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Corticotropin-Releasing Factor (CRF) Neurocircuitry and Neuropharmacology in Alcohol Drinking

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Abstract

Alcohol use is pervasive in the United States. In the transition from non-hazardous drinking to hazardous drinking and alcohol use disorder, neuroadaptations occur within brain reward and brain stress systems. One brain signaling system that has received much attention in animal models of excessive alcohol drinking and alcohol dependence is corticotropin-releasing factor (CRF). The CRF system is composed of CRF, the urocortins, CRF-binding protein, and two receptors – CRF-type 1 and CRF-type 2. This review summarizes how acute, binge, and chronic alcohol dysregulate CRF signaling in hypothalamic and extra-hypothalamic brain regions, and how this dysregulation may contribute to changes in alcohol reinforcement, excessive alcohol consumption, symptoms of negative affect during withdrawal, and alcohol relapse. In addition, it summarizes clinical work examining CRF-type 1 receptor antagonists in humans and discusses why the brain CRF system is still relevant in alcohol research.

Keywords

Corticotropin-releasing factor; Alcohol Use Disorder; Binge Drinking; Alcohol Dependence; Urocortin

1.1 Problematic Alcohol Use in Humans

Alcohol use is pervasive in the United States, with ~88% of adults 18 years or older reporting alcohol use at some time during their life and ~55% of adults reporting alcohol use within the past month (CBHSQ 2016). With the high prevalence of alcohol drinking, it is unsurprising that alcohol accounts for ~4% of global disease burden and is the fourth leading preventable cause of death in the United States (Mokdad et al. 2004). Therefore, there is an urgent need to understand the neurobiological processes that underlie the transition from moderate controlled alcohol use to problematic alcohol use in humans.

In the transition from non-hazardous drinking to hazardous drinking and alcohol use disorder (AUD), neuroadaptations occur within brain reward and brain stress systems. Initial alcohol use is driven by positive reinforcement, that is, drinking for the euphoric or rewarding effects of alcohol, and brain reward pathways are predominantly activated in this stage of alcohol use (Koob 2003). Intermittent bouts of binge alcohol consumption occur during the transition from moderate use to alcohol dependence (Koob and Le Moal 1997). During this time, individuals transition from drinking alcohol for its positive reinforcing effects to drinking alcohol for its negative reinforcing effects, in many cases to relieve the negative affective symptoms that define alcohol withdrawal (Koob 2003; Koob and Le Moal 1997). One brain signaling system that has received much attention in animal models of excessive alcohol drinking and alcohol dependence is corticotropin-releasing factor (CRF), a pro-stress neuropeptide that is dysregulated by chronic high-dose alcohol exposure, and that appears to contribute to binge alcohol drinking, alcohol dependence, and alcohol relapse.

1.2 Introduction to Brain CRF System

1.2.1 CRF and Urocortins

Corticotropin-releasing factor (CRF)—CRF is a 41-amino acid neuropeptide that is evolutionarily conserved across species (Vale et al. 1981). Within the central nervous system, CRF acts as a neuromodulator at both pre- and post-synaptic sites (Lowry and Moore 2006). In general, neuromodulators (like CRF) work at G-protein coupled receptors and they have longer lasting effects than classical neurotransmitters (van den Pol 2012). Such neuromodulators may enhance or attenuate neuronal activity by modulating the activity of ion channels, or by increasing or decreasing the activity of classical neurotransmitters via direct actions on peptide receptors (van den Pol 2012). CRF is widely expressed in the brain, including in the cortex, hypothalamus, thalamus, hippocampus, midbrain, and locus coeruleus (LC) (Merchenthaler 1984; Peng et al. 2017). The highest density of CRF neurons is in the paraventricular nucleus of the hypothalamus (PVN) and the extended amygdala, particularly the central amygdala (CeA) and bed nucleus of the stria terminalis (BNST; Dunn and Berridge 1990; Merchenthaler 1984; Peng et al. 2017). Importantly, CRF neurons are heterogeneous, co-expressing different molecules, having different electrophysiological properties, and differing in soma shape depending on the region (Dabrowska et al. 2013a; Dabrowska et al. 2013b; Peng et al. 2017).

Hypothalamic CRF projections modulate endocrine and autonomic responses to stress. CRF released from glutamatergic parvocellular neurons in the PVN is the primary activator of the hypothalamic-pituitary-adrenal (HPA) axis (Rivier and Vale 1983; Vale et al. 1981). Acting as a hormone, CRF is released from the PVN into the median eminence, where it travels to the pituitary, and binds receptors on corticotrophs, thereby increasing the synthesis and release of adrenocorticophic hormone (ACTH). ACTH travels in systemic circulation and increases glucocorticoid synthesis and release from the adrenal gland, thereby initiating the endocrine stress response. After initiation of the endocrine stress response, glucocorticoids feed back onto CRF cells in the PVN and other brain regions (e.g., hippocampus and cortex) to decrease CRF production. During stress, hypothalamic CRF neurons can synthesize and co-release arginine vasopressin (AVP), which can increase ACTH release from corticotrophs

in the anterior pituitary (Sawchenko et al. 1984). In addition, a majority of CRF neurons in the PVN express transcripts for oxytocin, suggesting that these neurons have multiple effects according to physiological demand (Dabrowska et al. 2013a).

Extended amygdala CRF pools participate in coordination of visceral, behavioral, and emotional responses to stress. CRF is particularly abundant in the lateral division of the CeA and the dorsolateral division of the BNST (Figure 1; Pomrenze et al. 2015; Shimada et al. 1989). CeA and oval BNST (oBNST) CRF neurons share many similarities with each other, and appear to be different from CRF neurons in the PVN. For example, while glucocorticoids negatively regulate CRF transcription in the PVN, glucocorticoids increase CRF production in a positive feedback loop in the CeA and BNST (Shepard et al. 2000). CRF is typically expressed in GABAergic interneurons with medium spiny neuron morphology in both the CeA and the BNST (Phelix and Paull 1990). Interestingly, CRF neurons in the CeA project to BNST, CRF neurons in the BNST project to CeA, and these two CRF neuron pools project to many of the same downstream regions including the lateral hypothalamus, ventrolateral periaqueductal grey (PAG), dorsal raphe nucleus (DRN), and the ventral tegmental area (VTA) (Dabrowska et al. 2016; Pomrenze et al. 2015). CRF neurons in CeA and BNST also co-express overlapping molecules, including but not limited to dynorphin, neurotensin, and somatostatin (Pomrenze et al. 2015; Shimada et al. 1989). In addition, CRF neurons in CeA and oBNST both co-express striatal-enriched protein tyrosine phosphatase (STEP; Dabrowska et al. 2013b). STEP may function as an indirect marker of neuronal activation in the CeA and BNST because it de-phosphorylates neuronal activation markers (e.g., pERK), and it is expressed in 98% of CRF neurons in the oBNST and 94% of CRF neurons in the CeA (Dabrowska et al. 2013b).

Urocortins (Ucns)—The urocortins (Ucns) are more recently discovered components of the CRF system. Urocortin 1, 2, and 3 (Ucn1, Ucn2, and Ucn3) share a highly conserved structural homology, but each molecule has a unique distribution and function in the mammalian brain. Although the exact physiological function of the Ucn system remains unclear, it appears to be involved in modulation of physiological processes that include stress response, osmoregulation, energy expenditure, food intake, and immune function (Fekete and Zorrilla 2007).

Ucn1 is expressed primarily in non-cholinergic cells in the centrally-projecting Edinger-Westphal nucleus (EWcp), a subdivision of the Edinger-Westphal nucleus that is not involved in autonomic responses (Bittencourt et al. 1999; Ryabinin et al. 2012). Ucn1 fibers project to lateral septum, DRN, supraoptic nucleus, PVN, PAG, Edinger-Westphal nucleus, BNST, CeA, and medial amygdala (MeA; Figure 1; Fekete and Zorrilla 2007; Pan and Kastin 2008; Ryabinin et al. 2012). In response to acute stress, Ucn1 shows rapid induction that is mediated by glucocorticoids (Koob and Heinrichs 1999; Weninger et al. 2000). Because Ucn1 binds to both CRF receptor subtypes (discussed below), its physiological function is not completely understood. It has been hypothesized that midbrain Ucn1 neurons play a role in sympathetic-mediated behavioral responses to stress, including increases in anxiety-like behavior and decreases in food consumption (Koob and Heinrichs 1999; Pan and Kastin 2008). Others have postulated that Ucn1 expression may be important for balancing activation of CRF receptor subtypes during stress (Ryabinin et al. 2012). In this

hypothesis, CRF and Ucn1 signaling at CRFR1 initiate the sympathetic, endocrine, and behavioral responses to stress, and Ucn1 signaling at CRFR2 may also mediate the later adaptive phases of stress (Ryabinin et al. 2012)

Ucn2 (also known as stresscopin-related peptide) is expressed in the PVN, supraoptic nucleus, LC, trigeminal, facial, and hypoglossal motor nuclei and the meninges (Dunn and Berridge 1990; Reyes et al. 2001; Ryabinin et al. 2012). Projection targets of Ucn2 fibers are not known (Fekete and Zorrilla 2007), but it is hypothesized that Ucn2 projections from LC to DRN increase depressive-like behavior by modulating serotonergic signaling (Fekete and Zorrilla 2007). In addition, Ucn2 may modulate basal HPA circadian amplitude in females by modulating AVP levels (Chen et al. 2006).

