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Review

Preclinical evidence implicating corticotropin-releasing factor signaling in ethanol consumption and neuroadaptation

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The results of many studies support the influence of the corticotropin-releasing factor (CRF) system on ethanol (EtOH) consumption and EtOH-induced neuroadaptations that are critical in the addiction process. This review summarizes the preclinical data in this area after first providing an overview of the components of the CRF system. This complex system involves hypothalamic and extra-hypothalamic mechanisms that play a role in the central and peripheral consequences of stressors, including EtOH and other drugs of abuse. In addition, several endogenous ligands and targets make up this system and show differences in their involvement in EtOH drinking and in the effects of chronic or repeated EtOH treatment. In general, genetic and pharmacological approaches paint a consistent picture of the importance of CRF signaling via type 1 CRF receptors (CRF_1) in EtOH-induced neuroadaptations that result in higher levels of intake, encourage alcohol seeking during abstinence and alter EtOH sensitivity. Furthermore, genetic findings in rodents, non-human primates and humans have provided some evidence of associations of genetic polymorphisms in CRF-related genes with EtOH drinking, although additional data are needed. These results suggest that CRF_1 antagonists have potential as pharmacotherapeutics for alcohol use disorders. However, given the broad and important role of these receptors in adaptation to environmental and other challenges, full antagonist effects may be too profound and consideration should be given to treatments with modulatory effects.

Keywords: Alcohol, alcohol use disorder, ethanol drinking, ethanol seeking, genetic animal model, HPA axis, knockout, pharmacology, pharmacotherapy, sensitization, stress, urocortin

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Interest in stress and stress-associated pathways for their roles in alcohol (ethanol, EtOH) use and related symptoms has a long history. The focus has evolved over time from examination of behavioral effects of stressors on EtOH-associated traits and effects of EtOH on stress-axis measures, such as corticosterone (CORT) and adrenocorticotropin hormone (ACTH) levels, to investigation of the relevance of central and peripheral peptides and receptors. In the last decade, a number of excellent reviews have described much of this literature and have influenced the authors' perspectives (Allen *et al.* 2011; Armario 2010; Burke & Miczek 2014; Ciccocioppo *et al.* 2009; Clapp *et al.* 2008; Crabbe *et al.* 2006; Gilpin 2012; Griffin 2014; Heilig & Koob 2007; Heilig *et al.* 2010; Koob 2013; Leggio *et al.* 2010; Lowery & Thiele 2010; Lu & Richardson 2014; Martin-Fardon *et al.* 2010; Rivier 2014; Roberto *et al.* 2012; Ryabinin & Weitemier 2006; Ryabinin *et al.* 2012; Shalev *et al.* 2010; Silberman *et al.* 2009; Sommer & Saavedra 2008; Spanagel *et al.* 2014; Sprow & Thiele 2012; Thiele 2012; Weiss *et al.* 2001; Wong & Schumann 2012; Zorrilla *et al.* 2013, 2014). Because there is already an excellent, recent literature in this area, we do not comprehensively repeat this information in the current review. Rather, this article reviews the preclinical literature investigating the importance of the corticotropin-releasing factor (CRF) system specifically in EtOH consumption and neuroadaptation-related behaviors. We also include comments on pertinent human data and suggest future perspectives.

The CRF System

Corticotropin-releasing factor has also been known as corticotropin-releasing hormone or CRH and is a 41-amino acid neuropeptide critically involved in the regulation of neuroendocrine and behavioral responses to stress. An intricate CRF-mediated system, involving hypothalamic and extra-hypothalamic mechanisms, regulates peripheral and central actions that allow for preparation and adaptation to environmental challenges or stressors (Bale & Vale 2004; de Kloet 2013; Hauger *et al.* 2006). The seminal work of

Vale and colleagues identified CRF as the primary molecule responsible for the activation of this neuroendocrine stress cascade, the hypothalamic–pituitary–adrenal (HPA) axis (Bale & Chen 2012; Rivier & Vale 1983a,1983b; Rivier *et al.* 1982; Spiess *et al.* 1981; Swanson *et al.* 1983; Vale *et al.* 1981). Activation of the HPA axis is triggered by neurons of the medial dorsal parvocellular region of the paraventricular nucleus (PVN) of the hypothalamus (Armario 2006, 2010; Herman *et al.* 2003). This region is rich in CRF and other neuropeptides, such as vasopressin (arginine-vasopressin; AVP). Although the role of AVP in activating the HPA axis *per se* appears to be limited, AVP can significantly increase the effects of CRF (Rivier & Vale 1983a,1983b; Sawchenko *et al.* 1984; Vale *et al.* 1981, 1983).

Paraventricular nucleus neurons release CRF at the level of the median eminence, inducing (via the hypophyseal portal system) the release of ACTH by corticotrope cells of the anterior pituitary. In turn, ACTH activates the secretion of the glucocorticoid, CORT (cortisol in humans) from the zona fasciculata of the adrenal cortex. Corticosterone plays an important role in regulating a number of physiological functions and modulates CRF signaling via a hypothalamic negative feedback mechanism that decreases CRF-mediated HPA axis activation; CORT also regulates an extra-hypothalamic positive regulatory mechanism that increases CRF activity (Bale & Vale 2004; Shepard *et al.* 2006). In the mammalian brain, CRF is identified in the PVN, but high levels of CRF are also found outside of the hypothalamus in structures such as the central nucleus of the amygdala (CeA), bed nucleus of the stria terminalis (BNST), hippocampus, thalamus, midbrain and locus coeruleus (Merchenthaler *et al.* 1982, 1984; Morin *et al.* 1999; Steckler & Holsboer 1999; Swanson *et al.* 1983). Glucocorticoid-induced increases in CRF activity have been particularly well characterized in the CeA and BNST (Shepard *et al.* 2006; Tran & Greenwood-Van Meerveld 2012). The extra-hypothalamic, neuroregulatory actions of CRF contribute to the integration of endocrine, sympathetic, behavioral and cognitive responses to stress, and are particularly involved in the emotional component of stress (Gilpin 2012; Hauger *et al.* 2006; Müller *et al.* 2003; Walker & Davis 2008; Walker *et al.* 2009). Although stressors initiate a series of CRF-mediated neuronal responses that can be beneficial and adaptive, dysregulation of CRF systems can be deleterious, and has been linked to a wide range of disorders including anxiety, depression, obsessive-compulsive disorder, post-traumatic stress disorder and addiction (Cador *et al.* 1993; Cole *et al.* 1990; Haass-Koffler & Bartlett 2012; Heilig & Egli 2006; Koob & Kreek 2007; Koob & Le Moal 2001; Sarnyai *et al.* 2001).

CRF system endogenous ligands, binding targets and pathways

Figure 1 summarizes the endogenous ligands, targets and target distribution, and illustrates the affinity of each CRF family neuropeptide for each CRF-related binding target. CRF actions are exerted through two Gs-protein coupled receptors, CRF type-1 (CRF_1) and type-2 (CRF_2), which share about 70% amino acid sequence identity (Bale & Vale 2004; Hauger *et al.* 2006). Corticotropin-releasing factor shows greater affinity for CRF_1 (Herman *et al.* 2003; Vaughan *et al.* 1995) and CRF-initiated activation of the HPA axis is mediated by CRF_1 (Armario 2006; Bale & Vale 2004). Corticotropin-releasing factor type-1 expression is found in many brain regions (Justice *et al.* 2008; Korosi *et al.* 2006, 2007; Kühne *et al.* 2012; Van Pett *et al.* 2000). In the cortex and hippocampus, CRF_1 is present on glutamatergic neurons, whereas CRF_1 is found on γ-aminobutyric acid (GABA) neurons in the striatum (including the nucleus accumbens; NAcc) and dopamine (DA) neurons in the midbrain (including the ventral tegmental area; VTA) (Bonfiglio *et al.* 2011; Lemos *et al.* 2012; Refojo *et al.* 2011). Corticotropin-releasing factor type-2 is also widely expressed in the central nervous system and found peripherally (Bittencourt & Sawchenko 2000; Korosi *et al.* 2006, 2007; Lukkes *et al.* 2011; Palchaudhuri *et al.* 1999; Van Pett *et al.* 2000). Although there is significant overlap in brain distribution of CRF_1 and CRF_2 (Hauger *et al.* 2006; Lukkes *et al.* 2011), important differences in distribution have also been found. For example, CRF_2, but not CRF_1, is present in the ventromedial and medial preoptic nuclei of the hypothalamus; CRF_1, but not CRF_2, is expressed in the NAcc and the CeA; and both CRF_1 and CRF_2 are present in the medial nucleus of the amygdala (Bittencourt & Sawchenko 2000; Hauger *et al.* 2006; Van Pett *et al.* 2000). A primary CRF_2-mediated regulation of serotonergic neurons in the dorsal raphe (DR), with implications for anxiety and depression, has also been described (Hauger *et al.* 2006; Meloni *et al.* 2008).

Corticotropin-releasing factor also binds to CRF-binding protein (CRF-BP), which is found centrally and peripherally (Alderman & Bernier 2007; Manuel *et al.* 2014; Potter *et al.* 1992). Several central locations are listed in Figure 1. The CeA is a particularly CRF-BP dense region (Alderman & Bernier 2007; Potter *et al.* 1992). Some of the proposed functions of CRF-BP are to restrict transport/release of CRF in some centrally located pathways (Potter *et al.* 1992), aid in protecting CRF from degradation once it has been released (Seasholtz *et al.* 2002) and modulate CRF-induced potentiation of glutamate receptor function via CRF_2 actions (Ungless *et al.* 2003).

The complexity of the CRF system is further increased by the existence of additional endogenous agonists. Corticotropin-releasing factor receptors can be activated by the urocortin (Ucn) family of neuropeptides: Ucn_1, Ucn_2 and Ucn_3. $Urocortin_1$ binds with similar affinity to CRF_1, CRF_2 and CRF-BP, whereas Ucn_2 and Ucn_3 bind primarily to CRF_2 (Bittencourt *et al.* 1999; Lewis *et al.* 2001; Reyes *et al.* 2001; Ryabinin *et al.* 2012; Vaughan *et al.* 1995). $Urocortin_1$ is predominantly expressed in the centrally projecting Edinger–Westphal (EWcp) nucleus (Bittencourt *et al.* 1999; Kozicz *et al.* 1998; Ryabinin *et al.* 2005; Vaughan *et al.* 1995). Note that two divisions of the EW have been named EWcp and EWpg (preganglionic), based on cell groups and projections (Kozicz *et al.* 2011). Cells in the EWcp contain stress- and feeding-related neuropeptides, such as Ucn_1, whereas the EWpg contains neurons that control oculomotor function and send cholinergic inputs to the ciliary ganglion. $Urocortin_2$ and Ucn_3 are more widely distributed than Ucn_1. Among other structures, Ucn_2 is present in the hypothalamus (PVN

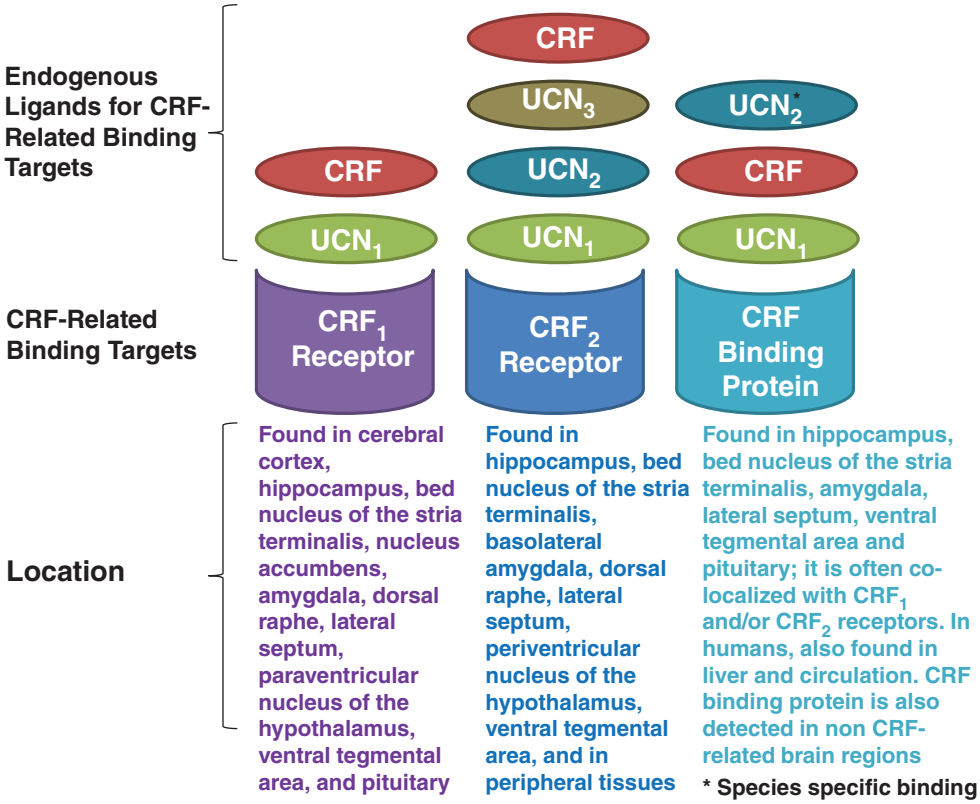

Figure 1: Binding relationships of CRF-family peptides and their targets. CRF binds with high affinity to CRF_1 and CRF-BP and with lower affinity to CRF_2. Ucn_1 binds with high affinity to CRF_1, CRF_2 and CRF-BP. Ucn_2 and Ucn_3 are selective for CRF_2 in all species; Ucn_2 has an affinity for CRF-BP in certain species. Those with the highest affinity for the binding target are placed closest to that target while those with the lowest affinity are placed farthest away. The locations shown for CRF_1, CRF_2 and CRF-BP are not inclusive, but are those most relevant to this review. For additional information, see De Souza (1995); Hauger *et al.* (2006); Huising *et al.* (2008); Kühne *et al.* (2012).

and arcuate nucleus) and the locus coeruleus, and Ucn_3 is expressed in several brain structures, including the BNST and the medial nucleus of the amygdala (Cavalcante *et al.* 2006; Deussing *et al.* 2010; Lewis *et al.* 2001; Li *et al.* 2002; Reyes *et al.* 2001; Tanaka *et al.* 2003).

