2004)
		Increased drinking in control animals (intra-CeA)	(Funk et al., 2007)
CRF_2 receptor	Antagonist (astressin-2B)	Decreased alcohol intake (intra-VTA) No effect (intra-CeA)	(Albrechet-Souza et al., 2015)
CRF-BP	Antagonist ($CRF_{[6-33]}$)		
CRF_1/CRF_2 receptor	Antagonist (α-helical CRF)	Decreased anxiety-like behavior in elevated plus maze during acute alcohol withdrawal (i.c.v.)	(Baldwin et al., 1991)
		No change in anxiogenic-like effect of acute alcohol withdrawal (i.c.v.)	(Rassnick et al., 1993)
		Increased alcohol preference in low-alcohol-preferring mice but not high-alcohol-preferring mice (i.c.v.)	(O'Callaghan et al., 2005)
		Decreased alcohol intake in DID paradigm (i.c.v.)	(Lowery and Thiele, 2010)
		Decreased anxiety-like behavior in elevated plus maze during acute alcohol withdrawal (intra-CeA)	(Rassnick et al., 1993)
CRF_1 receptor	Antagonist (LWH-63)	Decreased withdrawal-induced alcohol self-administration (s.c.)	(Sabino et al., 2006)
		Decreased alcohol intake and blood alcohol levels (s.c.)	(Lowery-Gionta et al., 2012)
CRF_1 receptor	Antagonist (antalarmin)	Decreased acquisition of alcohol drinking and alcohol consumption (i.p.)	(Lodge and Lawrence, 2003)
		Decreased alcohol self-administration and blocked footshock-induced reinstatement in Marchigian Sardinian alcohol-preferring rats (i.p.)	(Hansson et al., 2006)
		Decreased withdrawal-induced alcohol self-administration (i.p.)	(Chu et al., 2007)
		Decreased withdrawal-induced alcohol self-administration in dependent but not nondependent rats (i.p.)	(Funk et al., 2007)

		Decreased stress (yohimbine)-induced alcohol self-administration (i.p.)	(Marinelli et al., 2007) (Yang et al., 2008) (Ayanwuyi et al., 2013)
CRF_1 receptor	Antagonist (CP-154,526)	Decreased alcohol withdrawal-induced stress, measured by social interaction (i.p.)	(Overstreet et al., 2004b) (Overstreet et al., 2004a) (Breese et al., 2005) (Wills et al., 2009)
		Decreased swim stress-induced alcohol intake in mice (i.p.)	(Lowery et al., 2008)
CRF_1 receptor	Antagonist (CP-37,395)	Decreased alcohol intake (i.p.)	(Giardino and Ryabinin, 2013) (Simms et al., 2014)
CRF_1 receptor	Antagonist (CRA-1000)	Decreased alcohol withdrawal-induced stress, measured by social interaction (i.p.)	(Knapp et al., 2004)
CRF_1 receptor	Antagonist (MJL-1-109-2)	Decreased withdrawal-induced alcohol self-administration in dependent but not nondependent rats (i.p.)	(Funk et al., 2007)
CRF_1 receptor	Antagonist (MPZP)	Decreased withdrawal-induced alcohol self-administration in dependent but not nondependent rats (i.p.)	(Gilpin et al., 2008)
		Decreased withdrawal-induced alcohol self-administration in dependent but not nondependent rats (s.c.)	(Richardson et al., 2008)
CRF-BP	Antagonist ($CRF_{[6-33]}$)	Intra-VTA but not intra-CeA injections decreased alcohol intake	(Albrechet-Souza et al., 2015)

BP, binding protein; KO, knockout; WT, wildtype; CeA, central nucleus of the amygdala; BNST, bed nucleus of the stria terminalis; VTA, ventral tegmental area; DID, drinking-in-the-dark; HPA, hypothalamic-pituitary-adrenal; MRN, median raphe nucleus.

Table 3. Small-molecule manipulations of the CRF system in nicotine use.

Target	Drug/compound	Result	Reference
CRF	Viral overexpression (AAV2/5-CRF)	Increased CRF_2/CRF_1 gene expression ratio and decreased dysphoria during nicotine withdrawal (bilateral intra-VNST)	(Qi et al., 2016)
		Increased nicotine self-administration and CRF_1/CRF_2 gene expression in females compared with males (bilateral intra-NAc)	(Uribe et al., 2019)
CRF_1 receptor	Antagonist (R278995/CRA0450)	Prevented elevation of brain reward thresholds in withdrawal- and stress-induced reinstatement of nicotine seeking (i.c.v.)	(Bruijnzeel et al., 2009)
		Prevented elevation of brain reward thresholds during withdrawal (bilateral intra-CeA)	(Bruijnzeel, 2012b)
CRF_1 receptor	Antagonist (R121919)	Decreased thermal hyperalgesia during nicotine withdrawal and reinstatement of nicotine seeking (s.c.)	(Baiamonte et al., 2014)