Ucn3 (i.e., stresscopin) is the most widely expressed urocortin. It is expressed in the medial preoptic area, prefrontal area, BNST, MeA, ventral premammillary nucleus, superior olivary nucleus, and the parabrachial nucleus (Ryabinin et al. 2012). Projection targets for Ucn3 cells include the lateral septum and ventromedial hypothalamus, both of which contain high levels of CRF receptor-type 2 (Hillhouse and Grammatopoulos 2006). Ucn3 modulates food intake and basal neuroendocrine regulation (Hillhouse and Grammatopoulos 2006).

1.2.2 CRF Receptors

There are two CRF receptor subtypes in the mammalian central nervous system – CRF type-1 receptor (CRFR1) and CRF type-2 receptor (CRFR2). Both CRFR1 and CRFR2 are Gs-protein coupled receptors, and binding of endogenous ligands to these receptors activates adenylate cyclase, increases cAMP, and increases protein kinase A (PKA) signaling (Chen and Du 1996). In addition, these receptors are bound to various structural proteins that modulate CRF signaling according to brain region and physiological state (Henckens et al. 2016).

CRFR1—CRFR1s are widely expressed, with high concentrations in the anterior hypophysis, cerebral cortex, cerebellum, BNST, CeA, MeA, BLA, hippocampus, globus pallidus, and VTA (Figure 1; Chalmers et al. 1995; Henckens et al. 2016; Van Pett et al. 2000). CRFR1 bind both CRF and Ucn1 with high affinity (Bittencourt et al. 1999), and its expression corresponds to areas where there is high expression of CRF and Ucn1 cell bodies and projection fibers. Canonically, CRFR1 has been considered to be “pro-stress,” because increases in CRFR1 signaling are anxiogenic (Dunn and Berridge 1990), whereas antagonizing or knocking out CRFR1 reduces anxiety-like behavior (Henckens et al. 2016; Muller et al. 2003; Timpl et al. 1998; Zorrilla et al. 2002).

CRFR2—CRFR2s exhibit expression that is restricted to subcortical brain regions including the amygdala, BNST, lateral septum, and DRN (Figure 1; Chalmers et al. 1995; Van Pett et al. 2000). CRF has a much lower affinity for CRFR2 than for CRFR1; however, the Ucn1s all show high affinity for CRFR2 and they appear to be the primary endogenous ligand for this receptor. As such, regions that show high CRFR2 expression also show high Ucn1 expression and/or receive projections from Ucn1-rich brain areas. Two hypotheses exist to explain the role of CRFR2 activation in anxiety-like behavior (Henckens et al. 2016): the first is that CRFR2 activation counteracts the initial stress response and maintains homeostasis

(Henckens et al. 2016; Hillhouse and Grammatopoulos 2006); the second is that CRFR1 and CRFR2 mediate different aspects of the stress response, with CRFR1 mediating active defensive behavior and CRFR2 mediating passive coping behavior and depression-like responses (Henckens et al. 2016).

1.2.3 CRF-Binding Protein

CRF-binding protein (CRF-BP) is a 37kd secreted glycoprotein that binds CRF and Ucn1. In humans, CRF-BP binds 40-90% of CRF and its expression is 10-fold higher than CRF levels in most regions of the human brain (Hillhouse and Grammatopoulos 2006; Suda et al. 1988). CRF-BP expression and synthesis is regulated by stress, CRF, and glucocorticoids (Westphal and Seasholtz 2006). In addition, CRF-BP is often co-localized with CRF or CRFRs in the brain, with particularly high concentrations in CeA and BNST (Figure 1; Chan et al. 2000; Potter et al. 1992; Westphal and Seasholtz 2006).

Once CRF-BP binds CRF, it can inhibit or facilitate CRF activity. For example, CRF-BP can reduce CRFR activation by sequestering CRF or Ucn1 and/or targeting them for degradation (Ketchesin and Seasholtz 2015). Conversely, CRF-BP can increase CRF signaling by binding CRF and interacting with CRFRs, as recently shown for CRFR2 in the VTA (Albrechet-Souza et al. 2015; Ungless et al. 2003). In addition to modulating the activity of CRF and its receptors, CRF-BP may affect neuronal activity independent of CRF and CRFRs. For example, intraventricular administration of CRF-BP increases neuronal activation in CRFR-expressing cells, but also in CRF-BP-expressing cells that do not co-express CRF or CRFR (Chan et al. 2000).

1.3 Alcohol Effects on CRF Signaling

1.3.1 Acute Alcohol Effects on the CRF System

CRF—Acute alcohol activates the HPA axis by inducing CRF cell activation in the hypothalamus (Rivier and Lee 1996). Acute alcohol increases CRF heteronuclear RNA (hnRNA) in the PVN, suggesting increased CRF synthesis (Rivier and Lee 1996); however, there is not a clear increase in CRF mRNA following acute alcohol in vivo (Rivier and Lee 1996). The difference between hnRNA and mRNA could be due to presence of a large stable pool of CRF mRNA in parvocellular neurons of the PVN, which may make it hard to detect small changes, or due to unknown alcohol effects on events between gene transcription and detection of mRNA (Rivier and Lee 1996). Work done in hypothalamic cell culture has demonstrated acute alcohol-induced increases in CRF mRNA, CRF promoter activity, and CRF secretion via increases in cAMP and PKA (Li et al. 2005). In addition, in an in vitro hypothalamic preparation, acute alcohol exposure increased CRF release from neurons (Redei et al. 1988). See Table 1 for a summary of the effects of different alcohol exposure regimens on brain CRFR system signaling.

Within the CeA, high doses of acute alcohol increase CRF release 120-180 minutes later (Lam and Gianoulakis 2011). Interestingly, acute alcohol-induced increases in frequency of spontaneous mini inhibitory post-synaptic currents (IPSCs) in the CeA are mediated by CRFR1 signaling through both protein kinase C ϵ and PKA pathways (Bajo et al. 2008;

Cruz et al. 2012; Roberto et al. 2010). Therefore, acute alcohol increases CRF release in CeA, which in turn increases CeA inhibitory transmission, suggesting that CRF modulates acute alcohol effects on synaptic transmission in CeA.

Ucn1—The Ucn1 system is activated by acute alcohol, and may mediate acute alcohol effects on other brain signaling systems. Acute alcohol increases activation of Ucn1 cells in the EWcp, as measured by c-fos (Ryabinin et al. 1995; 1997; 2003). This acute alcohol-induced activation of Ucn1 neurons in the EWcp is slow to habituate to repeated bouts of alcohol exposure, as evidenced by increased c-fos expression in Ucn1 neurons in the EWcp following repeated alcohol self-administration (Ryabinin et al. 2003; Turek and Ryabinin 2005). The observed lack of tolerance to acute alcohol effects suggests that Ucn1 neurons may be involved in behaviors that accompany prolonged alcohol exposure (Weitemier and Ryabinin 2005). See Table 3 for a summary of the effects of different alcohol exposure regimens on brain Ucn system signaling.

Overall, acute alcohol exposure leads to activation of hypothalamic and extra-hypothalamic CRF systems and activation of Ucn1 cells within the EWcp. More work is needed to determine if acute alcohol effects on brain CRF signaling change after repeated low-level exposures and if/how they contribute to escalation of alcohol use.

1.3.2 Binge Alcohol Effects on the CRF System

The National Institute of Alcohol Abuse and Alcoholism (NIAAA) defines binge drinking as a pattern of ethanol consumption that leads to blood alcohol concentrations (BACs) of 80 mg/dl or above (NIH-NIAAA, 2004), which is usually about 4 drinks in about 2 hours for women and 5 or more drinks for men. Binge alcohol consumption is associated with increased risk to develop AUD, and is observed in populations that do and do not meet criteria for an AUD diagnosis (Deas and Brown 2006; Lai et al. 2012). Binge alcohol consumption affects brain CRF signaling acutely post-binge, and it is hypothesized that binge alcohol drinking may induce plasticity in brain CRF systems that becomes more robust and more rigid with repeated binge-like drinking episodes (Lowery-Gionta et al. 2012).

CRF—Different models of binge drinking have produced conflicting results regarding binge alcohol effects on hypothalamic CRF and HPA activity. Binge-like alcohol consumption in the Drinking in the Dark (DID) mouse model does not change HPA axis activity following one DID session (Lowery et al. 2010). Furthermore, repeated DID cycles do not alter CRF-immunoreactivity in the PVN, although corticosterone levels were not measured in this study (Lowery-Gionta et al. 2012). In contrast, in a model of intermittent alcohol homecage drinking in which rats achieve high levels of alcohol consumption punctuated by intermittent periods of abstinence, alcohol consumption decreases CRF binding and downstream signaling in the hypothalamus (Simms et al. 2014). This effect was attributed to the binge-like pattern of alcohol consumption in intermittent drinkers, rather than overall amount of alcohol consumed, as animals with continuous access to alcohol had higher lifetime alcohol consumption but did not display the same changes in hypothalamic CRF binding and downstream G-protein coupled signaling (Nielsen et al. 2012; Simms et al. 2014). The

difference between the DID model and escalation model may have to do with the pattern of EtOH consumption as well as the different species used for each model. Future work will determine how repeated binge-like sessions of alcohol drinking alter hypothalamic CRF signaling and HPA activity, and how these changes potentially contribute to excessive alcohol drinking and/or impact the transition to alcohol dependence.