The CRF system has a key role in mood disorders (Aubry 2013; Kormos & Gaszner 2013). Activation of CRF_1 and CRF_2 has been associated with negative emotionality, anxiety-like behavior and the behavioral responses to stress, with CRF_1 thought to be responsible for the initiation of such responses and CRF_2 mediating termination and recovery (Coste *et al.* 2006; Hauger *et al.* 2006; Janssen & Kozicz 2013). The roles of CRF_1 and CRF_2 in behavior have also been interpreted with regard to their involvement in responses to (real or perceived) escapable vs. inescapable stressors. For example, CRF_1 mediates active defensive responses to escapable stressors, and CRF_2 mediates responses to inescapable, uncontrollable stressors that could be associated with anxiety and depression vulnerability (Hauger *et al.* 2006). The involvement of CRF and Ucn peptides in stress-induced feeding behavior has received considerable attention (Stengel & Tache 2014), and mounting evidence is supporting involvement of CRF and Ucns in different aspects of social behavior (for a review, see Hostetler & Ryabinin 2013).

Overall, CRF systems play an important role in regulating a number of functions with key implications for adaptive behavior, motivation and emotion. Over the last three decades, special emphasis has been placed on the understanding of the behavioral relevance of stress- and drug-induced long-term changes in CRF system neurophysiology. This brief description does not do justice to a rich literature pertaining to a wide range of behaviors that involve CRF_1 and CRF_2 signaling, and the reader is referred to the reviews cited above. In the next section, we focus on the role of CRF and its receptor in the context of addiction, and specifically its importance in EtOH intake, changes in intake and behavioral traits that reflect neuroplasticity induced by chronic EtOH exposure. Figure 2 illustrates some of the central CRF-related neurocircuitry that may be involved in EtOH-related phenotypes discussed in this review. For example, the CeA and BNST play important roles in negative emotional states that drive chronic EtOH use in some individuals; the basolateral amygdala (BLA) further affects this circuit. The periaqueductal gray, in its role as an important functional interface between

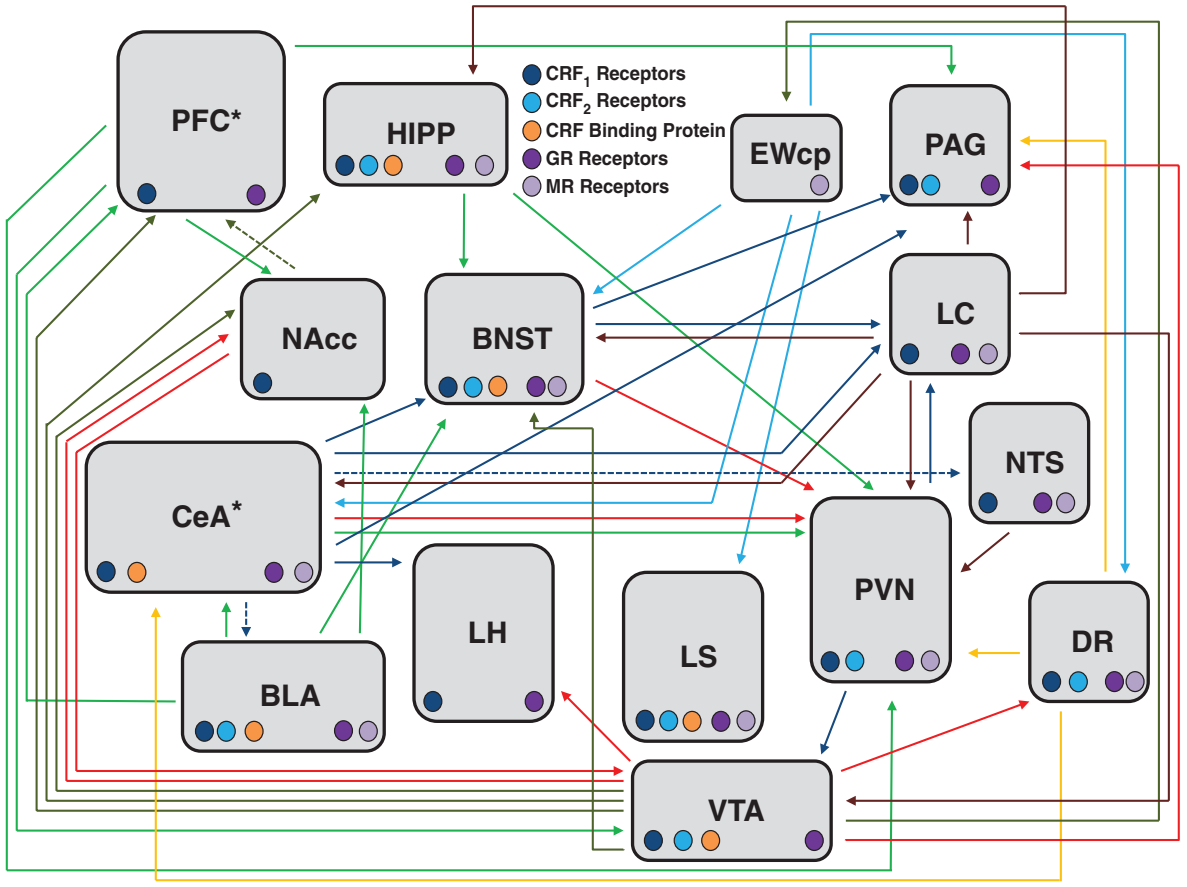

Figure 2: Diagram of central CRF-related neurocircuitry and interactions with other neurotransmitter systems. In this figure, we concentrate on the CRF neurocircuitry that we discuss in this article in relationship to EtOH drinking and neuroadaptation-related phenotypes; however, not all potentially relevant regions and pathways are represented. Colored circles within each brain region denote the CRF-related receptor or CRF-BP that is found in that region, with colors defined in the figure. Lines and arrows indicate the projections from one specific brain region to another, with the color denoting the primary transmitter or peptide. CRF projection, solid dark blue line and arrow; speculated CRF projections, dashed dark blue line and arrow; DA projection, solid dark green line and arrow; GABA projection, solid red line and arrow; glutamate projection, solid green line and arrow; norepinephrine projection, solid brown line and arrow; serotonin projection, solid yellow line and arrow; Ucn_1 projection, solid light blue line and arrow. Brain regions: BLA, basolateral nucleus of the amygdala; HIPP, hippocampus; LH, lateral hypothalamus; LS, lateral septum; NTS, nucleus of the solitary tract; PFC, prefrontal cortex; . * denotes that there are multiple divisions within this region that contain varying levels of each of the noted binding targets. These subdivisions may inferentially alter the roles CRF plays in EtOH-related behaviors. For additional information, see Ahima *et al.* (1991); Bittencourt *et al*. (1999); Brown (1986); Cowen *et al.* (2004); Duvarci and Pare (2014); George and Koob (2010); Gilpin (2012); Gray and Magnuson (1992); Haass-Koffler and Bartlett (2012); Handa and Weiser (2014); Hauger *et al*. (2006); Justice *et al*. (2008); Korosi *et al*. (2006); Kühne *et al.* (2012); Lu and Richardson (2014); Myers *et al*. (2014); Pitts *et al.* (2009); Potter *et al*. (1992); Radley (2012); Reul and Holsboer (2002); Reyes *et al.* (2008); Ryabinin and Weitemier (2006); Silberman and Winder (2013); Silberman *et al*. (2013); Sinha (2008); Van Pett *et al.* (2000); Wise and Morales (2010).

the forebrain and lower brainstem, has a probable effect as an integrator of behavioral responses to stressors, both internal and external. The prefrontal cortex has well-known executive functions that affect not only craving and habit formation via interactions with other brain nuclei, such as the dorsal striatum (not shown here), but also basic reinforcement and conditioned reinforcement via the NAcc shell and core, respectively, which sustain use and impact relapse. Also, receptor types found in the included brain regions and transmitters in neural pathways that direct communication are included in Fig. 2. For example, the PVN is a critical regulator of stress responses and is modulated by a serotonergic projection from the DR. For additional important information, the reader is referred to papers and figures that consider disorders that are co-morbid with addiction (Gilpin 2014; Reul & Holsboer 2002) and articles that discuss important functional differences of sub-regions of structures, such as the prefrontal cortex (George & Koob 2010; Lu & Richardson 2014; Marchant *et al.* 2014) and CeA (Duvarci & Pare 2014; Gilpin 2014).

Addiction and the CRF system: a common pathway for drugs of abuse

One psychopathology commonly associated with CRF dysregulation and stress is drug addiction. All drugs of abuse, regardless of specific mechanism of action, induce activation of CRF signaling and the HPA axis (for reviews, see Armario 2010; McReynolds *et al.* 2014), and their effects are modulated by stress (Aguilar *et al.* 2013; Picetti *et al.* 2013; Roberts *et al.* 1995; Stephens & Wand 2012). Additionally, these addictive substances produce important CRF-mediated and stress-influenced long-lasting neuroadaptations that have been suggested to explain key aspects of the development and maintenance of the addictive phenotype (Koob 2013; Koob & Le Moal 2001; Leyton & Vezina 2014; Robinson & Berridge 1993, 2008; Wise & Koob 2014; Zorrilla *et al.* 2014).

All abused drugs sensitize mesolimbic DA mechanisms and induce behavioral sensitization to their stimulant effects; in fact, behavioral, or psychomotor, sensitization has been used extensively as a measurable phenotype of such underlying neuroplasticity (Robinson & Berridge 1993, 2008; Sanchis-Segura & Spanagel 2006). Mesocorticolimbic DA signaling has been associated with different components of positive reinforcement and reward processes, including activation, motivation, incentive salience, 'wanting' (but not necessarily 'liking'), effort, goal-directed behavior and reward-related learning (Berridge & Kringelbach 2013; Salamone & Correa 2012; Schultz 2013; but also see Wise 2008). Long-lasting upregulation of DA mechanisms has been linked to unmanageable pathological motivation and compulsive drug seeking and taking characteristic of addiction. Evidence indicates that stress produces a CRF-mediated activation of DA systems that is comparable to that induced by addictive substances (Sinha 2008). This is, moreover, an effect that appears to be especially critical during adolescence, a time when maturing DA systems show increased sensitivity to stress hormones (Burke & Miczek 2014; Sinclair *et al.* 2014). Cross-sensitization between stressors and addictive drugs, including EtOH (Roberts *et al.* 1995), has also been described. Additionally, research on humans has shown that stress elevates striatal extracellular DA levels (Adler *et al.* 2000; Soliman *et al.* 2008). Stress and CRF activation can therefore be understood as key facilitators of drug-induced neuroplasticity in mesocorticolimbic DA systems associated with dysregulation of positive reinforcement mechanisms in addiction.

Abused drugs and stress also produce enduring changes within CRF systems. Long-lasting dysregulation of extra-hypothalamic CRF mechanisms (primarily extended amygdala, and also prefrontal cortex neurocircuitry) have been linked to the negative emotionality, anxiety and vulnerability to stress seen in addicts (Gerra *et al.* 2014; Thorberg & Lyvers 2006; Valdez & Koob 2004). As discussed in greater detail below for EtOH, drug-induced activation of brain stress response systems sensitizes over time, especially with repeated withdrawal, and this sensitization has been seen to persevere into protracted abstinence, critically contributing to the persistence of relapse. Extra-hypothalamic CRF dysregulation is a key biological mechanism underlying manifestation of negative emotional states associated with drug abstinence, even well beyond the time when physical symptoms of withdrawal are seen (Koob 2014; Koob & Le Moal 2008). However, important HPA axis-dependent effects, such as upregulation of glucocorticoid receptors (GRs) in the CeA, associated with protracted EtOH abstinence, indicate that both hypothalamic and extra-hypothalamic mechanisms interact and participate in critical aspects of long-lasting drug-induced neuroadaptation (Vendruscolo *et al.* 2012). Overall, strong scientific support suggests that dysregulation of positive and negative reinforcement mechanisms, which underlie pathological motivation associated with drug craving and increased negative emotionality and vulnerability to stress, critically involve the CRF system.

Corticotropin-releasing factor and stress-axis involvement have received particular attention in the context of the investigation of the neurobiological effects of EtOH. At a neurophysiological level, laboratory animal and human research show that systemic administration of EtOH increases CRF and induces HPA axis activation (Jenkins & Connolly 1968; Pastor *et al.* 2008, 2011; Rivier 1996). Although the precise mechanism by which EtOH stimulates stress systems and hormones is yet to be fully described, growing evidence indicates that this is a central, CRF/CRF_1-mediated effect (recently reviewed by Armario 2010). Convincing support for this conclusion arises from a number of studies showing HPA axis activation with systemic or intracerebroventricular (ICV) administration of EtOH (Lee *et al.* 2004; Ogilvie *et al.* 1998), as well as a blunted HPA response to EtOH in CRF_1 null mutant mice (Lee *et al.* 2001b; Pastor *et al.* 2008) or after administration of a CRF antiserum (Rivier *et al.* 1984).