CRF_1 receptor	Antagonist (MPZP)	Prevented nociceptive hypersensitivity and increased nicotine intake following abstinence (bilateral intra-CeA)	(Cohen et al., 2015)
		Prevented aversive effects of nicotine withdrawal and limited escalation of intake (bilateral intra-VTA)	(Grieder et al., 2014)
		Blocked anxiety-like behavior during withdrawal and decreased nicotine self-administration (s.c.)	(George et al., 2007)
CRF_1 receptor	Antagonist (antalarmin)	Alleviated anxiety-like behavior and decreased neuronal activation (intra-IPN)	(Zhao-Shea et al., 2015)
CRF_1/CRF_2 receptor	Antagonist (D-Phe CRF_{12-41})	Decreased stress-induced reinstatement of nicotine seeking (i.c.v.)	(Zislis et al., 2007)
		Prevented elevation of brain reward thresholds during nicotine withdrawal (i.c.v.)	(Bruijnzeel et al., 2007)
		Intra-NAc and intra-CeA but not intra-BNST administration prevented withdrawal-induced elevation of brain reward thresholds	(Marcinkiewcz et al., 2009)
CRF_2 receptor	Agonist (UCN2, UCN3)	Decreased anxiety-like behavior following acute nicotine withdrawal (i.c.v.)	(Bagosi et al., 2016)
CRF_2 receptor	Antagonist (astressin-2B)	Did not prevent elevation of brain reward thresholds or stress-induced reinstatement of nicotine seeking (i.c.v.)	(Bruijnzeel et al., 2009)
CRF-BP	Antagonist ($CRF_{[6-33]}$)	Blunted weight gain during nicotine withdrawal (i.c.v.)	(Heinrichs et al., 1996)

BP, binding protein; BNST, bed nucleus of the stria terminalis; NAc, nucleus accumbens; IPN, interpeduncular nucleus.

Figure Legend

Figure 1. **Distribution of corticotropin-releasing factor (CRF) and its receptors, CRF_1 and CRF_2 in the rat brain.** Corticotropin-releasing factor-positive regions are shown in green. CRF_1 receptor-positive regions are shown in blue. CRF_2 receptor-positive regions are shown in pink. A1, auditory cortex 1; A5, auditory cortex 5; Arc, arcuate nucleus; BAR, Barrington's nucleus; BLA, basolateral amygdala; BNST, bed nucleus of the stria terminalis; CeA, central nucleus of the amygdala; CB, cerebellum; CingCx, cingulate cortex; CoA, cortical amygdala; CPu, caudate putamen; DMH, dorsomedial hypothalamus; GPe, globus pallidus; Hip, hippocampus; IC, inferior colliculus; IO, inferior olive; IPN, interpeduncular nucleus; LC, locus coeruleus; LDTg, laterodorsal tegmental nucleus; LHA, lateral hypothalamus; LS, lateral septum; MeA, medial amygdala; MGN, medial geniculate nucleus; MV, medial vestibular nucleus]; NAc, nucleus accumbens; NTS, nucleus of the solitary tract; OB, olfactory bulb; OccCx, occipital cortex; PAG, periaqueductal gray; ParCx, parietal cortex; PBn, parabrachial nucleus; PFC, prefrontal cortex; PG, pontine gray; Pir, piriform cortex; PPTg, pedunculopontine tegmental nucleus; PVN, paraventricular nucleus of the hypothalamus; R, red nucleus; RN, raphe nucleus; RTN, reticular thalamic nucleus; SC, superior colliculus; SNr, substantia nigra; SON, supraoptic nucleus; SP5n, spinal trigeminus nucleus; VMH, ventromedial hypothalamus; VTA, ventral tegmental area. Adapted from Henckens et al. (Henckens et al., 2016) and Valadas et al. (Valadas et al., 2012).

Figure 2. Intracellular signaling pathways for CRF_1 and CRF_2. CRF and CRF-BP as well as related urocortins (Ucn1, Ucn2, Ucn2) signaling is depicted. Thick lines denote preferential signaling / higher binding affinity, while dotted lines represent less preferential signaling / lower

binding affinity. CRF_1 exhibits differences in sex with females preferentially signaling through GαS in situations with excess CRF, while males preferentially signal through β-arrestin 2. CRF-BP is capable of interacting with CRF and Ucn1. CRF, corticotrophin-releasing factor; CRF-BP, corticotrophin-releasing factor binding protein; Ucn1, urocortin1; Ucn2 urocortin2; Ucn3, urocortin3; PLC, phospholipase C; IP3, inositol triphosphate; Ca+, calcium; PKC, protein kinase C; GαS, Gs protein alpha subunit; PKA, protein kinase A; β-arrestin, beta-arrestin 2; ERK, extracellular signal regulated-kinase; MAPK, mitogen activated protein kinase; Rho, Rho GTPase; SRC, Src kinase.