The effects of binge-like alcohol drinking on the extra-hypothalamic CRF system depend on the age at time of alcohol exposure. Adolescence is a time of particularly high vulnerability to alcohol effects on the brain, and heavy onset early drinking is one of the strongest predictors of lifetime AUD (Chou and Pickering 1992). Binge drinking is highly prevalent in adolescents, and binge alcohol effects on adolescent brain CRF systems may affect subsequent alcohol-related behaviors (Gilpin et al. 2012). Following 14 days of binge-like alcohol consumption, adolescent male and female rats exhibit reductions in CRF cell number in the CeA, with no changes in CRF cell number in the BNST (Karanikas et al. 2013). Interestingly, adult male rats with a history of voluntary binge drinking in adolescence also exhibit reductions in CRF immunoreactivity in the CeA (Gilpin et al. 2012). This suggests that adolescent binge alcohol effects on the CeA CRF system last into adulthood (Gilpin et al. 2012), and may contribute to increased AUD vulnerability in adolescent binge drinkers (Chou and Pickering 1992).

Although binge alcohol drinking is common in adolescents, it is not a pattern of drinking seen only in adolescents. In fact, a large proportion of adults engage in binge drinking behavior (SAHMSA, 2016), and binge drinking is associated with negative consequences and increased risk for AUD in adults as well as adolescents (Jennison 2004; SAHMSA, 2016). In adult mice, after 1 and 6 cycles of DID, CRF-ir is increased in the CeA of mice with a history of binge-like alcohol drinking when compared to sucrose controls, suggesting that contrary to prior dogma, the CeA CRF system is recruited during early binge-like drinking episodes in animals without a chronic alcohol history (Lowery-Gionta et al. 2012). This increase in CeA CRF-ir persists 18-24 hours post-binge, well after alcohol has been cleared from blood and brain (Lowery-Gionta et al. 2012). This increase in CRF immunoreactivity may not be due to higher local CRF synthesis, as CRF mRNA was unchanged in the CeA 24 hours after the last of 3 DID cycles (Ketchesin et al. 2016). Additionally, repeated bouts of binge-like alcohol drinking result in a reduction in the ability of CRF to enhance GABAergic transmission in the CeA (Lowery-Gionta et al. 2012), which differs from alcohol dependence effects on CRF modulation of CeA GABAergic transmission (see below). This suggests a unique functional neuroadaptation in the CeA following repeated binge cycles that may contribute to escalated alcohol use and maintenance of excessive binge-like alcohol intake. Similar to adolescents, repeated cycles of DID do not alter CRF-ir or CRF mRNA outside the CeA (i.e., in the BNST, BLA, MeA, NAc core and shell, lateral hypothalamus, or lateral septum; Ketchesin et al. 2016; Lowery-Gionta et al. 2012). Interestingly, the VTA exhibits transient increases in CRF and decreases in CRF-BP levels following acute binge cycles that normalize after repeated binge exposures (Ketchesin et al. 2016; Lowery-Gionta et al. 2012; Rinker et al. 2017). More specifically, CRF mRNA and CRF-ir in VTA are increased following 1 cycle of DID, but CRF-ir normalizes after 6 cycles of DID (CRF mRNA was not measured after 6 cycles; Lowery-Gionta et al. 2012; Rinker et al. 2017). CRF-BP mRNA is decreased following 3 cycles of

DID, but, like CRF-ir, returns to normal after 6 cycles of DID (Ketchesin et al. 2016; Lowery-Gionta et al. 2012). Overall, this suggests that binge alcohol transiently increases CRF availability and/or activity in the VTA, which may represent a point of interaction between brain stress and reward systems, and which may contribute to the transition to alcohol dependence.

Ucn1—The effect of binge drinking on the Ucn1 system has not been extensively studied, although recent work suggests that Ucn1 neurons within the EWcp play a role in the maintenance of high levels of alcohol consumption (Giardino et al. 2017). Following long-term intermittent alcohol drinking in which mice escalate alcohol intake to binge-like levels, mice exhibit increased mRNA levels of Ucn1 and CRF-BP in the EWcp (Giardino et al. 2017). Furthermore, in that study, alcohol intake levels were positively correlated with fos mRNA levels in EWcp (Giardino et al. 2017). Overall, these results suggest that binge alcohol increases activity of the brain Ucn1 system.

CRFBP—Similar to Ucn1, it is largely unknown how binge-like alcohol consumption alters CRF-BP levels and its function in different brain regions. One study did not find binge alcohol effects on CRF-BP mRNA in the extended amygdala, but did find decreased CRF-BP transcript in extra-hypothalamic regions including the VTA (as mentioned earlier) and the mPFC (Ketchesin et al. 2016). Three cycles of DID binge-like alcohol drinking reduce CRF-BP mRNA in both the prelimbic (PrL) and infralimbic (IL) subdivisions of the mPFC, but this decrease is more persistent in the PrL than in the IL (Ketchesin et al. 2016). The same procedure did not alter CRF mRNA in the brain regions tested. Further work is needed to determine whether CRF-BP in mPFC plays a causal role in alcohol-related behavioral dysregulation.

Overall, binge-like alcohol consumption alters CRF signaling differently depending on the model, the brain region, and the age of exposure, with the CeA exhibiting the largest, most lasting effects. Higher CRF (and possibly Ucn1) signaling after binge-like alcohol consumption in brain reward and stress regions may contribute to increased vulnerability to addiction. Much remains unknown regarding the relationship between binge-like alcohol consumption and brain CRF signaling, including potential differences between adolescents and adults, potential lasting effects of binge alcohol drinking on brain CRF signaling, and how this latter effect may contribute to the transition to alcohol dependence.

1.3.3 Chronic Alcohol (i.e. dependence) Effects on the CRF system

CRF—Extensive work has detailed the effects of chronic alcohol on HPA axis activity in humans with AUD (Adinoff et al. 1998; Stephens and Wand 2012). In general, humans with AUD display elevated basal ACTH and cortisol, and hyporeactive HPA response to acute alcohol (as reviewed in Blaine et al. 2016). During early abstinence from alcohol, humans with AUD have low cortisol production and a blunted cortisol response to stress (Stephens and Wand 2012). Animals exposed to chronic alcohol liquid diet exhibit alterations in HPA axis function similar to what is seen in humans, with basally increased corticosterone levels during alcohol dependence, and lower HPA activity after alcohol is removed, which persists 3 weeks into alcohol withdrawal (Rasmussen et al., 2000). Exposure to alcohol for 3-7 days

leads to increased CRF gene expression and biosynthesis immediately after the alcohol exposure (Rivier et al. 1990). During acute withdrawal, chronic alcohol exposure also decreases CRF mRNA content in hypothalamic neurons without changing CRF release characteristics, and decreases anterior pituitary corticotroph responses to CRF by decreasing CRF binding and adenylate cyclase in the pituitary of chronic alcohol-exposed rats (Richardson et al. 2008; Redei et al. 1988). Downregulation of CRF in the hypothalamus may be explained by negative feedback from higher circulating cortisol levels, whereas high levels of circulating cortisol increase CRF levels in the extended amygdala and may “sensitize” the extended amygdala to the effects of chronic alcohol (Koob 2010; Shepard et al. 2000).

Many studies have examined chronic alcohol effects on CRF in the CeA. Immediately following chronic ingestion of alcohol liquid diet, CRF mRNA in the CeA is increased (Lack et al. 2005). Various studies have examined CeA CRF during alcohol withdrawal, and collectively report that alcohol withdrawal leads to decreased CRF-immunoreactivity and increased CRF release, as measured by microdialysis, in CeA (Funk et al. 2006; Merlo Pich et al. 1995; Zorrilla et al. 2001). Specifically, CRF release peaks ~10-12 hours into withdrawal in alcohol-dependent rats (Merlo Pich et al. 1995). This increase in CRF release corresponds to a decrease in CRF-ir seen during the first day of withdrawal from alcohol liquid diet or alcohol vapor, interpreted by the authors to reflect depletion of peptide in the cell due to increased CRF release (Funk et al. 2006; Zorrilla et al. 2001). Furthermore, following the development of alcohol dependence, the CeA becomes sensitized to CRF effects, such that the ability of CRF to augment mIPSC frequency is increased and CRFR1 antagonists have a greater suppressive effect on basal inhibitory transmission in dependent vs. non-dependent rats (Roberto et al. 2010). Overall, this suggests that heightened CRF signaling during acute alcohol withdrawal possibly contributes to escalated alcohol self-administration during withdrawal. During protracted withdrawal, neuroadaptations to CRF signaling in the CeA may continue to occur. Three weeks after cessation of alcohol vapor, CRF mRNA is increased in dependent rats compared to alcohol-naïve controls (Sommer et al. 2008), and after 6 weeks of alcohol withdrawal, alcohol-dependent rats have increased CRF tissue levels compared to alcohol-naïve rats (Zorrilla et al. 2001). Overall, this suggests that chronic alcohol effects on CRF signaling in the CeA last long after alcohol exposure is terminated.

A large population of CRF neurons also exists within the BNST, and these neurons are also affected by alcohol dependence and withdrawal. Like what is observed in the CeA, there is a trend toward a decrease in CRF-ir in alcohol-dependent animals compared to non-dependent controls (Funk et al. 2006). During alcohol withdrawal, there is an increase in extracellular CRF in the BNST (Olive et al. 2002). Interestingly, this putative increase in CRF release in the BNST is normalized by oral alcohol consumption (Olive et al. 2002). Recent work suggests that during acute withdrawal, increases in extracellular CRF in BNST activate glutamatergic neurons that project from the BNST to the VTA (Silberman et al. 2013). This is another example of how CRF signaling in brain stress regions impacts brain reward signaling after chronic alcohol exposure, which may be important for mediating escalated alcohol drinking, which may in turn normalize dysregulated CRF signaling.