Hypothalamic regulation of glucocorticoids is altered by a history of EtOH exposure; human and rodent data show that repeated EtOH produces an increase in baseline levels of CORT, with a flattening of natural glucocorticoid circadian level variations and a diminished response to stress challenges (Errico *et al.* 1993; Lee & Rivier 1997; Lee *et al.* 2001a; Rasmussen *et al.* 2000; Wand & Dobs 1991). However, the effects of chronic EtOH and EtOH withdrawal on CRF systems are complex and depend on EtOH administration procedures, time of measurement and whether other stressful stimuli are included in the study design (for a review, see Allen *et al.* 2011). For example, one study examined the effect of 14 days of continuous EtOH vapor exposure in Sprague–Dawley rats and found a decrease in the number of CRF-binding sites in the pituitary when tissue was taken immediately after withdrawal from EtOH (Dave *et al.* 1986). However, in another study, again in Sprague–Dawley rats, 7 days of continuous vapor exposure were associated with decreased hypothalamic CRF content in tissue obtained immediately after withdrawal (Rivier *et al.* 1984). A change in the same direction in these two studies suggests that the decrease in receptors was not a compensatory change; however, the methods were not identical in the two studies, complicating interpretation. A number of studies examining CRF-related effects have used intermittent, rather than continuous, vapor exposure procedures. For example, using a 6 h/day, 8-day EtOH vapor exposure period,

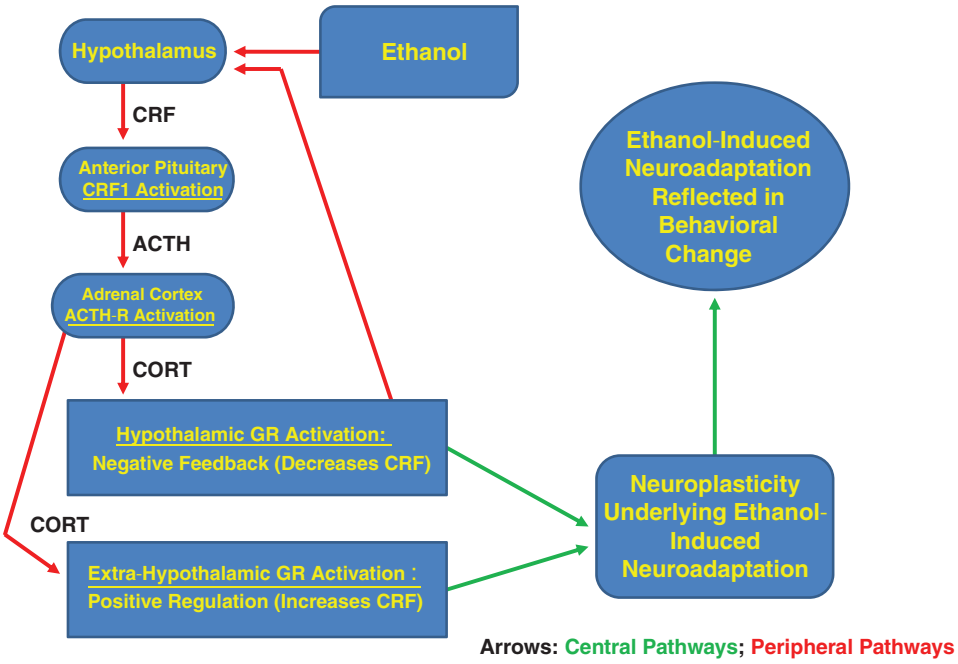

Figure 3: The HPA axis and central CRF processes in EtOH-induced neuroadaptations. EtOH activates the HPA axis and induces a well-known cascade of events: CRF is released from the hypothalamus and binds to CRF_1 in the anterior pituitary, resulting in ACTH release; ACTH receptor (ACTH-R) activation results in CORT release from the adrenal cortex. Hypothalamic GR activation reduces CRF release via a negative feedback loop. CORT also regulates an extra-hypothalamic positive regulatory mechanism that increases CRF activity. GR activation plays a role in EtOH-induced neuroadaptation, with a role for long-lasting changes in hypothalamic and extra-hypothalamic structures.

CRF stores in the external zone of the median eminence of Sprague–Dawley rats were decreased; tissue was taken ~12 h after withdrawal (Lee *et al.* 2000). Criado *et al.* (2011) exposed Wistar rats to EtOH vapor for 14 h/day for either 2 or 8 weeks and then examined CRF immunoreactivity in the amygdala, frontal cortex, hippocampus and parietal cortex immediately, 24 h or 2 weeks after withdrawal from EtOH vapors. No significant effects were found in the rats exposed for 2 weeks. However, increased CRF immunoreactivity was found in the hippocampus and parietal cortex of rats exposed for 8 weeks, when examined 24 h or 2 weeks, but not immediately, after withdrawal. These and other data not reviewed here (Koob & Zorrilla 2010; Läck et al. 2005; Richardson et al. 2008a; Sommer et al. 2008; Uhart & Wand 2009) show that effects of chronic EtOH exposure on CRF systems cannot be defined without careful consideration of methodological details. Furthermore, factors such as sex, species and age of stress or EtOH exposure should be considered (Logrip *et al.* 2013; Przybycien-Szymanska *et al.* 2010, 2011; Silva *et al.* 2009; Van Waes *et al.* 2011).

At a behavioral level, EtOH consumption, abuse and relapse have been observed to be critically modulated by CRF and stress, and there is a high incidence of co-morbidity between alcoholism and stress-associated disorders such as anxiety and depression (Boden & Fergusson 2011; Haass-Koffler *et al.* 2014; Lijffijt *et al.* 2014). Furthermore, abnormally high levels of CORT, a condition known as pseudo-Cushing's syndrome (Kirkman & Nelson 1988), are frequently found in alcoholics. Laboratory animal research has provided support for participation of the CRF system in stress-induced changes in EtOH effects and in EtOH-induced neuroadaptations that are reflected in behavioral changes (Fig. 3). In the following sections, we review literature focusing on the involvement of stress, and components of the CRF system, in EtOH consumption and the behavioral aspects of EtOH-induced neuroadaptation. Tables 1 and 2 list many of the studies in these areas, with Table 1 providing references, trait information and results for knockout (KO) and transgenic mouse studies, and Table 2 providing detailed information for pharmacological studies. We do not exhaustively review the literature on the effects of EtOH on CRF-related peptide levels; that is beyond the scope of this review. We refer to specific literature, but the reader is referred to the tables for details such as animal species, genotype and methods associated with EtOH and other drug treatments.

The role of CRF systems in EtOH intake

A large number of studies have supported a role for CRF and CRF-related systems in EtOH intake, which has led to considerable interest in the potential of CRF-related pharmaceuticals as treatments for alcohol use disorders (Egli 2005; Heilig & Koob 2007; Zorrilla *et al.* 2013). EtOH intake has been examined using multiple procedures, including two-bottle choice continuous access, operant self-administration to get

Table 1: Studies of EtOH drinking and neuroadaptation in KO and transgenic mice

Gene	Reference	Sex/background	Trait	Results
CRF	Olive *et al.* (2003)	129S2/SvPas × C57BL/6J	EtOH drinking; two-bottle choice, 23 h/day for 16 days (2–10% EtOH); or 2 h choice (10% EtOH) under 22 h/day fluid restriction for 3 days; EtOH-induced conditioned place preference for four EtOH conditioning trials (2 or 3 g/kg EtOH)	CRF KO mice consumed more EtOH than WT control mice in both 23 and 2 h access conditions. The conditioned rewarding effect of 2 g/kg EtOH was absent in KO mice, but present in WT. The genotypes showed equivalent conditioned rewarding effects of 3 g/kg EtOH
CRF	Kaur *et al.* (2012)	129S2/SvPas × C57BL/6J; male and female	EtOH drinking; single-bottle DID 2 h/day for 3 days, then 4 h/day on day 4 (20% EtOH)	CRF KO mice had reduced EtOH intake and BEC, compared with WT controls
CRF	Pastor *et al.* (2012)	129Sv/J × C57BL/6J	EtOH-induced locomotor sensitization; IP 2.5 g/kg EtOH once daily for 10 days, then IP 1.5 g/kg EtOH challenge and locomotor test; BEC and CORT levels	CRF KO mice did not develop EtOH-induced locomotor sensitization, whereas WT mice did; CRF KO mice had drastically reduced CORT plasma levels, compared with WT controls. BEC levels did not differ
CRF overexpression transgenic	Palmer *et al.* (2004)	C57BL/6J × SJL	EtOH drinking; two-bottle choice continuous access for 16 days (3–20% EtOH)	Transgenic mice consumed significantly less EtOH than their non-transgenic littermates. Older transgenic mice drank less EtOH than younger transgenic mice
CRF_1	Sillaber *et al.* (2002)	129/SvJ × 129/Ola × CD1; male	EtOH drinking; two-bottle choice continuous access (2–8% EtOH for 18 days; then 8% EtOH for up to 9 months); exposure to swim and social defeat stress at 2 and 3 months	There was no initial difference in EtOH consumption between KO and WT mice; KO mice exposed to stress at 2 and 3 months consumed more EtOH than WT mice at 4–9 months. There was no stress effect on WT mice
CRF_1	Nie *et al.* (2004)	C57BL/6J × 129Sv	GABA neurotransmission; brain slice electrophysiology	CRF (100 nM) or EtOH (44 mM) did not enhance GABA-mediated neurotransmission in the CeA in CRF_1 KO mice, but did in WT mice
CRF_1	Chu *et al.* (2007)	129/Ola × CD1	EtOH self-administration training then EtOH liquid diet for 14 days (2–4% EtOH); EtOH WD effects on operant EtOH self-administration for 10 subsequent days	CRF_1 KO mice did not display EtOH WD-induced increases in EtOH self-administration, but WT mice did
CRF_1	Pastor *et al.* (2008)	129SV/J × C57BL/6J	EtOH-induced locomotor sensitization; IP 2.5 g/kg EtOH once daily for 10 days, then IP 1.5 g/kg EtOH challenge and locomotor test; BEC and CORT levels	CRF_1 KO mice did not show the EtOH-induced locomotor sensitization seen in WT mice, and had a blunted CORT response to EtOH. BEC levels did not differ

Table 1: Continued

Gene	Reference	Sex/background	Trait	Results
CRF_1	Pastor *et al.* (2011)	129SV/J × C57BL/6J	EtOH drinking; two-bottle choice continuous access for 16 days (3–20% EtOH); in a separate study, two-bottle intermittent access for 47 days (3–10% EtOH, and 21 h/day); swim stress effects on EtOH drinking	EtOH intake (20% EtOH concentration only) was lower in CRF_1 KO mice compared with WT during continuous access; repeated swim stress, but not acute swim stress, resulted in higher levels of 21 h/day EtOH consumption in WT mice, but not CRF_1 KO mice
CRF_1	Molander *et al.* (2012)	129/SvJ × 129/Ola × CD1; male	EtOH drinking; two-bottle choice continuous access for ~5 months (2–8% EtOH); EtOH vapor (four cycles of 16 h/day exposure); swim and social defeat stress effects on EtOH drinking	There was no initial difference in EtOH consumption between KO and WT mice; CRF_1 KO mice displayed greater social defeat-induced, but not forced swim stress-induced, increased EtOH intake, as well as greater EtOH WD-induced increases in EtOH intake, compared with WT controls
CRF_1	Kaur *et al.* (2012)	129/Ola × CD1; male and female	EtOH drinking; single-bottle DID 2 h/day for 3 days, then 4 h/day on day 4 (20% EtOH)	CRF_1 KO mice had lower EtOH intake and BEC, compared with WT mice
CRF_1	Giardino and Ryabinin (2013)	129/Ola × CD1 backcrossed to C57BL/6J	EtOH drinking; two-bottle DID 2 h/day for 3 days, then 4 h/day on day 4 (15% EtOH); water and food intake	EtOH intake was lower in CRF_1 KO mice, compared with WT mice; water intake and total caloric intake were also lower
$CRF_1^{NestinCre}$	Molander *et al.* (2012)	129S2/Sv × SJL × C57BL/6J	EtOH drinking; two-bottle choice continuous access for ~5 months (2–8% EtOH); EtOH vapor (four cycles of 16 h/day exposure); swim and social defeat stress effects on EtOH drinking	There was no initial difference in EtOH consumption between $CRF_1^{NestinCre}$ KO and WT mice. Stress-induced increases in EtOH consumption were lower in $CRF_1^{NestinCre}$ KO, compared with controls, and $CRF_1^{NestinCre}$ KO mice did not display EtOH WD-induced increases in EtOH intake, whereas controls did
CRF_2	Nie *et al.* (2004)	C57BL/6J × 129	GABA neurotransmission; brain slice electrophysiology	CRF (100 nM) and EtOH (44 mM) each enhanced GABA-mediated neurotransmission in the CeA in both WT and CRF_2 KO mice
CRF_2	Sharpe *et al.* (2005)	129X1/SvJ × C57BL/6J	EtOH drinking; two-bottle choice continuous access for 16 days (3–20% EtOH); in a separate study, single-bottle DID (0.6–10% EtOH; 30 min/day for first 14 days and then 2 h/day for 6 days at 10% EtOH)	EtOH consumption was slightly reduced in CRF_2 mice, compared with WT littermates, at 7.5% and 10% concentrations, during limited access only
CRF_2	Giardino *et al.* (2011)	129X1/SvJ x C57BL/6J	EtOH-induced conditioned place preference (IP 2 g/kg EtOH for 4 EtOH conditioning trials)	The conditioned rewarding effect of EtOH was absent in CRF_2 KO mice, compared with WT
CRF_2	Kaur *et al.* (2012)	129X1/SvJ × C57BL/6J; male and female	EtOH drinking; single-bottle DID 2 h/day for 3 days, then 4 h/day on day 4 (20% EtOH)	CRF_2 KO mice had slightly reduced EtOH intake on the first day, compared with WT mice; this difference was not sustained on subsequent days and not accompanied by differences in BEC

Table 1: Continued

Gene	Reference	Sex/background	Trait	Results
$CRF_{1/2}$	Pastor *et al.* (2008)	C57BL/6J × 129SV/J	EtOH-induced locomotor sensitization; IP 2.5 g/kg EtOH once daily for 10 days, then IP 1.5 g/kg EtOH challenge and locomotor test; BEC and CORT levels	$CRF_{1/2}$ KO mice did not show the EtOH-induced locomotor sensitization seen in WT mice and had a blunted CORT response to EtOH. BEC levels did not differ
$CRF_{1/2}$	Pastor *et al.* (2011)	C57BL/6J × 129SV/J	EtOH drinking; two-bottle choice for 47 days (3–10% EtOH, 21 h/day); swim stress effects on EtOH drinking	Repeated swim stress, but not acute swim stress, resulted in higher levels of EtOH consumption in WT, but not in $CRF_{1/2}$ KO mice.
Ucn_1	Pastor *et al.* (2008)	C57BL/6J × 129SV/J	EtOH-induced locomotor sensitization; IP 2.5 g/kg EtOH once daily for 10 days, then IP 1.5 g/kg EtOH challenge and locomotor test	Ucn_1 KO mice displayed normal EtOH-induced locomotor sensitization
Ucn_1	Giardino *et al.* (2011)	129X1/SvJ × C57BL/6J	EtOH drinking; two-bottle choice continuous access for 16 days (3–10% EtOH)	Ucn_1 KO mice consumed less of a 6%, but not 3% or 10%, solution, compared with WT mice; KO mice showed reduced preference for both the 6% and 10% EtOH concentrations, compared with WT mice
Ucn_1	Giardino *et al.* (2011)	129X1/SvJ × C57BL/6J	EtOH-induced conditioned place preference and aversion (IP 2 g/kg EtOH for four EtOH conditioning trials)	The conditioned rewarding effect of EtOH was absent in Ucn_1 KO mice, compared with WT; sensitivity to the conditioned aversive effect of EtOH was equivalent in the KO and WT mice
Ucn_1	Kaur *et al.* (2012)	129X1/SvJ × C57BL/6J; male and female	EtOH drinking; single-bottle DID 2 h/day for 3 days, then 4 h/day on day 4 (20% EtOH)	Ucn_1 KO mice did not differ from WT mice in EtOH intake or BEC

BEC, blood ethanol concentration; WD, withdrawal.

at strength of reinforcement and reinstatement of EtOH seeking (discussed in greater detail in the section on the role of CRF systems in EtOH-induced neuroadaptation), and limited access 'drinking in the dark' (DID) procedures to obtain binge-like levels of intake. Papers and findings are listed in Tables 1 and 2, and several reviews have covered much of the literature (see reviews cited above). We highlight some of the findings here.

Single gene mutant mice

Only four papers had been published using single gene manipulations in mice to examine the influence of CRF-related genes on EtOH intake by the time of a 2006 general review of EtOH-related genes (Crabbe *et al.* 2006). Since then, many additional papers have appeared (Table 1). In the initial study examining EtOH intake in CRF KO mice, KO mice consumed more EtOH than did wild-type (WT) mice in both a 24-h continuous access procedure and a limited access procedure (Olive *et al.* 2003). The opposite phenotype was found in CRF overexpression mice that were examined for their continuous access EtOH drinking phenotype (Palmer *et al.* 2004). However, more recently, Kaur *et al.* (2012) reported reduced EtOH intake in CRF KO mice in a binge-like DID study. The opposite findings in CRF KO mice could be related to the role of CRF in procedures in which EtOH intake is generally lower (Olive *et al.* 2003) vs. higher (Kaur *et al.* 2012).

Several studies have examined the role of CRF_1 using KO mice, and results have not been entirely in agreement. The first study examining EtOH intake in constitutive CRF_1 KO mice found no initial effect, but reported a long-term increase in continuous access EtOH consumption in CRF_1 KO mice after repeated stress exposure that was not seen in their WT controls (Sillaber *et al.* 2002). In that report, initial EtOH intake levels were low (~1 g/kg/24 h) and stress-induced levels remained relatively low (<4 g/kg/24 h). A more recent paper, using the same KO mice, obtained data that are in agreement with those findings (Molander *et al.* 2012). However, other studies have found reduced EtOH intake in constitutive CRF_1 KO mice, specifically when EtOH was offered at higher

Table 2: Pharmacological studies involving the CRF system and EtOH drinking and neuroadaptation

Receptor, drug type	Drug	Reference	Model	Treatment or trait	Finding
$CRF_{1/2}$, agonist	CRF	Ehlers *et al.* (1992)	P and NP rats	No EtOH treatment; drug given 25 min before EEG recording	ICV CRF (0.15 nmol, once) to P rats resulted in a significant increase in EEG theta waves in the frontal cortex, compared with data after vehicle. This was not seen in NP rats. When compared with NP rats, P rats had lower concentrations of CRF in the hypothalamus, amygdala, prefrontal cortex and cingulate cortex
	CRF	Bell *et al.* (1998)	Long Evans rats	EtOH drinking; single-bottle 1 h/day for 38 days (2–8% EtOH), then two-bottle choice for 1 h/day for 7 days (8% EtOH)	ICV CRF (0.5 and 5 μg/1.5 μl) dose-dependently decreased EtOH consumption during the two-bottle choice limited access procedure; 24-h food intake was also reduced
	CRF	Le *et al.* (2002)	Wistar rats	EtOH drinking; two-bottle choice for 30 min/day for 25 days (3–12% EtOH); then operant EtOH self-administration (12% EtOH) for 1 h/day for 18–25 days; extinction for 5–9 days; CRF given 15 min before reinstatement session	ICV (300 and 1000 ng) or intra-MRN (3 and 10 ng) CRF reinstated EtOH seeking
	CRF	Overstreet *et al.* (2004)	Sprague–Dawley rats	EtOH liquid diet for 5 days (7% EtOH); CRF treatment occurred twice, 6 days and 1 day prior to EtOH liquid diet exposure; social interaction testing occurred 30 min after the first CRF exposure and 5–6 h after WD	Social interaction is used to index anxiety-related behavior and is sensitive to EtOH WD and stress. ICV CRF (1 μg) reduced social interaction after the first treatment, prior to EtOH exposure; ICV CRF also reduced social interaction in EtOH WD rats
	CRF	O'Callaghan *et al.* (2005)	'High preference' and 'low preference' mice	EtOH drinking; 24-h two-bottle choice for 3 weeks (8% EtOH) to establish drinking, then CRF was given and intake was measured after 12, 24 and 36 h	ICV CRF (5 μg in 2 μl, once) did not alter EtOH preference in high or low preference mice
	CRF	Thorsell *et al.* (2005)	Wistar rats	Single-bottle 25 min/day EtOH access (2–10% EtOH), using sucrose fading for 3–4 weeks; EtOH vapor 14 h/day for 8 weeks; reestablishment of EtOH drinking for 7–10 sessions, then treatment effects examined 30 min after CRF administration	ICV CRF (1 μg in 5 μl) reduced EtOH intake in both control and EtOH vapor exposed rats
	CRF	Gabriel *et al.* (2006)	Sprague–Dawley rats	Prenatal EtOH liquid diet exposure throughout gestation (36% EtOH-derived calories); elevated plus maze testing at 60–90 days of age, 30 min after CRF infusion	Treatment with CRF (0.75–2 μg) had anxiogenic effects across prenatal treatment groups, with some effects being more profound in rats prenatally exposed to EtOH
	CRF	Weitemier and Ryabinin (2006)	C57/BL6J mice	EtOH drinking for one week; two-bottle choice continuous access (2.5% and then 5%); then cannulation surgery; then re-establishment of EtOH drinking (5%); effect of CRF 2 h after infusion	Intra-DR CRF (20 pmol in 50 nl) had no effect on the intake of EtOH, food or water during a 14-h period, beginning 2 h after treatment

Table 2: Continued

Receptor, drug type	Drug	Reference	Model	Treatment or trait	Finding
	CRF	Ryabinin *et al.* (2008)	C57BL/6J mice	EtOH drinking; single-bottle DID 2 h/day for 3 days (20% EtOH); CRF effect examined on day 3 of EtOH drinking ~15 min after infusion	Intra-lateral septum CRF (6–60 pmol) dose-dependently suppressed both water and EtOH intake
	CRF	Huang *et al.* (2010)	Sprague–Dawley rats	CRF infused on days 6 and 12, while on control diet, followed by EtOH liquid diet for 5 days (4.5% EtOH); social interaction test and locomotor activity assessed 5 h after EtOH WD	Social interaction is used to index anxiety-related behavior and is sensitive to EtOH WD and stress. CRF (0.045–0.5 μg/site in 0.5 μl) microinjected into the CeA, BLA, DRN, or dorsal BNST, exacerbated reductions in social interactions seen in EtOH WD rats; there were no effects in non-EtOH exposed rats. This effect was not found for several other brain regions. Locomotor activity was not affected
	CRF	Knapp *et al.* (2011a)	Inbred alcohol-preferring (iP) rats	EtOH drinking; single-bottle continuous access for 3 days (10% EtOH) then two-bottle choice continuous access for 15 days or 15 days with 2 days of WD every 5 days (10% EtOH); CRF given ~4 h after WD during the first two EtOH WD periods; drinking examined in subsequent 5-day periods; social interaction measured 5–6 h after the final (third) EtOH WD	Social interaction is used to index anxiety-related behavior and is sensitive to EtOH WD and stress. Intra-NAcc CRF (0.5 μg) exacerbated WD-increased drinking; this effect was not seen for the CeA, DRN, VTA or PVN. Intra-amygdala and intra-DRN CRF reduced social interaction time during EtOH WD; this effect was not seen for the NAcc, VTA or PVN
	CRF	Zhao *et al.* (2013)	Sprague–Dawley rats	IP 3 g/kg/day EtOH for 28 days; beginning 3 days after EtOH WD, acupuncture given for three consecutive days; CRF infused 5 min after third acupuncture treatment and rats tested on the elevated plus maze for 5 min, beginning 1 min after infusion	EtOH WD group spent less time on the open arms of the plus maze; acupuncture increased open arm time to non-WD group levels; intra-CeA CRF (0.2 μg in 0.2 μl/side) blocked this anxiolytic effect of acupuncture during EtOH WD
$CRF_{1/2}$, agonist	Ucn_1	Weitemier and Ryabinin (2006)	C57/BL6J mice	EtOH drinking for 1 week; two-bottle choice continuous access (2.5% and then 5%); then cannulation surgery; then re-establishment of EtOH drinking (5%); effect of Ucn_1 2 h after infusion	Intra-DR Ucn_1 (20 pmol in 50 nl) had no effect on EtOH intake, but reduced food and water intake; reduced body weight gain was also seen
	Ucn_1	Ryabinin *et al.* (2008)	C57BL/6J mice	EtOH drinking; single-bottle DID 2 h/day for 3 days (20% EtOH); effect of Ucn_1 examined on day 3 of EtOH drinking ~15 min after infusion; separate study examined Ucn_1 effect on acquisition of EtOH drinking; Ucn_1 was infused immediately before EtOH access on all 3 days	Intra-lateral septum Ucn_1 (6–60 pmol) reduced established EtOH intake, but not water intake, with little dose-dependence. Repeated Ucn_1 (0.1–40 pmol) dose-dependently attenuated the acquisition of EtOH drinking