Similar to binge drinking, chronic intermittent alcohol vapor exposure alters CRF expression in the mPFC. Twenty-four hours into withdrawal from chronic alcohol vapor, there are more CRF-positive cells in the mPFC; however, these cells are not more highly activated, as measured by c-fos, despite a large increase in c-fos expression in mPFC GABAergic interneurons at the same time point (George et al. 2012). Overall, this suggests that chronic alcohol dysregulates CRF within the mPFC, (George et al. 2012), but a functional role for mPFC CRF signaling in alcohol dependence-related behavior has not yet been established.

Ucn1—Chronic alcohol does not alter Ucn1 levels in the EWcp, but does affect Ucn1 circuit function (Weitemier & Ryabinin, 2005); more specifically, chronic alcohol decreases the number of Ucn1 fibers projecting to the lateral septum and the DRN (Weitemier and Ryabinin 2005). This change is associated with increased CRFR2 binding in the lateral septum and DRN after chronic alcohol (Weitemier and Ryabinin 2005), suggesting a potential role for this circuit in mediating behavioral change after chronic alcohol exposure. More work is needed to understand precisely how alcohol dependence and withdrawal affect Ucn1 levels and circuit function, and the impact of those changes on behavior.

CRF Receptors—Chronic alcohol alters CRF receptor expression and function in specific ways according to receptor subtype and brain region. Acute alcohol increases CRFR1 hnRNA in the PVN (Lee et al., 2001), but this increase is blunted in rats with a history of chronic alcohol, and this blunting effect, which lasts at least 7 days, may be important for mediating altered HPA activity during alcohol dependence (Lee et al. 2001). During acute withdrawal from alcohol vapor, CRFR1 mRNA trends to be unregulated in the CeA, but not the BLA or NAc (Roberto et al. 2010). Functionally, this same study demonstrated increased CRFR1 enhancement of GABA release in the CeA during acute withdrawal (Roberto et al. 2010). Two-week withdrawal from alcohol vapor produces robust increases in CRFR1 mRNA in the CeA, but not the MeA or BLA of mice (Eisenhardt et al. 2015). Interestingly, in rats the opposite is true, with three weeks of withdrawal from alcohol vapor increasing CRFR1 gene expression in the BLA and MeA, but not the CeA or BNST (Sommer et al. 2008). The reason for the difference in CRFR1 gene expression in mice compared to rats is unclear, but may be attributable to the amount of time between ethanol vapor and sacrifice in those studies.

Chronic alcohol affects CRFR2 gene expression in a brain region-dependent manner. During protracted withdrawal from alcohol vapor, CRFR2 gene expression is downregulated in the BLA of rats (Sommer et al. 2008); however, chronic alcohol exposure does not significantly change CRFR2 gene expression in the CeA, MeA, or BNST (Eisenhardt et al. 2015; Sommer et al. 2008). In mice, chronic alcohol injections increase CRFR2 binding in the DRN, a region that receives strong Ucn1 inputs from the EWcp (Weitemier and Ryabinin 2005). Also, alcohol-preferring rats show decreased CRFR2 expression compared to alcohol non-preferring rats in hypothalamus, amygdala, and caudate putamen. Finally, alcohol-preferring rats (iP rats) have a polymorphism in the CRFR2 gene that is associated with lower CRFR2 binding in the amygdala, which may contribute to the increased alcohol drinking behavior observed in those animals (Yong et al. 2014).

Overall, alcohol dependence and withdrawal leads to increases in extended amygdala CRF signaling (Table 1) that are hypothesized to functionally contribute to dependence-induced increases in alcohol consumption and negative affect (discussed below). In addition, this is associated with potentially decreased signaling of Ucn1 in the lateral septum and DRN, suggesting a potentially dysregulated balance between CRFR1 and CRFR2 signaling. It is not yet clear how alcohol dependence changes CRF signaling in brain reward regions, especially in the VTA, which exhibits transient increases in CRF signaling after binge-like alcohol consumption. In addition, little work has been done to examine how alcohol dependence and withdrawal affect CRF-BP levels, CRF-BP function, and Ucn2/3-CRFR2 signaling.

1.4 Brain Region-Specific CRFR1 and CRFR2 effects on Alcohol-Related Outcomes

1.4.1 Alcohol Effects at the Synapse

The relationship between brain CRF signaling and alcohol effects on neurotransmission has been extensively studied in the extended amygdala. Within the CeA, acute alcohol increases GABAergic transmission, and antagonizing CRFR1 blocks this effect (Nie et al. 2004; Nie et al. 2009; Roberto et al. 2010). In alcohol-dependent rats, although there is not tolerance to the effect of acute alcohol on GABA release, antagonizing CRFR1 more effectively reduces basal and alcohol-induced increases in GABAergic transmission in CeA (Roberto et al. 2010). In addition, chronic CRFR1 blockade in CeA blocks the transition to dependence-induced escalation of alcohol drinking (Roberto et al. 2010). Overall, these findings suggest that the transition to alcohol dependence is characterized in part by alcohol-induced neuroadaptations in CeA CRF-CRFR1 signaling, and that these neuroadaptations mediate some of the behavioral changes seen during and following the transition to alcohol dependence.

In the BNST, protracted withdrawal from alcohol vapor impairs long-term potentiation (LTP) induction (Francesconi et al. 2009.) This impairment appears to be mediated by CRF, as repeated systemic administration of CRFR1 antagonist during withdrawal abolished the impairment in LTP induction, and repeated but not acute CRF administration mimicked the withdrawal-induced impairment (Francesconi et al. 2009). Repeated administration of CRFR2 agonist during withdrawal had no effect on LTP induction in alcohol dependent rats during protracted withdrawal (Francesconi et al. 2009). Overall, these results suggest that alcohol withdrawal produces neuroadaptations in BNST CRF-CRFR1 signaling that may be important for mediating behavioral change.

1.4.2 Alcohol Reinforcement

The role of CRF in the positive reinforcing effects of alcohol has not been extensively studied, but brain CRF signaling alters the rewarding properties of alcohol, as measured by alcohol conditioned place preference (CPP). Conditioned place preference is an indirect way of testing the reinforcing properties of a drug by pairing the drug with specific external stimuli; animals express their preference or aversion for drug-paired stimuli by approaching them or avoiding them, respectively, and this behavioral readout is thought to reflect drug

reward or aversion. Although CRF is not often tested for its role in the positive reinforcing effects of alcohol (and other drugs), CRF deficient mice fail to show an alcohol CPP at 2 g/kg alcohol, but do exhibit alcohol CPP at 3 g/kg alcohol (Olive et al. 2003). Experimental inhibition of glucocorticoid synthesis or secretion does not alter the acquisition or expression of an alcohol CPP, suggesting that the change in alcohol CPP in CRF KO rats is due to extra-hypothalamic processes (Chester and Cunningham 1998). Similar to CRF KO mice, Ucn1 and CRFR2 KO mice fail to show alcohol CPP at 2 g/kg alcohol (Giardino et al. 2011); however, a higher dose of alcohol was not tested, so it remains to be seen if alterations in alcohol CPP in these knockout strains is dose-dependent, similar to what is seen in CRF-deficient mice. These whole-brain knockout mice suggest that brain CRF signaling is involved in alcohol reinforcement, but the brain-region specific roles of CRF, the Ucn1, and their receptors have not been tested. Furthermore, it is unknown how CRF modulation of the positive reinforcing effects of alcohol may change during the transition to dependence.

The negative reinforcing effects of alcohol are often tested by examining negative affective symptoms and increased alcohol drinking during withdrawal (discussed below). Place conditioning can also be used to assess aversion associated with alcohol and/or alcohol withdrawal. Rats show conditioned place aversion (CPA) to a chamber paired with acute withdrawal from high doses of acute alcohol (Morse et al., 2000). Similarly, injecting mice with alcohol immediately after removal from the conditioning chamber produces a CPA that is observed in wild-type and Ucn1 knockout mice (Giardino et al. 2011), suggesting that Ucn1 signaling via CRFR1 may not mediate the aversive and/or negative reinforcing effects of alcohol (i.e., perhaps this effect is mediated by CRF signaling). In support of this, rats show a conditioned place aversion to a chamber paired with CRF infused into the ventricles, the vmPFC, or the CeA (Cador et al. 1992; Itoga et al. 2016; Schreiber et al. 2017), which suggests that excess brain CRF signaling is aversive, and may support the notion that CRF mediates the aversive effects of alcohol.

1.4.3 Alcohol Consumption

Low-level Alcohol Consumption—During consumption of low alcohol quantities by a non-dependent individual, the positive reinforcing effects of alcohol drive alcohol intake, and there is limited engagement of brain stress systems (Koob 2003; Koob and Le Moal 1997). As chronicity and quantity of alcohol consumption increases and withdrawals are experienced, brain stress systems are increasingly engaged and become important for mediating escalated alcohol consumption (Koob 2003). As mentioned above, although brain CRF signaling is not typically assigned a major role in mediating the positive reinforcing effects of low doses, non-binge, and non-dependent levels of alcohol, brain CRF signaling may not be without a role in maintenance of low-level alcohol drinking (see Table 2 for a summary of the effects of brain region-specific CRF system manipulations and Table 3 for a summary of Ucn system manipulations on different types of alcohol drinking).

CRF: CRF may play a role in non-escalated alcohol consumption, because CRF knockout mice drink twice as much alcohol as their wildtype counterparts (Olive et al. 2003), and CRF overexpressing mice show reduced alcohol consumption and preference (Palmer et al.

2004). In addition, acute intra-ventricular CRF reduces acute alcohol drinking in mice (Bell et al. 1998). It is not clear why the direction of whole-animal and ventricular CRF effects on low-level alcohol drinking in mice is opposite what is typically observed in procedures that engender high levels of alcohol consumption in rats and mice. Regardless, it is likely that chronically escalated alcohol consumption and/or repeated withdrawal produces neuroadaptations that fundamentally change the role of brain CRF signaling in alcohol-related behaviors.

Ucn1: Ucn1 neurons in the EWcp modulate acute low-level alcohol drinking in a concentration-dependent manner. At low alcohol concentrations (3-10% v/v), lesion of the EWcp decreases alcohol preference and consumption in a two-bottle choice continuous access paradigm (Bachtell et al. 2004), but at higher alcohol concentrations (20% v/v), lesions of the EWcp do not affect alcohol consumption (Bachtell et al. 2004). Interestingly, the role of EWcp Ucn1 neurons in alcohol drinking seems to differ from the role of the EWcp as a whole. In a two-bottle choice continuous access paradigm, Ucn1 knockout mice do not differ from wildtype controls in levels of alcohol consumption or alcohol preference of 10% (v/v) alcohol (Giardino et al. 2017). Despite being activated by moderate doses of alcohol, it does not appear that Ucn1 signaling modulates moderate alcohol consumption, and the effects of EWcp lesion on decreasing alcohol consumption in mice may be attributable to other neuropeptides and signaling pathways.

CRFR1: CRFR1 modulation of non-dependent alcohol drinking is contingent on the concentration of alcohol and the amount of alcohol the animal consumes. In non-dependent animals consuming alcohol at low concentrations (<20%), systemic antagonism of CRFR1 or CRFR1 knockout has no effect on alcohol consumption (Chu et al. 2007; Gehlert et al. 2007; Roberto et al. 2010; Sabino et al. 2006). In addition, when mice do not drink in a binge-like manner or reach binge levels of alcohol consumption (BACs of <40 mg/dl), systemic CRFR1 antagonism does not alter alcohol consumption (Lowery-Gionta et al. 2012; Sparta et al. 2008). Collectively, these data suggest that CRFR1 signaling does not modulate low-level alcohol consumption.

In non-dependent animals, CRFR1 signaling mediates consumption of high alcohol concentrations or high quantities of alcohol. In non-dependent rats, systemic CRFR1 antagonism does not affect low-level operant self-administration without induction of dependence (Funk et al. 2006; Gehlert et al. 2007; Gilpin et al. 2008). For this reason, the effects of brain-region specific CRFR1 manipulations on low-level alcohol drinking have not been extensively studied, except in control groups in alcohol dependence studies. Those studies have shown that CRFR1 antagonism in either the CeA or BNST does not affect alcohol drinking in non-dependent drinkers (Finn et al. 2007; Funk et al. 2006). However, as animals drink more alcohol or the concentration of alcohol is increased, CRFR1 signaling plays a larger role in maintenance of alcohol consumption in non-dependent animals. The effects of CRFR1 antagonism in non-dependent rats are sensitive to alcohol concentration, reducing alcohol consumption at 20% v/v alcohol, but not at lower concentrations, in a continuous access drinking procedure (Cippitelli et al. 2014). In a rat model of escalating alcohol consumption, CRFR1 antagonists reduce alcohol consumption in animals that

consume the highest quantities of alcohol (Simms et al. 2014). Overall, these data suggest that CRFR1 signaling is recruited as levels of alcohol intake increase over time, even in non-dependent animals.

In non-dependent animals drinking low quantities of alcohol, basal CRF-CRFR1 signaling does not appear to modulate alcohol consumption, but brain CRF system signaling may modulate alcohol drinking in non-dependent animals consuming high alcohol concentrations and/or quantities.

Binge-Like Alcohol Consumption—Brain CRF signaling is increased during repeated binge-like alcohol intake, and pharmacologic manipulations of CRF signaling during binge-like alcohol consumption may alter the transition to alcohol dependence (Lowery-Gionta et al. 2012; see Table 2).

CRF: Repeated cycles of binge-like alcohol consumption increase CRF-ir in the extended amygdala, suggesting a role of CRF signaling in mediating binge-like alcohol drinking. In support of this hypothesis, whole-brain CRF knockout mice consume less alcohol over all four days of the DID procedure (Kaur et al. 2012). In particular, CRF neurons in the BNST that project locally and those that project out of the BNST mediate binge-like alcohol consumption (Pleil et al. 2015; Rinker et al. 2017). Chemogenetic inhibition of all BNST local and projection CRF neurons reduces binge drinking in the DID procedure (Pleil et al. 2015). BNST CRF neurons project to the VTA, a brain region critical for alcohol reward and binge-like alcohol drinking (Dabrowska et al. 2016). Indeed, specific inhibition of VTA-projecting CRF projection neurons in BNST reduces binge-like alcohol drinking (Rinker et al. 2017). Interestingly, inhibition of CRF neurons originating in VTA does not affect binge-like alcohol drinking, suggesting that BNST CRF inputs to VTA, but not local VTA CRF neurons, are important for mediating binge-like alcohol drinking in mice (Rinker et al. 2017). It also once again suggests that brain stress systems interact with brain reward systems, and that this interaction may 1) increase with heavy bouts of alcohol consumption, and 2) mediate the transition to alcohol dependence.

Ucn1: Ucn1 signaling may mediate excessive binge-like alcohol consumption, although its potential role is not completely understood. Whole brain Ucn knockout mice do not show a change in alcohol consumption in the DID procedure (Kaur et al. 2012). However, in an escalating continuous-access drinking procedure, whole-brain knockout of Ucn1 decreases consumption of high alcohol concentrations (40% v/v), such that knockout mice fail to reach intoxicating binge-like intake levels and exhibit lower BACs than wildtype controls (Giardino et al. 2017). This effect was alcohol-specific, as Ucn1 knockout mice did not exhibit altered sweet or bitter taste reactivity (Giardino et al. 2017). In contrast to the DID procedure, where mice achieve binge-like alcohol consumption in a 4-day procedure without escalating concentrations, this escalating continuous-access model increases the alcohol concentration from 10% v/v to 40% v/v over the course of 12 days (Giardino et al. 2017). The difference in these studies suggests that Ucn1 contributes to escalation of alcohol intake over time resulting in binge-like levels of alcohol consumption, but may not contribute to non-escalating binge-like alcohol drinking as modeled in the DID procedure.

CRFR1: Systemic CRFR1 antagonism and whole-brain CRFR1 knockout decrease binge-like alcohol drinking in the DID model (Kaur et al. 2012; Lowery et al. 2010; Lowery-Gionta et al. 2012; Sparta et al. 2008) and in modified 2-bottle choice drinking paradigms where animals reach binge-like levels of alcohol consumption (BACs >80 mg/dl; (Cippitelli et al. 2014; Simms et al. 2014). As mentioned above, CRF-CRFR1 signaling is increased in CeA during repeated binge-like alcohol consumption (Lowery-Gionta et al. 2012), and antagonizing CRFR1 in the CeA reduces binge-like alcohol drinking in the DID procedure (Lowery-Gionta et al. 2012). CRFR1 antagonism in VTA also decreases binge-like alcohol drinking in an intermittent access drinking model in rats and in high-alcohol-drinking mice (Hwa et al. 2013), and CRFR1 antagonism in DRN decreases binge-like levels of alcohol drinking in mice and rats (Hwa et al. 2013). These data clearly delineate a role for CRFR1 in binge alcohol drinking, perhaps via interactions with brain DA and/or 5-HT systems.

Ucn2/3-CRFR2: The role of Ucn2/3-CRFR2 signaling in binge-like alcohol drinking depends on the brain region and the animal model of alcohol consumption. Whole-brain deletion of CRFR2 increases alcohol intake in a limited-access model of alcohol consumption (Sharpe et al. 2005), but does not affect binge-like alcohol consumption in the DID model (Kaur et al. 2012). Conversely, intra-ventricular administration of Ucn3 decreases binge-like alcohol drinking in the DID model (Lowery et al. 2010). Overall this suggests that activation of whole brain Ucn3-CRFR2 signaling protects against excessive alcohol drinking. Interestingly, antagonizing CRFR2 specifically in the VTA decreases binge-like alcohol drinking in mice (Albrechet-Souza et al. 2015), suggesting that VTA CRFR2 signaling may have a unique role in mediating excessive alcohol intake.

CRF-BP: Similar to CRFR2, CRF-BP effects on binge-like alcohol consumption depend on the brain region being tested. Whole brain CRF-BP knockout in mice does not alter binge-like alcohol drinking in the DID model (Ketchesin et al. 2016). In the CeA, inhibition of CRF-BP does not affect binge-like alcohol drinking, however, as with CRFR2, inhibition of CRF-BP in the VTA decreases binge-like alcohol drinking (Albrechet-Souza et al. 2015). Furthermore, antagonizing both CRF-BP and CRFR2 decreases binge-like alcohol consumption to a greater degree than antagonizing CRFR2 alone, suggesting that in the VTA, CRF-BP facilitates CRF signaling, potentially through an association with CRFR2 (Albrechet-Souza et al. 2015). More work needs to be done to determine how CRF-BP signaling site-specifically influences binge-like alcohol consumption.