Table 2: Continued

Receptor, drug type	Drug	Reference	Model	Treatment or trait	Finding
$CRF_{1/2}$, antagonist	α-Helical $CRF_{(9-41)}$	Baldwin *et al.* (1991)	Wistar rats	EtOH liquid diet for 2–3 weeks (8.5–11.5% EtOH); 8 h after WD, CRF was infused and elevated plus maze test was conducted 30 min later for 5 min	ICV α-helical $CRF_{(9-41)}$ (5 or 25 μg) blocked the anxiogenic-like effects of EtOH WD on the elevated plus maze, seen in comparison with rats that received non-EtOH control diet; general WD symptoms were unaffected
	α-Helical $CRF_{(9-41)}$	Rassnick *et al.* (1993)	Wistar rats	EtOH liquid diet for 16 days (8.7% EtOH); 8 h after WD, CRF was infused and elevated plus maze test was conducted 30 min later for 5 min	Intra-CeA α-helical $CRF_{(9-41)}$ (250 ng, but not 500 ng, given bilaterally in 0.5 μl) blocked the decrease in percent time in the open arms of the elevated plus maze seen during EtOH WD. ICV α-helical $CRF_{(9-41)}$ (250 ng) had no significant effect
	α-Helical $CRF_{(9-41)}$	O'Callaghan *et al.* (2005)	High preference and low preference mice	EtOH drinking; 24-h two-bottle choice for 3 weeks (8% EtOH) to establish drinking, then treatment was given and intake was after measured 12, 24 and 36 h	ICV α-helical $CRF_{(9-41)}$ (5 μg in 2 μl) had no effect on EtOH preference in high preference mice, but increased EtOH preference in low preference mice
	α-Helical $CRF_{(9-41)}$	Lowery *et al.* (2010)	C57BL/6J mice	EtOH drinking; sucrose drinking; single-bottle DID 2 h/day for 3 days, then for 4 h on day 4 (20% EtOH); same procedure for 10% sucrose; drug given 60 min before day 4 EtOH or sucrose access; BEC	Only the lowest dose of ICV α-helical $CRF_{(9-41)}$ (1, 5 or 10 μg in 1 μl) attenuated EtOH consumption. BEC was significantly reduced after treatment with both the 1 and 10 μg doses. There was no effect on sucrose drinking (only 1 μg dose tested)
$CRF_{1/2}$, antagonist	D-Phe-$CRF_{(12-41)}$	Le *et al.* (2000)	Wistar rats	EtOH drinking; two-bottle choice intermittent access for 30 days (3–12% EtOH; 30 min/day); then operant self-administration for ~24 days (12% EtOH; 1 h/day); extinction for 5–8 days; treatment given 15 min prior to reinstatement session or 15 min prior to intermittent footshock, which was administered just before reinstatement session	ICV D-Phe-$CRF_{(12-41)}$ (0.3 or 1 μg in 2 μl) dose-dependently attenuated footshock-induced reinstatement of EtOH seeking. The highest dose increased responding in the no-shock condition
	D-Phe-$CRF_{(12-41)}$	Le *et al.* (2002)	Wistar rats	EtOH drinking; two-bottle choice for 30 min/day for 25 days (3–12% EtOH); then operant EtOH self-administration for 1 h/day for 18–25 days (12% EtOH); extinction for 5–9 days; treatment given 15 min prior to reinstatement session or 15 min prior to intermittent footshock, which was administered just before reinstatement session	Intra-MRN D-Phe-$CRF_{(12-41)}$ (50 ng) blocked footshock-induced reinstatement. There was no significant effect on the no-shock condition

Table 2: Continued

Receptor, drug type	Drug	Reference	Model	Treatment or trait	Finding
	D-Phe-CRF$_{(12-41)}$	Liu and Weiss (2002)	Wistar rats	Operant self-administration for ~ 9 weeks (5–10% EtOH; 30 min access), then continuous EtOH vapor for 10 days; then EtOH vapor for 12 h/day alternating with 12 h/day self-administration for 3 days; extinction beginning 7–10 days later for an average of 19 days; drug given 30 min before reinstatement test	ICV D-Phe-CRF$_{(12-41)}$ (1 or 10 μg in 5 μl) dose-dependently reduced footshock-induced, but not EtOH-conditioned cue-induced reinstatement of EtOH seeking in post-EtOH dependent rats; it also reduced stress plus EtOH cue-induced reinstatement; non-dependent rats were not studied
	D-Phe-CRF$_{(12-41)}$	Valdez *et al.* (2002)	Wistar rats	Operant self-administration for ~ 9 weeks (5–10% EtOH; 30–60 min access), then continuous EtOH vapor for 3 weeks; effect of drug on operant EtOH self-administration was assessed 2 h or 2–5 weeks after WD, with sessions initiated 10 min after infusion	ICV D-Phe-CRF$_{(12-41)}$ (1, 5 or 10 μg in 5 μl) dose-dependently attenuated WD-induced increases in EtOH seeking at both 2 h and 2–5 weeks after WD
	D-Phe-CRF$_{(12-41)}$	Valdez *et al.* (2003)	Wistar rats	EtOH liquid diet for 21 days (8.7%); elevated plus maze test 6 weeks after WD, with drug given 15 min before the test in combination with restraint stress or no stress	ICV D-Phe-CRF$_{(12-41)}$ (10 μg in 5 μl) attenuated anxiogenic effects of combined EtOH WD and restraint stress
	D-Phe-CRF$_{(12-41)}$	Funk *et al.* (2006)	Wistar rats	Operant self-administration for ~25 days (10% EtOH; 30 min access), then intermittent EtOH vapor for 14 h/day for 4 weeks; then drug was infused 5 min before operant self-administration, which was examined 2 h after WD	Intra-CeA D-Phe-CRF$_{(12-41)}$ (0.125, 0.25 or 0.5 μg/μl) dose-dependently reduced EtOH seeking in dependent, but not in non-dependent, rats. This effect was absent for other brain regions (BNST and NAcc shell). CRF immunoreactivity was significantly reduced in CeA of EtOH-dependent and withdrawn rats, but not in other brain regions
	D-Phe-CRF$_{(12-41)}$	Finn *et al.* (2007)	C57BL/6J mice	EtOH drinking; two-bottle DID for 2 h/day for 1 week (15% EtOH); EtOH vapor for 16 h/day for 4 days; then WD for 2 weeks, followed by 1 week of 15% EtOH access for 2 h/day and another 4-day vapor exposure; then 1 week of 15% EtOH drinking; the effect of drug was examined 15 min after infusion in a single drinking session	Intra-CeA D-Phe-CRF$_{(12-41)}$ (0.25 μg/0.5μl/side) decreased EtOH WD-induced increases in EtOH intake, compared with vehicle treatment; it had no effect on non-dependent controls
	D-Phe-CRF$_{(12-41)}$	Bruijnzeel *et al.* (2010)	Wistar rats	EtOH liquid diet for 12 weeks (5.7% EtOH); drug given 15 min before intracranial self-stimulation test that occurred 8 h after EtOH WD	ICV D-Phe CRF$_{(14-21)}$ (10 or 20 μg in 5 μl) dose-dependently prevented the elevation of brain reward self-stimulation thresholds associated with EtOH WD

Table 2: Continued

Receptor, drug type	Drug	Reference	Model	Treatment or trait	Finding
	D-Phe-$CRF_{(12–41)}$	Le *et al.* (2013)	Wistar rats	EtOH drinking; two-bottle choice for 3 weeks; then operant EtOH self-administration for 3 weeks (12% EtOH; 1 h access); effect of D-Phe-$CRF_{(12–41)}$, given 15 min before vehicle or yohimbine, was examined on active self-administration; effect also examined after extinction (5–9 days) to test reinstatement	Yohimbine (1.25 mg/kg) increased active EtOH self-administration but intra-MRN D-Phe-$CRF_{(12–41)}$ (25 or 50 ng in 0.5 μl) had no effect. Intra-MRN D-Phe-$CRF_{(12–41)}$ (25 or 50 ng in 0.5 μl) dose-dependently reduced yohimbine-induced reinstatement of EtOH seeking
CRF_1, antagonist	LWH-63 (NIH-3)	Sabino *et al.* (2006)	sP rats	Study one: EtOH drinking; continuous access two-bottle choice for 3 weeks (10% EtOH); then limited access for 6 days (1 h/day); then operant EtOH self-administration training (10% EtOH, 30 min/day); vapor exposure for 14 h/day for 5–7 weeks; drug was given 1 h before self-administration session, which was initiated 6 h after EtOH WD Study two: EtOH drinking; continuous access two-bottle choice for 4 weeks (10% EtOH); then EtOH DID with 2 h/day EtOH access (10% EtOH) until stable; drug effect on EtOH intake assessed 1 h after treatment	Study one: SC LWH-63 (5, 10 or 20 mg/kg) reduced WD-associated self-administration behavior in dependent rats, but had no effect on non-dependent rats Study two: LWH-63 (5, 10 or 20 mg/kg) increased both EtOH and water intake in the DID study
	LWH-63	Lowery-Gionta *et al.* (2012)	C57BL/6J mice	EtOH drinking and BEC; single-bottle DID 2 h/day for 3 days, then 4 h on day 4 (20% EtOH); same procedure for 10% sucrose; also assessed drinking in the light using same procedure as above. Drug was given 30 min prior to EtOH access	IP LWH-63 (10, 30 or 60 mg/kg) dose-dependently attenuated DID EtOH consumption and BEC, but not sucrose intake or EtOH consumption in the light
CRF_1, antagonist	Antalarmin	Lodge and Lawrence (2003)	Fawn-Hooded rats	Drug given on 3 days to examine effect on acquisition of EtOH drinking; two-bottle choice continuous access for 10 days (5% EtOH); drug then given twice daily for 10 days to examine effects on established EtOH drinking	IP Antalarmin (20 mg/kg) reduced the acquisition of EtOH drinking, and reduced established EtOH consumption
	Antalarmin	Hansson *et al.* 2006	msP and Wistar rats	Operant self-administration for 15 days (10% EtOH 30/min/day); extinction for 15 days; drug given 30 min before self-administration or footshock-induced reinstatement test	IP Antalarmin (10 or 20 mg/kg) dose-dependently attenuated EtOH lever pressing and blocked footshock-induced reinstatement of EtOH seeking in msP rats, but had no effect on Wistar rats
	Antalarmin	Chu *et al.* (2007)	C57BL/6J mice	Operant self-administration training, then EtOH vapor for 3 weeks (14 h/day); drug was given 1 h before self-administration session, which was assessed 10 days after WD	IP Antalarmin (30 mg/kg) blocked WD-induced increases in EtOH self-administration behavior (number of reinforcers)

Table 2: Continued

Receptor, drug type	Drug	Reference	Model	Treatment or trait	Finding
	Antalarmin	Funk *et al.* (2007)	Wistar rats	Operant self-administration for ~25 days (10% EtOH; 30 min access), then intermittent EtOH vapor 14 h/day for 4 weeks; drug was given 1 h prior to operant self-administration session that was initiated 2 h after WD	IP Antalarmin (10 or 20 mg/kg) dose-dependently reduced WD-induced increases in EtOH lever presses in dependent rats; there was no effect on non-dependent rats
	Antalarmin	Marinelli *et al.* (2007)	Wistar rats	EtOH drinking; two-bottle choice EtOH continuous access for 20 days (3–12% EtOH); then operant EtOH self-administration for 1 h/day for ~16 days (12% EtOH); extinction for 13 days; reinstatement induced by yohimbine stress; antalarmin was given 15 min before yohimbine and EtOH self-administration was measured 45 min later	IP Antalarmin (10 or 20 mg/kg) dose-dependently attenuated yohimbine (1.25 mg/kg) stressor-induced increases in EtOH self-administration behavior and intake, as well as yohimbine-induced reinstatement of EtOH seeking. Antalarmin had no effect on non-yohimbine-treated rats and no effect on yohimbine-induced CORT levels
	Antalarmin	Yang *et al.* (2008)	129SVEV mice	EtOH drinking; two-bottle choice continuous access for 11 days (3–12% EtOH); restraint stress twice daily for 60 min on days 4–7; drug given 30 min prior to each restraint stress	IP Antalarmin (20 mg/kg) did not block stress-induced increases in EtOH intake or EtOH intake in non-stressed mice
	Antalarmin	Cippitelli *et al.* (2012)	Wistar rats	EtOH drinking; two-bottle choice intermittent access (10% or 20% EtOH every other day for 24 h/day for 40 days and thus, 20 days of EtOH exposure) or continuous access (10% or 20% EtOH for 24 h/day for 20 days); drug given 30 min prior to EtOH access after the 20-day exposure period	IP Antalarmin (10 or 20 mg/kg) dose-dependently reduced EtOH intake in intermittent access condition for both 10% and 20% EtOH, but only for 20% EtOH in continuous access condition
	Antalarmin	Lowery-Gionta *et al.* (2012)	C57BL/6J mice	EtOH drinking and BEC; single-bottle DID 2 h/day for 3 days, then 4 h on day 4 (20% EtOH); same procedure for 10% sucrose; also assessed drinking in the light using same procedure as above. Drug was given 30 min prior to EtOH access	IP Antalarmin (30 mg/kg) attenuated DID EtOH consumption and BEC, but not EtOH consumption in the light; intra-CeA Antalarmin (1 μg in 0.5 μl, 1 h before EtOH access) attenuated DID EtOH consumption and BEC, but not sucrose consumption
	Antalarmin	Molander *et al.* (2012)	Wistar rats	EtOH drinking; two-bottle choice continuous access for 8 weeks (5–20% EtOH); the EtOH access removed for 3 weeks; antalarmin was given on five consecutive days and EtOH intake was measured for 1 week, beginning after the second injection	IP Antalarmin (20 mg/kg) did not alter increased EtOH intake seen after the EtOH deprivation period

Table 2: Continued

Receptor, drug type	Drug	Reference	Model	Treatment or trait	Finding
	Antalarmin	Ayanwuyi *et al.* (2013)	msP rats with CRF point mutations associated with CRF over-expression (genotype AA or GG); Wistar rats	EtOH drinking; two-bottle choice continuous access for 15 days (10% EtOH), then operant self-administration until stable (10%, 30 min/day); antalarmin given 30-min prior to operant session or 30 min prior to yohimbine stress	IP Antalarmin (5, 10 or 20 mg/kg) reduced the number of EtOH-rewards earned in Wistar and msP-AA rats, but not in msP-GG rats; effect occurred at a lower dose in msP-AA than Wistar; IP Antalarmin (10 mg/kg) blocked the yohimbine-induced increase in EtOH-rewards earned in all three rat lines
CRF_1, antagonist	CP-154,526	Le *et al.* (2000)	Wistar rats	EtOH drinking; two-bottle choice intermittent access for 30 days (3–12% EtOH; 30 min/day); then operant self-administration for ~24 days (12% EtOH; 1 h/day); extinction for 5–8 days; treatment given 15 min prior to reinstatement session or 15 min prior to intermittent footshock, which was administered just before reinstatement session	IP CP-154,526 (15, 30 or 45 mg/kg) dose-dependently attenuated footshock-induced reinstatement of EtOH seeking. There was no significant effect on the no-shock condition
	CP-154,526	Overstreet *et al.* (2004)	Sprague–Dawley rats	EtOH liquid diet for 15 days with 2 days of WD every 5 days (7% EtOH); drug given during the first two WD periods and social interaction measured 5 h after the final (third) EtOH WD	Social interaction is used to index anxiety-related behavior and is sensitive to EtOH WD and stress. IP CP-154,526 (10 mg/kg) blocked the repeated EtOH WD-associated reduction in social interaction
	CP-154,526	Overstreet *et al.* (2005)	P rats	EtOH liquid diet for 15 days with 2 days of WD every 5 days (4.5% EtOH); drug given during the first two WD periods and social interaction measured 5 h after the final (third) EtOH WD	Social interaction was used as a measure of behavioral change sensitive to EtOH WD and stress. IP CP-154,526 (10 mg/kg) blocked the repeated EtOH WD-associated reduction in social interaction
	CP-154,526	Breese *et al.* (2005b)	Sprague–Dawley rats	EtOH liquid diet for 15 days with 2 days of WD every 5 days (4.5% EtOH); drug given during the first two WD periods and social interaction measured 30 min after restraint stress applied three days after the final (third) WD; in another group drug was given 30 min prior to the restraint stress exposure	Social interaction is used to index anxiety-related behavior and is sensitive to EtOH WD and stress. IP CP-154,526 (10 mg/kg) attenuated the reduced social interaction induced by EtOH WD plus stress when given at either time point
	CP-154,526	Fee *et al.* (2007)	DBA/2J mice	EtOH-induced locomotor sensitization; acute test after IP 1.5 g/kg EtOH, then mice treated with IP 2.5 g/kg EtOH for 10 days, then challenge locomotor test performed after IP 1.5 g/kg EtOH; for acquisition study, drug was given 30 min prior to each 2.5 g/kg EtOH treatment; for expression study, drug was given 30 min prior to the final 1.5 g/kg EtOH challenge	IP CP-154,526 (5 or 10 mg/kg) had no effect on the acquisition of sensitization, but dose-dependently prevented the expression of EtOH-induced locomotor sensitization

Table 2: Continued

Receptor, drug type	Drug	Reference	Model	Treatment or trait	Finding
	CP-154,526	Overstreet *et al.* (2007)	P rats	EtOH drinking; two-bottle choice continuous access for 15 days with 2 days of WD every 5 days (10% EtOH); drug given 30 min before a 1 h restraint stress applied during the first two EtOH WD periods; social interaction measured 5 h after the final (third) EtOH WD	Social interaction is used to index anxiety-related behavior and is sensitive to EtOH WD and stress. IP CP-154,526 (10 mg/kg) reduced the increase in EtOH intake seen after repeated EtOH WD and stress; it also attenuated the reduced social interaction seen after repeated EtOH WD and stress
	CP-154,526	Lowery *et al.* (2008)	C57BL/6N and BALB/cJ mice	EtOH drinking; two-bottle choice continuous access for 16 days (2–8% EtOH); then drug given 30 min before 5-min forced swim stress, followed by EtOH intake measured for 4 weeks	IP CP-154,526 (10 mg/kg) blunted swim stress-induced increased EtOH intake in BALB/cJ mice, with no significant effect on non-stressed mice. Swim stress did not alter EtOH intake in C57BL/6N mice
	CP-154,526	Pastor *et al.* (2008)	DBA/2J mice	EtOH-induced locomotor sensitization; IP 1.5 g/kg EtOH once daily for 10 days, then 1.5 g/kg EtOH challenge and locomotor test; for acquisition study, drug was given 30 min prior to each 1.5 g/kg EtOH treatment except on the final challenge day; for expression study, drug was given 30 min prior to the final 1.5 g/kg EtOH challenge only	IP CP-154,526 (15 or 30 mg/kg, dose-dependently attenuated the acquisition of sensitization and prevented the expression of EtOH-induced locomotor sensitization
	CP-154,526	Sparta *et al.* (2008)	C57BL/6J mice	EtOH drinking; single-bottle DID 4 h/day for all 4 days or 2 h/day on days 1–3 and then 4 h on day 4 (20% EtOH); drug given 30 min before EtOH access on day 4; sucrose consumption using the same 2 h/4 h procedure	IP CP-154,526 (1, 3 or 10 mg/kg) reduced EtOH intake in the 2-h then 4 h DID, but not in the all 4-h DID procedure; sucrose consumption was not affected
	CP-154,526	Sparta *et al.* (2009)	C57BL/6J mice	Operant self-administration for 2 weeks to establish stable responding (2–10% EtOH 16 h/day); EtOH was also available in the home cage; 4-day EtOH deprivation period, then drug 30 min before 2 h operant session	IP CP-154,526 (10 or 20 mg/kg) dose-dependently blocked EtOH deprivation-induced increase in EtOH-associated lever responding and EtOH intake; there were also effects at some doses on water responding and consumption
	CP-154,526	Wills *et al.* (2009)	Sprague–Dawley rats; adult and adolescent	EtOH liquid diet for 15 days with 2 days of WD every 5 days (2.5% or 3.5% EtOH); drug given 4 h after WD during the first two WD periods; social interaction measured 5 h after the final (third) EtOH WD	Social interaction is used to index anxiety-related behavior and is sensitive to EtOH WD and stress. IP CP-154,526 (10 mg/kg) attenuated the reduced social interaction induced by repeated EtOH WD

Table 2: Continued

Receptor, drug type	Drug	Reference	Model	Treatment or trait	Finding
	CP-154,526	Lowery *et al.* (2010)	C57BL/6J mice	EtOH drinking; single-bottle DID for 3 days, then for 4 h on day 4 (20% EtOH, 2 h/day); drug given 60 min before day 4 EtOH access; adrenalectomy or sham surgery	IP CP-154,526 (10 or 15 mg/kg) dose-dependently reduced EtOH intake and BEC in both sham and adrenalectomized mice
	CP-154,526	Hwa *et al.* (2013)	C57BL/6J mice; Long Evans rats	EtOH drinking; two-bottle choice for 4 weeks, 3 days a week (20% EtOH, 24 h/day); for one study, mice were given continuous daily EtOH access for similar period (20% EtOH, 24 h/day); drug infused 10 min before measuring EtOH drinking	Intra-VTA and intra-DRN CP-154,526 (0.3 or 0.6 μg) dose-dependently decreased EtOH intake in both mice and rats, under both drinking conditions; there were also some effects on water intake
	CP-154,526	Sparta *et al.* (2013)	C57BL/6J mice	EtOH drinking; single-bottle DID 2 h/day on days 1–3 and then 4 h on day 4 (20% EtOH); drug infused 30 min before EtOH access on day 4; also sucrose consumption using the 2 h/4 h procedure; electrophysiology in slice preparation	Intra-VTA CP-154,526 (1 μg, 0.5 μl/side) reduced EtOH intake; DID enhanced CRF-mediated potentiation of VTA DA neuron *N*-methyl-D-aspartate receptor currents; this effect was blocked by CP-154,526
CRF_1, antagonist	CP-376,395	Giardino and Ryabinin (2013)	C57BL/6J mice	EtOH drinking; two-bottle DID 2 h/day for 3 days, then for 4 h on day 4 (15% EtOH); also sucrose consumption using the same procedure; water and food intake; drug was given 30 min prior to measurements	IP CP-376,395 (10 or 20 mg/kg) reduced EtOH intake and food intake; it increased sucrose intake
	CP-376,395	Simms *et al.* (2014)	Long Evans rats	EtOH drinking; two-bottle choice continuous access every day or every other day for 9 weeks (20% EtOH); drug given 30 min prior to drinking session	IP CP-376,395 (5 or 10 mg/kg) reduced EtOH intake in every other day access, but not in every day access, condition.
CRF_1, antagonist	CRA-1000	Breese *et al.* (2004)	Sprague–Dawley and P rats	Restraint stress for 1 h on days 6 and 11 (on control diet) and then 4.5% EtOH liquid diet for 5 days; social interaction measured 5 h after WD; drug given 30 min prior to each restraint stress	Social interaction is used to index anxiety-related behavior and is sensitive to EtOH WD and stress. IP CRA-1000 (3 mg/kg) attenuated the reduced social interaction induced by stress in EtOH WD rats
	CRA1000	Knapp *et al.* (2004)	Sprague–Dawley rats	EtOH liquid diet for 17 days (7% EtOH); drug given 30 min before social interaction test which was administered 5–6 h after WD	Social interaction is used to index anxiety-related behavior and is sensitive to EtOH WD and stress. IP CRA1000 (0.25, 0.5 or 1 mg/kg) dose-dependently attenuated the decrease in social interaction seen during EtOH WD without altering locomotor activity

Table 2: Continued

Receptor, drug type	Drug	Reference	Model	Treatment or trait	Finding
	CRA1000	Overstreet *et al.* (2004)	Sprague–Dawley rats	EtOH liquid diet for 15 days with 2 days of WD every 5 days (7% EtOH); drug given 4 h after EtOH WD during the first two WD periods and social interaction measured 5 h after the final (third) EtOH WD; in another group, drug given only 30 min before social interaction test	Social interaction was used to measure a behavioral change associated with EtOH exposure and WD. IP CRA1000 (3 mg/kg) given during the first two WD periods blocked the repeated EtOH WD-associated reduction in social interaction. IP CRA1000 (1 mg/kg) given prior to the social interaction test also blocked this effect of EtOH WD
	CRA1000	Overstreet *et al.* (2007)	P rats	EtOH drinking; two-bottle choice continuous access for 15 days with 2 days of WD every 5 days (10% EtOH); drug given 30 min before a 1 h restraint stress applied during the first two EtOH WD periods; social interaction measured 5 h after the final (third) EtOH WD	Social interaction is used to index anxiety-related behavior and is sensitive to EtOH WD and stress. IP CRA1000 (3 mg/kg) reduced the increase in EtOH intake seen after repeated EtOH WD and stress; it also attenuated the reduced social interaction seen after repeated EtOH WD and stress
CRF_1, antagonist	MJL-1-109-2	Funk *et al.* (2007)	Wistar rats	Operant self-administration for ~25 days (10% EtOH; 30 min/day), then intermittent EtOH vapor 14 h/day for 4 weeks; drug was given 1 h prior to operant self-administration session that was initiated 2 h after WD	IP MJL-1-109 (0.6, 1.25 or 5 mg/kg) dose-dependently reduced WD-induced increases in EtOH lever presses in dependent rats; there was no effect on non-dependent rats
	MJL-1-109-2	Sabino *et al.* (2006)	sP rats	EtOH drinking; two-bottle choice continuous access for 4 weeks (10% EtOH); drug effect on EtOH intake assessed 1 h after treatment	IP MJL-1-109 (1.25, 2.5 or 10 mg/kg) had not effect on EtOH intake
CRF_1, antagonist	MPZP	Gilpin *et al.* 2008	P rats	Operant self-administration for ~25 days (10% EtOH; 30 min/day), then intermittent EtOH vapor for 10 weeks (14 h/day); drug was given 1 h before operant self-administration, which was examined 6 h after WD	SC MPZP (5, 10 or 20 mg/kg) attenuated WD-induced increases in EtOH lever presses and intake in dependent rats; there was no effect on non-dependent rats
	MPZP	Ji *et al.* (2008)	Wistar rats	Two-bottle choice sweetened EtOH DID for 9 days (10% EtOH in 'supersac' 30 min/day); also operant sweetened EtOH self-administration for 17 days (10% EtOH, 30 min/day); then drug given 1 h prior to subsequent drinking or operant session	SC MPZP (5, 10 or 20 mg/kg) had no effect on two-bottle choice sweetened EtOH intake; a small increase in sweetened EtOH intake was seen with increasing dose of SC MPZP in the operant study