In summary, CRF signaling plays a major role in mediating binge-like alcohol consumption, especially in the VTA. CRF projections from the BNST to the VTA mediate binge-like alcohol consumption, perhaps via signaling at both CRFR1 and CRFR2. Ucn1 signaling contributes to binge-like escalation of alcohol consumption, while Ucn3 signaling may be protective against binge-like alcohol drinking behavior. Future work should determine the relative roles of CRF and Ucn signaling in mediating binge drinking, and also how connections between brain stress and reward circuits mediate binge-like alcohol drinking.

Dependence-Induced Increases in Alcohol Consumption—Brain CRF signaling plays a key role in dependence-induced escalation of alcohol drinking. In rodent models of chronic high-dose alcohol exposure, excessive alcohol drinking during acute and protracted

withdrawal is a key sign of alcohol dependence (Edwards et al. 2012; Gilpin et al. 2008), and is mediated, at least in part, by brain CRF signaling, especially in the CeA and neighboring regions.

CRF-CRFR1: Dependence-induced escalation of alcohol drinking is highly contingent on CRFR1 signaling, and CRF-CRFR1 signaling is recruited during the transition to alcohol dependence. Whole-brain CRF knockout mice do not increase alcohol intake after induction of alcohol dependence (Chu et al. 2007), suggesting that CRF is necessary for the dependence-induced escalation of alcohol drinking. Conversely, a non-specific CRFR antagonist injected into the ventricles decreases dependence-induced increases in alcohol self-administration 2 hours and 5 weeks into forced abstinence (Valdez et al. 2002). In addition, chronic systemic injection of a CRFR1 antagonist during alcohol withdrawal periods blocks escalation of alcohol self-administration, relative to alcohol-dependent rats treated chronically with vehicle (Roberto et al. 2010). In this study, at least 24 hours passed between CRFR1 antagonist injections and subsequent operant self-administration sessions, suggesting that CRFR1 antagonists may block neuroadaptations that normally accumulate with repeated withdrawals (Roberto et al. 2010). In addition, systemic CRFR1 antagonism decreases alcohol intake in alcohol-dependent rats during acute and protracted withdrawal (Chu et al. 2007; Funk et al. 2007; Gehlert et al. 2007; Gilpin et al. 2008; Roberto et al. 2010). The CeA is critical for mediating CRFR1 effects on escalated alcohol drinking, because antagonizing CRFR1 in the CeA, but not the BNST or nucleus accumbens shell, decreases operant alcohol self-administration in alcohol-dependent rats (Finn et al. 2007; Funk et al. 2006).

Ucn3-CRFR2: CRFR2 activation appears to have effects that are opposite of CRFR1 activation effects on dependence-induced escalation of alcohol drinking. Intra-ventricular administration of Ucn3 attenuates high alcohol drinking in alcohol-dependent rats (Valdez et al. 2004). Like CRFR1, the effect appears to be mediated in the CeA, since intra-CeA administration of Ucn3 also decreases dependence-induced alcohol drinking (Funk and Koob 2007). In light of the different effects of whole-brain and VTA modulation of CRFR2 signaling on binge-like alcohol drinking, it will be interesting to see how Ucn3-CRFR2 signaling in VTA modulates escalated alcohol drinking in alcohol-dependent animals.

In summary, CRFR1 and CRFR2 signaling have opposite effects on dependence-induced escalations in alcohol consumption. CRFR1 signaling increases alcohol consumption, and CRFR2 signaling may counteract this effect. Little work has examined a role for Ucn1/2 in dependence-induced escalation of alcohol drinking, and little is known about the CRF circuits mediating escalation of alcohol drinking during alcohol dependence.

1.4.4 Alcohol Relapse (e.g. Reinstatement)

AUD is defined as a chronically relapsing disorder. Environmental stimuli associated with alcohol (i.e., cues) and stressful events can each elicit relapse drinking after a period of alcohol abstinence (Sinha 2001). In rodents, reinstatement of alcohol-seeking can be induced by stress or an alcohol-paired cue, and is typically quantified as an increase in previously extinguished alcohol responding elicited by one of these stimuli.

Cue-Induced Reinstatement—Reinstatement of alcohol seeking in response to a cue formerly associated with alcohol reward, may or may not be mediated by brain CRF signaling. In one study, cue-induced reinstatement of alcohol seeking was not blocked by a non-specific peptide CRFR antagonist that targeted both CRFR1 and CRFR2 (Liu and Weiss 2003). But a more recent study suggests that CRFR1 might play a role in cue-induced reinstatement; that study showed that rats treated with a systemic CRFR1 antagonist respond less on an alcohol-paired lever after presentation of an alcohol-paired cue, relative to vehicle-treated rats (Galesi et al., 2016). This effect may be mediated by hypothalamic CRF signaling, because systemic CRFR1 antagonist effects were mimicked by systemic injection of metyrapone, a glucocorticoid synthesis inhibitor (Galesi et al. 2016). More studies are needed to clarify these apparently contradictory results, and to clarify the potential role for hypothalamic and extra-hypothalamic CRF signaling in cue-induced reinstatement of alcohol seeking.

Stress-Induced Reinstatement—Stress is a major trigger for relapse drinking in abstinent alcoholics (Sinha 2001). In animals, stress-induced reinstatement of alcohol-seeking is a paradigm in which acute stress promotes alcohol-seeking by increasing the frequency of a previously extinguished operant alcohol response. In rats, this is generally accomplished with footshock or with administration of yohimbine, an alpha-2 adrenergic receptor antagonist. Footshock reliably increases responding on a previously alcohol-paired (and previously extinguished) lever, and systemic yohimbine injection mimics this effect (Le et al. 2000).

CRF signaling is intimately involved in stress-induced reinstatement of alcohol seeking. A non-specific CRFR antagonist blocks reinstatement of alcohol-seeking induced by footshock in rats (Le et al. 2000; Liu and Weiss 2003). Furthermore, footshock-induced reinstatement can be mimicked by intra-ventricular CRF administration (Le et al. 2002). The role of CRF in stress-induced reinstatement seems primarily due to CRFR1 because systemic administration of a selective CRFR1 antagonist attenuates footshock stress- and yohimbine-induced reinstatement of alcohol seeking in rats (Gehlert et al. 2007; Le et al. 2000; Marinelli et al. 2007). In contrast to cue-induced reinstatement of alcohol self-administration, stress-induced reinstatement is likely mediated by extra-hypothalamic CRF signaling because adrenalectomy has no effect on stress-induced reinstatement or the ability of CRFR1 antagonism to decrease alcohol-seeking behavior following footshock (Le et al. 2000). Specifically, brain stem regions including the nucleus incertus (NI) and the median raphe nucleus (MRN) appear to have a prominent role in stress-induced reinstatement mediated by CRFR1. The NI is a brain region characterized by dense CRFR expression (Potter et al. 1994) and is sensitive to exogenous CRF administration (Bittencourt and Sawchenko 2000). Recently, its role in alcohol-related behaviors has begun to be explored. CRFR1 antagonism, but not CRFR2 antagonism, in the NI attenuates yohimbine-induced reinstatement of alcohol seeking in iP rats (Walker et al. 2016). Interestingly, unlike systemic CRFR1 antagonists, site-specific antagonism in the NI did not completely reverse stress-induced reinstatement of alcohol seeking, suggesting involvement of other brain regions and perhaps interaction with other neurotransmitter systems in this behavior. One such possible candidate site is the MRN, a brain region rich in serotonergic cells that express

CRFR1 and CRFR2 (Chalmers et al. 1995). Intra-MRN CRF infusion mimics footshock-induced reinstatement of alcohol seeking, and CRFR antagonism in the MRN blocks increased alcohol-seeking behavior following footshock in rats, perhaps suggesting a CRF-5-HT interaction in mediating alcohol relapse (Le et al. 2002). CRF may also interact with the kappa opioid system to induce relapse to alcohol seeking behavior, since systemic activation of kappa opioid receptors (KOR) increases alcohol-seeking behavior, and this effect is prevented by systemic injection of a CRFR1 antagonist (Funk et al. 2014). Although these results used systemic drug injection, it is tempting to speculate that the CRF-KOR interaction may occur in the extended amygdala, due to the high degree of co-localization of CRF and dynorphin in CeA and BNST (Pomrenze et al. 2015; Reyes et al. 2001), as well as CRF and dynorphin convergent effects on GABAergic transmission in CeA (Gilpin et al. 2014; Roberto et al. 2010). Emerging preliminary data suggests that CRF-KOR interactions also occur in VTA, with obvious potential implications for alcohol reward, consumption, and seeking.

Reinstatement of alcohol seeking appears to be at least partially mediated by CRFR1 signaling. Hypothalamic CRF signaling likely contributes to cue-induced reinstatement of alcohol seeking, whereas extra-hypothalamic CRF signaling, especially in the NI and MRN, likely contributes to stress-induced reinstatement of alcohol seeking. It is not known how the urocortins and/or CRFR2 may contribute to reinstatement of alcohol seeking.

1.4.5 Alcohol-Induced Negative Affect (e.g. anxiety, nociception)

Negative affect during acute withdrawal from chronic alcohol is hypothesized to promote escalation of alcohol drinking and relapse in alcohol-dependent individuals. In fact, negative affective symptoms are reported by alcohol-dependent humans to be one of the main reasons for continual drinking (Hershon 1977; Sinha 2001). In rodents, negative affect is measured as increases in anxiety-like behavior, behavioral sensitivity to stress, and increases in nociception (i.e., hyperalgesia/allodynia). Rats exposed to repeated cycles of intoxication and withdrawal exhibit increases in anxiety-like behavior that are not seen in rats continuously exposed to alcohol (Overstreet et al. 2002). Brain CRF signaling may become sensitized during repeated withdrawals and contribute to negative affect (Koob 2003). Although much work has been done examining the role of CRF, CRFR1, and CRFR2 in negative affect associated with chronic alcohol exposure, the potential role of Ucn3 and CRF-BP in negative affect has been less explored (these are not covered below).

CRF—Brain CRF signaling may be recruited during multiple withdrawals such that brain stress systems become sensitized with repeated withdrawals and contribute to negative affect in the absence of alcohol. This hypothesis is supported by the fact that intra-ventricular infusions of CRF can substitute for alcohol withdrawals by mimicking repeated withdrawal-induced increases in anxiety-like behavior (Overstreet et al. 2004). This CRF effect appears to be mediated by extra-hypothalamic brain regions: repeated injections of CRF into the CeA, BLA, DRN, and dorsal BNST before initiation of alcohol liquid diet increase anxiety-like behavior during subsequent alcohol withdrawals, but CRF microinjections in the PVN, ventral BNST, or CA1 of the hippocampus do not (Huang et al. 2010). This is specific to a CRF-alcohol interaction because injections of CRF before a control diet do not alter anxiety-

like behavior (Huang et al. 2010). These data support the hypothesis that repeated alcohol withdrawals sensitize the brain to the effects of future alcohol withdrawals through a brain CRF signaling mechanism. In addition, non-specific CRF receptor antagonism blocks restraint stress-induced increases in anxiety-like behavior after six weeks of alcohol deprivation in previously alcohol-dependent rats, suggesting a role for CRF in enhanced responsiveness to stress during protracted withdrawal from alcohol (Valdez et al. 2003).

CRFR1—Activation of CRFR1 during withdrawal is critical for many aspects of negative affect including anxiety-like behavior, sensitization of anxiety-like behavior during repeated alcohol withdrawals, and hyperalgesia. Systemic CRFR1 antagonism before the first and second withdrawals of a multiple-withdrawal protocol prevented increases in anxiety-like behavior normally observed during subsequent alcohol withdrawals (Overstreet et al. 2007; Breese et al. 2004). Site-specific CRFR1 antagonism in the CeA, DRN, and dorsal BNST blocks CRF-induced sensitization of withdrawal anxiety-like behavior, suggesting that stress-induced sensitization during withdrawal is mediated by extra-hypothalamic brain regions (Huang et al. 2010). Alcohol-dependent animals typically exhibit withdrawal-induced increases in anxiety-like behavior that can be blocked with systemic CRFR1 antagonism or whole-brain CRFR1 knockout (Rassnick et al. 1993; Sommer et al. 2008; Timpl et al. 1998). As with dependence-induced increases in alcohol self-administration, intra-CeA CRFR1 antagonism reverses alcohol withdrawal-induced increases in anxiety-like behavior in alcohol-dependent animals (Baldwin et al. 1991; Rassnick et al. 1993). Similarly, systemic CRFR1 antagonism decreases allodynia in alcohol-dependent rats, although the specific brain regions mediating this effect have yet to be determined (Edwards et al. 2012). Overall, CRFR1 signaling in CeA is recruited during repeated withdrawals, and contributes to withdrawal-induced negative affect and drives the negative reinforcing effects of alcohol.

CRFR2—In stark contrast to CRFR1, CRFR2 activation decreases negative affective symptoms in alcohol dependence. Intra-ventricular Ucn3 decreases dependence-induced anxiety-like behavior (Valdez et al. 2004). The brain region mediating Ucn3-CRFR2 signaling-induced decreases in negative affect have not yet been determined, although Ucn3-CRFR2 signaling in the DRN and dorsal BNST, brain regions implicated in withdrawal-induced sensitization of negative affect, does not significantly contribute to decreases in anxiety-like behavior induced by dependence (Huang et al. 2010). Intra-CeA Ucn3-CRFR2 manipulations have not been tested for their effects on alcohol withdrawal-related negative affective behaviors, but Ucn3-CRFR2 signaling may have a role since intra-CeA injection of a CRFR2 agonist decreases alcohol withdrawal-induced increases in alcohol self-administration (Funk and Koob 2007). More work is needed to clearly delineate the role of Ucn3-CRFR2 in mediating alcohol dependence-induced negative affect, excessive alcohol drinking, and relapse.

Similar to escalations in alcohol drinking, negative affect in the alcohol-dependent organism is mediated by CRF-CRFR1 signaling, and it is counteracted by Ucn3-CRFR2 signaling. It will be interesting to see what role if any Ucn1 and Ucn2 play in negative affect, and also

which brain networks contribute to the negative affect observed in alcohol-dependent animals during withdrawal.

1.5 Human Studies

1.5.1 Status of Clinical Trials

Extensive pre-clinical work examining the role of CRF-CRFR1 signaling in alcohol-related behavior suggests that CRFR1 antagonists may have therapeutic potential in humans with AUD, specifically to reduce excessive alcohol drinking, symptoms of withdrawal, and prevent relapse in those individuals. Although numerous small molecule CRFR1 antagonists have been developed for clinical trials, the results have been overwhelmingly negative. One of the first molecules tested, R121919, showed some promise in depression, but development of the drug was suspended due to elevations in liver enzymes (Zobel et al. 2000). Other small molecule CRFR1 antagonists have been tested in depression, anxiety, human fear lab studies, and PTSD but all have yielded negative results (Binneman et al. 2008; Coric et al. 2010; Dunlop et al. 2017; Grillon et al. 2015). Two studies have examined the efficacy of CRFR1 antagonists for decreasing craving in anxious adults with AUD. The first study tested pexacerfont, an orally available, brain-penetrant CRFR1 antagonist (Kwako et al. 2015), but that study reported no effect on alcohol craving following alcohol or stress cues, or following a Trier Social Stress Test (TSST; Kwako et al. 2015). In addition, pexacerfont had no effect on measured neuroendocrine outcomes, including cortisol and ACTH levels following stress (Kwako et al. 2015). The negative results in this study were thought to be due to the binding kinetics of pexacerfont, which has a fast receptor off-rate (Fleck et al. 2012). Therefore, a follow-up study examined therapeutic potential of CRFR1 antagonism in anxious AUD females, this time using verucerfont (Schwandt et al. 2016). Verucerfont is an orally available, brain-penetrant potentially selective CRFR1 antagonist with a similar structure to compounds with slow off-kinetics, suggesting increased efficacy compared to pexacerfont (Schwandt et al. 2016). Indeed, verucerfont blunted HPA axis activity during a dexamethasone-CRF challenge, suggesting that verucerfont is more active than pexacerfont (Schwandt et al. 2016). However, like pexacerfont, verucerfont failed to reduce craving after stress cues or alcohol cues (Schwandt et al. 2016). In fact, verucerfont significantly increased anxiety in the TSST (relative to placebo) without significantly affecting alcohol craving or HPA axis activation after TSST (Schwandt et al. 2016). Overall, these data suggest that antagonism of CRFR1 in anxious humans with AUD does not affect stress- or cue-induced increases in alcohol craving, despite having activity on the HPA axis. That said, the preponderance of pre-clinical data strongly implicate CRFR1 signaling in mediating escalated alcohol drinking and negative affective symptoms seen during early withdrawal, which were not measured in these two clinical studies. In addition, these studies may have missed the temporal window in which CRFR1 antagonists might be expected to have efficacy, because they were completed after withdrawal symptoms subsided. Therefore, CRFR1 antagonism may still be therapeutically relevant for reducing alcohol drinking or negative affective symptoms, but likely not craving, in people with AUD.

1.5.2 Why the CRF system is still important in AUD Research

The CRF system is still important in AUD research despite negative effects in two clinical trials examining CRFR1 antagonist effects on alcohol craving. The negative results of the clinical trials may be attributable to any combination of the following: limitations of stress-induced alcohol reinstatement in animal studies relative to the human craving it models, mismatched timing of drug delivery in animal and human studies, differences between brain CRF systems in rodents versus humans, or the possibility that CRFR1 antagonists would only be therapeutically effective in an as yet unidentified sub-group of AUD patients (Spierling and Zorrilla 2017).

The two alcohol craving clinical trials described above targeted CRFR1 signaling, but pre-clinical literature supports a potential role for CRFR2, the Ucn3, and CRF-BP in mediating various aspects of alcohol use. A recent study in non-human primates postulated that in primates, CRFR2 in the amygdala might play an important role in anxiety-like responses (Kalin et al. 2016). Indeed, compared to rodents, where there is limited CRFR2 expression in the CeA (Van Pett et al. 2000), primates express high density of CRFR2 in the CeA (Sanchez et al. 1999), although the functional relevance of this has not yet been determined. CRFBP in rodents was recently shown to interact with CRFR2 in the VTA to influence binge-like alcohol drinking (Haass-Koffler et al. 2016). Therefore, a deeper understanding of the relationship between alcohol and brain CRF system plasticity and signaling, especially CRFR2 signaling and CRF-BP, may be necessary to effectively leverage the brain CRF system as a therapeutic target for reducing excessive alcohol drinking and negative affect in at least a subset of humans living with AUD.