Table 2: Continued

Receptor, drug type	Drug	Reference	Model	Treatment or trait	Finding
	MPZP	Richardson *et al.* (2008b)	Wistar rats	Operant self-administration for ~3 weeks (10% EtOH; 30 min/day), then intermittent EtOH vapor for 4 weeks (14 h/day); drug was given 1 h before operant session, which was initiated 6–8 h after WD	SC MPZP (5, 10 or 20 mg/kg) reduced increased EtOH intake associated with EtOH WD, but did not affect EtOH intake in non-dependent rats
CRF_1, antagonist	MTIP	Gehlert *et al.* (2007)	Sprague–Dawley, Wistar and msP rats	Operant self-administration for 15 days (10% EtOH, 30 min/day); after self-administration established, EtOH by gavage for 6 days to induce dependence in one group of Wistar rats (9–12 g/kg/day); then drug was given 30 min before self-administration session; extinction over 15 days and then drug effect on footshock-induced reinstatement	IP MTIP (3, 10 or 20 mg/kg) reduced EtOH self-administration and reinstatement of EtOH seeking in msP rats, and in EtOH-dependent, but not non-dependent, Wistar rats
	MTIP	Sommer *et al.* (2008)	Wistar rats	Intermittent EtOH vapor for 7 weeks (17 h/day); drug given 30 min prior to Vogel conflict test of punished drinking, which was examined 13 weeks after WD	IP MTIP (10 mg/kg) eliminated fear suppression of behavior in the Vogel conflict test in rats with and without a history of EtOH dependence
CRF_1, antagonist	NBI-27914	Lowery-Gionta *et al.* (2012)	C57BL/6J mice	EtOH drinking and BEC; single-bottle DID 2 h/day for 3 days, then 4 h on day 4 (20% EtOH); same procedure for 10% sucrose; also assessed drinking in the light using same procedure as above, drug was given 30 min prior to EtOH access	IP NBI-27914 (10, 30 or 60 mg/kg) dose-dependently attenuated DID EtOH consumption and BEC, but not sucrose intake or EtOH consumption in the light
	NBI-27914	Molander *et al.* (2012)	Wistar rats	EtOH drinking; two-bottle choice continuous access for 8 weeks (5–20% EtOH); then EtOH access removed for 3 weeks; antalarmin was given on 5 consecutive days and EtOH intake was measured for 1 week, beginning after the second injection	IP NBI-27914 (10 mg/kg) did not alter increased EtOH intake seen after the EtOH deprivation period
	NBI-27914	Giardino and Ryabinin (2013)	C57BL/6J mice	EtOH drinking; two-bottle DID 2 h/day for 3 days, then for 4 h on day 4 (15% EtOH); also sucrose consumption using the same procedure; water and food intake; drug was given 30 min prior to measurements	IP NBI-27914 (10 or 30 mg/kg) reduced EtOH, food and saccharin intake
CRF_1, antagonist	R121919	Sabino *et al.* (2006)	sP rats	Study one: Two-bottle choice continuous access (10% EtOH) for 4 weeks; drug effect on EtOH intake assessed 1 h after treatment Study two: Two-bottle choice as described above; then 2 h/day EtOH access (10% EtOH DID) until stable; drug effect on DID EtOH intake assessed 1 h after treatment Study three: same as Study two and then drug treated prior to novelty stress and then EtOH intake measured 1 h after drug	Study one: SC R121919 (10 mg/kg) had no effect on continuous access EtOH intake Study two: SC R121919 (5, 10 and 20 mg/kg) increased DID EtOH intake. Study three: SC R121919 (10 mg/kg) attenuated novelty stress-induced suppression of EtOH intake

Table 2: Continued

Receptor, drug type	Drug	Reference	Model	Treatment or trait	Finding
	R121919	Funk *et al.* (2007)	Wistar rats	Operant self-administration for ~25 days (10% EtOH; 30 min access), then intermittent EtOH vapor 14 h/day for 4 weeks; drug was given 1 h prior to operant self-administration session that was initiated 2 h after WD	SC R121919 (5, 10 or 20 mg/kg) dose-dependently reduced WD-induced increases in EtOH lever presses in dependent rats; there was no effect on non-dependent rats
	R121919	Yang *et al.* (2008)	129SVEV mice	EtOH drinking; two-bottle choice continuous access for 11 days (3–12%); restraint stress twice daily for 60 min on days 4–7; drug given 30 min prior to each restraint stress	IP R121919 (15 or 20 mg/kg) did not block stress-induced increases in EtOH intake or EtOH intake in non-stressed mice
	R121919	Roberto *et al.* (2010)	Sprague–Dawley rats	Operant self-administration for ~25 days (10% EtOH; 30 min/day), then intermittent EtOH vapor for 2–4 weeks (14 h/day); EtOH self-administration measured during this time, with drug given repeatedly on even numbered days and self-administration tested on odd numbered days; GABA neurotransmission; brain slice electrophysiology; *in vivo* microdialysis	Chronic SC R121919 (10 mg/kg) blocked the development of EtOH-dependence-induced increases in EtOH intake and also reduced EtOH intake in non-dependent rats; *in vitro* R121919 decreased basal GABAergic responses and blocked EtOH-induced increases in GABA transmission. SC R121919 decreased EtOH-induced increases in dialysate GABA in the CeA in both EtOH-dependent and non-dependent rats
	R121919	Jee *et al.* (2013)	C57BL/6J mice	Acute functional tolerance to ataxic effects of EtOH (IP 2 g/kg) (time of R121919 treatment unclear)	R121919 reduced the rate of recovery and the development of acute functional tolerance to the ataxic effects of EtOH
	R121919	Sabino *et al.* (2013)	sP rats	EtOH drinking; two-bottle choice continuous access for 7 weeks, every day (10% EtOH) or every other day (20% EtOH); then drug given 45 min before drinking test	SC R121919 (5, 10 or 20 mg/kg) had no effect on EtOH intake in either condition
	R121919	Roltsch *et al.* (2014)	Wistar rats	EtOH drinking; two-bottle choice continuous access for 1 day (10% EtOH), then operant self-administration for ~15 days (10% EtOH, 30 min/day); effect of drug given 2, 5, 8, 12, 15 and 19 days after predator odor stress on stress-induced increase in EtOH self-administration, measured 60 min after drug treatment	SC R121919 (10 mg/kg) reduced the increased EtOH intake seen in predator odor stress-exposed rats; the effect was not dependent on day
CRF_1, antagonist	SSR125543	Huang *et al.* (2010)	Sprague–Dawley rats	SSR125543 given 15 min before CRF or restraint stress on days 2 days, one week apart, while on control diet, then EtOH liquid diet for 5 days (4.5% EtOH); social interaction test and locomotor activity assessed 5 h after EtOH WD	Social interaction is used to index anxiety-related behavior and is sensitive to EtOH WD and stress. IP SSR-125543 (10 mg/kg) given prior to CRF or restraint stress (see Huang *et al.* 2010 entry above for CRF) prevented CRF-associated and stress-associated exacerbated reductions in social interactions seen in EtOH WD rats

Table 2: Continued

Receptor, drug type	Drug	Reference	Model	Treatment or trait	Finding
	SSR125543	Knapp *et al.* (2011a)	Inbred Alcohol-preferring (iP) rats	EtOH drinking; single-bottle continuous access for 3 days (10% EtOH) then two-bottle choice continuous access for 15 days or 15 days with 2 days of WD every 5 days (10% EtOH); drug given 15 min before a 1 h restraint stress (or without stress) applied during the first two EtOH WD periods; drinking examined in subsequent 5-day periods; social interaction measured 5–6 h after the final (third) EtOH WD	Social interaction is used to index anxiety-related behavior and is sensitive to EtOH WD and stress. Intra-NAcc SSR125543 (10 μg/0.5 μl) prevented stress and EtOH WD-associated increased EtOH intake, but not reduced social interaction seen in these rats; intra-amygdala or DRN SSR125543 prevented stress and WD effects on social interaction, but did not prevent the increase in EtOH intake
	SSR1235543	Knapp *et al.* (2011b)	Sprague–Dawley rats	SSR125543 given 15 min before cytokine or chemokine treatment 2 days, 1 week apart, while on control diet, then EtOH liquid diet for 5 days (4.5% or 7% EtOH); social interaction test and locomotor activity assessed 5 h after EtOH WD	Social interaction is used to index anxiety-related behavior and is sensitive to EtOH WD and stress. IP SSR125543 (10 mg/kg) given prior to the cytokine reversed the reduced social interaction time seen in cytokine- and chemokine-treated animals after EtOH WD
CRF_2, agonist	Ucn_3	Valdez *et al.* (2004)	Wistar rats	Operant self-administration for ~22 days (5–10%, 30 min/day); then EtOH liquid diet for 21 days (10%); drug given and self-administration and elevated plus maze behavior assessed 2 h after EtOH WD; drug was infused 10 min before the 5-min plus maze test or just before the operant test	ICV Ucn_3 (1 or 10 μg) attenuated anxiety-like behavior induced by WD from chronic liquid EtOH diet; ICV Ucn_3 (1 or 10 μg) reduced increased EtOH lever presses associated with repeated EtOH WD. It had no significant effect on control group animals in either study
	Ucn_3	Funk and Koob (2007)	Wistar rats	Operant self-administration (10% EtOH for ~25 days; 30 min access), then EtOH vapor (intermittent for 14 h/day for 4 weeks); then effect of drug on operant EtOH self-administration 2 h after WD	Intra-CeA Ucn_3 (0.02, 0.1, or 0.5 μg/μl) dose-dependently decreased EtOH lever presses in dependent rats; a small increase in EtOH lever presses was seen at the highest concentration of Ucn_3 in non-dependent rats; there was no effect on lever pressing for water
	Ucn_3	Sharpe and Phillips (2009)	C57BL/6J mice	EtOH drinking; two-bottle choice DID for ~ 40 days (10% EtOH); drug treatment occurred immediately before a drinking session	ICV Unc_3 (0.3, 1 or 3 nmol in 1–2 μl) dose-dependently reduced EtOH intake
	Ucn_3	Huang *et al.* (2010)	Sprague–Dawley rats	EtOH liquid diet (4.5% EtOH) for 5 days; social interaction test and locomotor activity assessed 5 h after EtOH WD	Social interaction is used to index anxiety-related behavior and is sensitive to EtOH WD and stress. ICV Ucn_3 (5 μg in 5 μl) or Ucn_3 (0.5 μg/site in 0.5 μl) infused into several brain regions did not affect social interaction in repeated EtOH WD or non-dependent rats. Locomotor activity was not affected

Table 2: Continued

Receptor, drug type	Drug	Reference	Model	Treatment or trait	Finding
	Ucn_3	Lowery *et al.* (2010)	C57BL/6J mice	EtOH drinking; sucrose drinking; single-bottle DID (20% EtOH 2 h/day for 3 days, then for 4 h on day 4; same procedure for 10% sucrose); drug given 90 min before day 4 EtOH or sucrose access; BEC	ICV Ucn_3 (0.05, 0.1 or 0.5 μg in 1 μl) dose-dependently reduced EtOH intake and BEC. Sucrose consumption was not affected
CRF_2, antagonist	Antisauvagine-30	Overstreet *et al.* (2004)	Sprague–Dawley rats	EtOH liquid diet (7% EtOH for 15 days with 2 days of WD every 5 days); drug given 4 h after EtOH WD during the first two WD periods and social interaction measured 5 h after the final (third) EtOH WD	Social interaction is used to index anxiety-related behavior and is sensitive to EtOH WD and stress. ICV Antisauvagine-30 (20 μg) had no effect on repeated EtOH WD-induced reductions in social interaction
	Antisauvagine-30	Weitemier and Ryabinin (2006)	C57/BL6J mice	EtOH drinking (2.5 and then 5%) for 1 week; two-bottle choice continuous access; then cannulation surgery; then re-establishment of EtOH drinking (5%)	Intra-DR Antisauvagine-30 (100 pmol) did not affect EtOH, food or total fluid intake, during a 14-h period, beginning 2 h after treatment
GR and mineralocorticoid receptor, agonist	CORT	Fahlke *et al.* (1996)	Wistar rats	EtOH drinking; two-bottle choice continuous access for 14 days (2–6% EtOH), then two-bottle choice at 6% EtOH for 3 weeks; adrenalectomy; CORT given for 2 weeks (3 times/week), during which time EtOH consumption was measured	Adrenalectomy decreased EtOH intake; ICV CORT (100 μg) restored EtOH intake; SC CORT (100 μg) did not restore EtOH intake; non-adrenalectomized individual rats with lower EtOH intake showed elevated intake after ICV (100 μg) CORT
	CORT	O'Callaghan *et al.* (2005)	High preference and low preference mice	EtOH drinking; 24-h two-bottle choice (8% EtOH) for 3 weeks to establish drinking, then treatment effects were examined	IP CORT (20 mg/kg, once daily for 7 days) did not affect EtOH preference in low preference mice; high preference mice were not studied
	CORT	Yang *et al.* (2008)	129SVEV and C57BL/6J mice	Two-bottle choice continuous access EtOH drinking (3-12% for 7 days); mice were adrenalectomized and given CORT replacement (10 mg concentration pellet) prior to EtOH drinking	Both strains of mice with higher CORT levels (>100 ng/ml) showed reduced EtOH consumption, compared with sham plus placebo and those with lower CORT levels (<100 ng/ml)
	CORT	Pastor *et al.* (2012)	DBA/2J mice	CORT-induced locomotor sensitization to EtOH (IP 1.5 g/kg)	Repeated IP CORT (10 or 20 mg/kg once daily for 10 days) induced sensitization to the locomotor stimulant effect of EtOH
Blocks CORT synthesis	Metyrapone	O'Callaghan *et al.* (2005)	High preference and low preference mice	EtOH drinking; 24-h two-bottle choice (8% EtOH) for 3 weeks to establish drinking, then treatment effects were examined	IP metyrapone (100 mg/kg, twice daily for 7 days) reduced EtOH preference in high preference mice and prevented increases in EtOH preference over time that were seen in low preference mice injected with vehicle