1.6 Future Directions and Conclusions

Animal studies clearly implicate the brain CRF system in mediating escalated alcohol drinking and negative affect observed in rodent models of AUD. Rodent models suggest that high-dose alcohol exposure, in the form of binge-like alcohol drinking or forced high-dose alcohol exposure (i.e., that which produces alcohol dependence), dysregulates CRF signaling in hypothalamic and extra-hypothalamic brain regions. This dysregulated CRF signaling, particularly CRF-CRFR1 signaling within the extended amygdala, are hypothesized to drive excessive alcohol drinking, negative affect, and stress-induced alcohol-seeking behaviors that are associated with alcohol dependence and binge-like alcohol drinking.

Although clinical studies have demonstrated a lack of efficacy of CRFR1 antagonists in decreasing craving in anxious adults with AUD, these studies don't entirely rule out other clinical endpoints (e.g., alcohol consumption) or other components of the brain CRF system as potential therapeutic targets for treating aspects of AUD. In addition, hypothalamic CRF signaling and the HPA axis may be of more importance in AUD than the pre-clinical literature suggests. In a recent study, glucocorticoid receptor antagonists decreased alcohol craving and alcohol drinking in treatment-seeking individuals with AUD (Vendruscolo et al. 2015).

Advances in basic science research technology, including optogenetics, chemogenetics, and transgenic rodent lines, allow for circuit-specific modulation of brain CRF signaling that

will greatly enhance our understanding of how, where, and when brain CRF signaling modulates escalated alcohol drinking, relapse-like behavior, and negative affect. In addition, future work should examine the potential roles of CRFR2, Ucns, and CRF-BP in binge-like alcohol drinking, negative affective states associated with alcohol withdrawal, and relapse, both in rodent models and in primate models to maximize the translational value of this work. The investigation of brain CRF system signaling remains important not only for potential therapeutic benefits, but also in the investigation of CRF receptors as gatekeepers on the function of brain circuits impacted by alcohol and drugs, and important for various behaviors, many of which extend beyond the addiction field (e.g., stress, fear, and pain).

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Figure 1. Distribution and projection of CRF, Ucn1, and CRFRs in alcohol-related regions
Corticotropin-releasing factor (CRF) is more widely expressed than urocortin 1 (Ucn1); CRF type 1 receptors (CRFR1; green triangle) are expressed widely throughout the brain, while CRF type 2 receptors (CRFR2; yellow triangle) have a more restricted distribution. Corticotropin-releasing factor binding protein (CRF-BP; red diamond) is expressed in most brain regions that express CRF, Ucn1, and CRFRs. CeA, central amygdala; DRN, dorsal raphe nucleus; EWcp, centrally projecting Edinger-Westphal nucleus; LHA, lateral hypothalamus; mPFC, medial prefrontal cortex; PAG, periaqueductal grey; PVN, paraventricular nucleus of the hypothalamus

Table 1

Alcohol Effects on CRF Signaling.

	PVN	CeA	BNST	VTA	mPFC	EWcp	Lateral Septum	DRN
<u>Low-Level/Acute Alcohol Exposure Effect on</u>								
CRF	↑ hnRNA, activity	↑ release	?	?	↔ cell number	?	?	?
<u>Binge-Like Alcohol Consumption Effect on</u>								
CRF	<u>Adolescents</u>	<u>Adolescents</u>	<u>Adolescents</u>	<u>Adolescents</u>	<u>Adolescents</u>	<u>Adolescents</u>	<u>Adolescents</u>	<u>Adolescents</u>
	?	↓ (operant binge) cell number	↔ (operant binge) cell number	?	?	?	?	?
	<u>Adults</u>	<u>Adults</u>	<u>Adults</u>	<u>Adults</u>	<u>Adults</u>	<u>Adults</u>	<u>Adults</u>	<u>Adults</u>
	↔ (DID) mRNA ↓ (escalation model) binding and signaling	↑ (DID) CRF-ir ↔ (DID) mRNA	↔ (DID) mRNA	↑ (DID) mRNA	?	?	↔ (DID) mRNA	?
CRF-BP	?	↔ (DID) mRNA	↔ (DID) mRNA	↓ (DID) mRNA	↓ (DID) mRNA	↑ (escalation model) mRNA	?	?
<u>Chronic Alcohol/Dependence Effect on</u>								
CRF	<u>Intoxication</u>	<u>Intoxication</u>	<u>Intoxication</u>	<u>Intoxication</u>	<u>Intoxication</u>	<u>Intoxication</u>	<u>Intoxication</u>	<u>Intoxication</u>
	↑ mRNA	↑ mRNA	↔ release	?	?	?	?	?
	<u>Acute WD</u>	<u>Acute WD</u>	<u>Acute WD</u>	<u>Acute WD</u>	<u>Acute WD</u>	<u>Acute WD</u>	<u>Acute WD</u>	<u>Acute WD</u>
	↓ content, mRNA	↑ release ↓ content	↑ release ↓ content	?	↑ cell number	?	?	?
CRF	<u>Protracted WD</u>	<u>Protracted WD</u>	<u>Protracted WD</u>	<u>Protracted WD</u>	<u>Protracted WD</u>	<u>Protracted WD</u>	<u>Protracted WD</u>	<u>Protracted WD</u>
	?	↑ mRNA	?	?	?	?	?	?
	↑ mRNA (Blunted compared to acute)	↑ mRNA (mice) ↔ mRNA (rats)	↔ mRNA (rats)	?	?	?	?	?
CRFR2	?	↔ mRNA	↔ mRNA	?	?	?	↑ mRNA	↑ mRNA

↑ - Increase in expression or signaling due to alcohol; ↔ - No change in expression or signaling due to alcohol; ↓ - Decrease in expression or signaling due to alcohol. CRF, corticotropin-releasing factor; CRF-BP, CRF-binding protein; CRFR1, CRF receptor type 1; CRFR2, CRF receptor type 2; PVN, paraventricular nucleus of the hypothalamus; CeA, central amygdala; BNST, bed nucleus of stria terminalis; VTA, ventral tegmental area; mPFC, medial prefrontal cortex; EWcp, centrally-projecting Edinger Westphal nucleus; DRN, dorsal raphe nucleus

Table 2

Brain Region-Specific CRF System Manipulations on Alcohol Consumption.

	Systemic/ICV	Intra-CeA	Intra-BNST	Intra-VTA	Intra-DRN
<u>Effects on Low-level Alcohol Consumption</u>					
CRF	↑ (KO) ↓ (Overexpress; ICV)	?	?	?	?
CRFR1 Antagonism	↔	↔	↔	?	?
<u>Effects on Binge-Like Alcohol Consumption</u>					
CRF	↓ (KO - DID)	?	↓ (Gi-BNST; DID) ↓ (Gi-BNST ^ VTA; DID)	↔ (Gi-VTA; DID)	?
CRFR1 Antagonism	↓ (DID) ↓ (escalation model)	↓ (DID) ? (escalation model)	? (DID) ? (escalation model)	? (DID) ↓ (escalation model)	? (DID) ↓ (escalation model)
CRFR2 KO/Antagonism	↔ (KO - DID) ↑ (KO - escalation model)	?	?	↓ (antagonism - DID)	?
CRFBP KO/Inhibition	↔ (KO - DID)	↔ (inhibition - DID)	?	↓ (inhibition - DID)	?
<u>Effects on Dependence-Induced increases in Alcohol Consumption</u>					
CRF	No escalation	?	?	?	?
CRFR1 Antagonism	↓	↓	↔	?	?

↑ - Increase alcohol consumption due to manipulation; ↔ - No change in alcohol consumption; ↓ - Decrease in alcohol consumption. CRF, corticotropin-releasing factor; CRF-BP, CRF-binding protein; CRFR1, CRF receptor type 1; CRFR2, CRF receptor type 2; ICV, intra-ventricular; CeA, central amygdala; BNST, bed nucleus of stria terminalis; VTA, ventral tegmental area; DRN, dorsal raphe nucleus

Table 3

Alcohol Interactions with the Urocortin System.

	Systemic/ICV	CeA	EWcp
Alcohol Effects on Ucn1			
Low-level/Acute EtOH (Ucn1)	N/A	?	↑ activation
Binge-Like Alcohol (Ucn1)	N/A	?	↑ (escalation model) mRNA
Chronic Alcohol (Ucn)	N/A	?	↔ content ↓ fibers projecting to lateral septum and DRN
Ucn Manipulation Effects on Alcohol Consumption			
Ucn1 Effect on Low-level Alcohol Consumption	↔ (KO – DID)	?	?
Ucn1 Effect on Binge-like Alcohol Consumption	↔ (KO – DID) ↓ (KO – escalation model)	?	?
Ucn3 Effect on Binge-like Alcohol Consumption	↓	?	?
Ucn3 Effects on Dependence-induced increases in Alcohol consumption	↓	↓	?

↑ - Increase; ↔ - No change; ↓ - Decrease. ICV, intra-ventricular; Ucn1, urocortin-1; Ucn3 – urocortin-3; CeA, central amygdala; EWcp, centrally projecting Edinger-Westphal nucleus