Table 2: Continued

Receptor, drug type	Drug	Reference	Model	Treatment or trait	Finding
	Metyrapone	Lowery *et al.* (2010)	C57BL/6J mice	EtOH drinking; sucrose drinking; single-bottle DID (20% EtOH 2 h/day for 3 days, then for 4 h on day 4; same procedure for 10% sucrose); drug given 30 min before day 4 EtOH or sucrose access; BEC	IP Metyrapone (50, 100 or 150 mg/kg) dose-dependently reduced EtOH intake, BEC and sucrose intake
	Metyrapone	Pastor *et al.* (2012)	DBA/2J mice	EtOH-induced locomotor sensitization; IP 1.5 g/kg EtOH once daily for 10 days, then IP 1.5 g/kg EtOH challenge and locomotor test; for acquisition study, drug was given 30 min prior to each 1.5 g/kg EtOH treatment except on the final challenge day; for expression study, drug was given 30 min prior to the final 1.5 g/kg EtOH challenge only; CORT levels	IP Metyrapone (25 or 50 mg/kg) blocked the acquisition of sensitization and reduced EtOH-induced increases in CORT. IP Metyrapone (25 or 50 mg/kg) did not block the expression of sensitization
GR, antagonist	Mifepristone (RU486)	Roberts *et al.* (1995)	DBA/2J mice	EtOH-induced locomotor sensitization; IP 1.5 g/kg EtOH once daily for 10 days, then 1.5 g/kg EtOH challenge and locomotor test; drug given 30 min before each EtOH treatment except the final challenge treatment	IP Mifepristone (20 mg/kg) attenuated the acquisition of EtOH-induced sensitization
	Mifepristone	Koenig and Olive (2004)	Long Evans rats	EtOH drinking; two-bottle choice limited access for 5 days/week (10% EtOH); drug was given just before a 1 h access session	IP Mifepristone (1, 5 or 20 mg/kg) dose-dependently reduced EtOH, but not water, intake
	Mifepristone	Yang *et al.* (2008)	129SVEV	Two-bottle choice continuous access EtOH drinking (3–12% for 11 days); restraint stress twice daily for 60 min on days 4–7; drug given 30 min prior to each restraint stress	IP Mifepristone (25 or 50 μg/kg) did not block stress-induced increases in EtOH intake or EtOH intake in non-stressed mice
	Mifepristone	Pastor *et al.* (2008)	DBA/2J mice	EtOH-induced locomotor sensitization; IP 1.5 g/kg EtOH once daily for 10 days, then IP 1.5 g/kg EtOH challenge and locomotor test; drug given 30 min prior to the EtOH challenge test	IP Mifepristone (10 or 20 mg/kg) did not prevent the expression of EtOH-induced locomotor sensitization
	Mifepristone	Lowery *et al.* (2010)	C57BL/6J mice	EtOH drinking; single-bottle DID (20% EtOH 2 h/day for 3 days, then for 4 h on day 4); drug given 30 min before day 4 EtOH access; BEC	IP Mifepristone (25, 50 and 100 mg/kg) did not affect EtOH intake
Mineralocorticoid receptor, antagonist	Spironolactone	Koenig and Olive (2004)	Long Evans rats	EtOH drinking; two-bottle choice limited access for 5 days/week (10% EtOH); drug was given just before a 1 h access session	IP Spironolactone (10, 25 or 50 mg/kg) had no effect on EtOH or water intake

Table 2: Continued

Receptor, drug type	Drug	Reference	Model	Treatment or trait	Finding
	Spironolactone	Pastor *et al.* (2012)	DBA/2J mice	EtOH-induced locomotor sensitization; IP 1.5 g/kg EtOH once daily for 10 days, then 1.5 g/kg EtOH challenge and locomotor test; for acquisition study, drug was given 30 min prior to each 1.5 g/kg EtOH treatment except on the final challenge day; for expression study, drug was given 30 min prior to the final 1.5 g/kg EtOH challenge only	IP Spironolactone (15 or 30 mg/kg) did not prevent acquisition of sensitization or block the expression of sensitization
GR, antagonist plus mineralocorticoid receptor, antagonist	Mifepristone plus RU28318	Fahlke *et al.* (1996)	Wistar rats	EtOH drinking; two-bottle choice continuous access for 14 days (2–6% EtOH), then two-bottle choice at 6% EtOH for ~3 weeks; drugs given for 2 weeks (three times/week), during which time EtOH drinking was assessed	ICV mifepristone (0.1 then 250 μg) plus RU28318 (0.1 then 250 μg) did not affect EtOH intake

BEC, blood ethanol concentration; DRN, dorsal raphe nucleus; EEG, electroencephalogram; IP, intraperitoneal; MPZP, *N*,*N*-bis(2-methoxyethyl)-3-(4-methoxy-2-methylphenyl)-2,5-dimethyl-pyrazolo[1,5-a] pyrimidin-7-amine; MRN, median raphe nucleus; MTIP, 3-(4-chloro-2-morpholin-4-yl-thiazol-5-yl)-8-(1-ethylpropyl)-2,6-dimethyl-imidazo[1,2-b]pyridazine; SC, subcutaneous; WD, withdrawal

concentrations (20%) or using a DID procedure, and thus, when EtOH intake was generally higher in the WT littermate mice (Giardino & Ryabinin 2013; Kaur *et al.* 2012; Pastor *et al.* 2011). In addition, also inconsistent with Sillaber *et al.* (2002), are studies in which forced swim or social defeat stress-induced increases in EtOH intake have been found to be absent or reduced in CRF_1 KO mice (Molander *et al.* 2012; Pastor *et al.* 2011); furthermore, EtOH withdrawal-induced increases in EtOH self-administration and intake were not seen in CRF_1 KO mice (Chu *et al.* 2007; Molander *et al.* 2012). One difference in the studies that have found reduced intake and a lack of stress response is that the KO mice were backcrossed onto the EtOH-preferring C57BL/6J mouse strain for several generations. In fact, Molander *et al.* (2012) also found that stress-induced increases in EtOH consumption were lower in a brain-specific CRF_1 KO that was on a mixed 129S2/Sv × C57BL/6J × SJL strain background. In general, the majority of the data suggest that adequate CRF_1 function is important for higher levels of EtOH intake and for stress-induced changes in EtOH intake.

Because receptor-specific antagonists for CRF_2 that can be administered peripherally are not available, information about the involvement of CRF_2 signaling in EtOH-related phenotypes has relied mostly on studies in CRF_2 KO mice. In general, data have suggested a modulatory role on the more significant involvement of CRF_1 in stress-related responses (Coste *et al.* 2000, 2006). The initial study in CRF_2 KO mice examined both continuous and limited access EtOH drinking. No effect of the mutation was found in the continuous access study. The limited access study included 30-min access periods as the EtOH concentration was increased, followed by 2-h access periods. A modest difference in intake (KO > WT) was found for some concentrations during the 30-min access phase that was not sustained when the access period was increased (Sharpe *et al.* 2005). In a more recent study, a small transient reduction in EtOH intake was seen in CRF_2 KO mice that appeared to be largely in males (Kaur *et al.* 2012). Therefore, this receptor subtype has not had a sustained effect on EtOH consumption in the studies that have been conducted thus far.

Pharmacological studies

Intracerebroventricular administration of CRF has been found to decrease EtOH consumption in rats and mice (Bell *et al.* 1998; Ryabinin *et al.* 2008; Thorsell *et al.* 2005), which is consistent with reduced EtOH intake in CRF overexpression mice (Palmer *et al.* 2004). However, some data suggest non-specific effects on fluid intake (Ryabinin *et al.* 2008). Centrally administered CRF has also been found to reinstate EtOH seeking behavior (Le *et al.* 2002), which is consistent with its role as a stressor. Results for the effect of the other endogenous $CRF_{1/2}$ agonist peptide, Ucn_1, on EtOH intake have been dependent upon brain region, as intra-DR application had no effect, but intra-lateral septum infusion reduced both established EtOH intake and the acquisition of EtOH drinking (Ryabinin *et al.* 2008). Overall, the majority of the data appear to indicate that drugs that have combined agonist actions at CRF_1 and CRF_2 receptors reduce EtOH intake (see CRF and Ucn_1 entries in Table 2).

On the other hand, there is a large body of data showing that reduced CRF_1 signaling via receptor antagonist administration also reduces EtOH intake. As this literature has evolved, it has become more apparent that CRF_1 antagonists have greater effects when EtOH intake levels are high. For example, subcutaneous administration of the CRF_1 antagonist, *N,N*-bis(2-methoxyethyl)-3-(4-methoxy-2-methylphenyl)-2,5-dimethyl-pyrazolo [1,5-a]pyrimidin-7-amine, attenuated elevated levels of EtOH intake seen in alcohol preferring (P) rats after dependence induction, while not affecting EtOH intake in non-dependent P rats (Gilpin *et al.* 2008). Similarly, operant responding for EtOH was decreased by several different CRF_1 (or $CRF_{1/2}$) antagonists in EtOH-dependent, but not in non-dependent, animals (Finn *et al.* 2007; Funk *et al.* 2006; Overstreet *et al.* 2007; Sabino *et al.* 2006), and these drugs tend to reduce binge-like or stress-induced heightened EtOH intake, with less consistent effects on more modest levels of intake (Cippitelli *et al.* 2012; Lowery *et al.* 2008; Lowery-Gionta *et al.* 2012; Simms *et al.* 2014). However, not all studies have consistently supported this generalization. For example, a significant restraint stress-induced increase in EtOH consumption in 129SVEV mice was not blocked by the CRF_1 antagonist, R121919 (Yang e*t al*. 2008), and CP-154,526 reduced intake under both higher and lower intake conditions in mice and rats (Hwa *et al.* 2013). Also, lesioning the CeA, which would be expected to affect neurons that are relevant to CRF-related pathways, did not prevent heightened levels of EtOH intake seen in C57BL/6J mice after dependence induction (Dhaher *et al*. 2008). Finally, Sharpe and Phillips (2009) showed that the selective CRF_2 agonist, Ucn_3, delivered centrally to non-dependent C57BL/6J mice, reduced 2-h limited access 10% EtOH consumption. This study used lickometers to investigate drinking patterns, and identified that reduced EtOH drinking by Ucn_3 was associated with a change in size of the largest drinking bouts. Lowery *et al.* (2010) also found that ICV infusions of Ucn_3 reduced binge-like EtOH drinking in C57BL/6J mice.

In conclusion, growing evidence from studies using both single gene mutant mice and pharmacology indicates that voluntary EtOH intake can be mediated by CRF signaling via CRF_1. CRF_1 appears to play a key role in acquisition of EtOH drinking when high levels of intake are achieved via binge-like drinking, genetic predisposition, exposure to high concentrations of EtOH or a combination of these conditions. Current literature also suggests that the enhancing effects of stress on EtOH drinking are mediated by CRF_1, although results may be influenced by species, genotype and methodological factors. A significant literature supports the view that increased EtOH drinking seen after long-term, dependency inducing periods of exposure to EtOH and EtOH-induced negative emotionality and anxiety associated with post-dependent states are mediated, at least in part, by CRF_1. CRF_2 appear to play a more minor role.

Effects of EtOH drinking on CRF and related molecules

We have focused on the ability to manipulate EtOH drinking and EtOH-induced neuroplasticity by genetically or pharmacologically altering relevant components of the CRF system. However, a few comments about the changes in this system induced by EtOH drinking are pertinent. A relatively early study examined the effect of different levels of voluntary EtOH drinking on brain CRF levels. Wistar rats were classified as low, moderate and high intake and then examined for CRF concentration in several brain regions. Rats classified as high drinkers had higher non-median eminence hypothalamic CRF concentrations, but lower neurointermediate pituitary and pons-medulla CRF concentrations (George *et al.* 1990). It should be noted that the different drinking levels in these Wistar rats could have had genetic, environmental or both types of influences as their source. In a more recent study, the number of CRF-positive cells in the CeA was higher in adult mice immediately after a binge-like EtOH drinking episode (Lowery-Gionta *et al.* 2012). This relationship appears to be altered when EtOH exposure occurs at an earlier, more distant time point, as CRF cell counts in the CeA were reduced, rather than increased, in adult rats that had a history of adolescent binge drinking (Gilpin *et al.* 2012), and so was CRF mRNA in the BLA (Falco *et al.* 2009). However, adolescent rats may have a higher basal level of CRF in some brain regions, including the CeA, compared with adult rats, which could affect the response of this system (Wills *et al.* 2010).

Some studies have examined the effect of pre-existing genetically-determined differences in EtOH preference. When the effect of voluntary EtOH drinking on CRF mRNA levels was examined in selectively bred Sardinian alcohol preferring (sP) rats, CRF mRNA levels were decreased in the CeA, but not in hypothalamus (Zhou *et al.* 2013). Furthermore, data for individual animals showed a significant negative correlation between intake and CRF mRNA level in the amygdala. Of course, it is impossible to compare this outcome to that in the oppositely selectively bred non-preferring line, because they will not voluntarily consume much EtOH. However, innate differences in pairs of selected lines can be examined. For example, when lines of rats bred for high and low EtOH drinking were compared in the EtOH-naïve state, CRF-positive cells and CRF mRNA were significantly lower in the CeA of alcohol preferring (P), compared with alcohol non-preferring (NP) rats, but not in the high alcohol drinking (HAD), compared with low alcohol drinking (LAD) rats (Hwang *et al.* 2004). Thus, the data are inconsistent with regard to levels of these CRF-related peptides as predictors of genetically determined tendency to consume EtOH. In addition, several lines of rats have been compared for native differences in Ucn_1-positive cells in the EWcp, with mixed findings; a greater number of Ucn_1 cells was found in the preferring line in two of the five surveyed pairs; a lower number in one preferring compared with non-preferring; and no difference was found in two of the five pairs (Turek *et al.* 2005). On the other hand, data from these rat lines were more consistent in showing a greater number of Ucn_1-positive projections to the lateral septum in association with EtOH preference (Turek *et al.* 2005). Furthermore, using immunohistochemistry, three sublines of alcohol-preferring rats were compared with control Wistar rats for Ucn_1-positive cells in the EWcp. The number of Ucn_1-positive cells was greater in male P, compared with Wistar rats; a similar non-significant trend was found in female animals (Fonareva *et al.* 2009).

Therefore, there are again contradictory findings, with regard to whether number of Ucn_1-positive cells serves as a marker for differences in genetically determined EtOH preference. Some of this variability in results could be related to the heterogeneous nature of the underlying genetic factors for EtOH drinking.

Other genetic findings

A few studies have provided evidence of associations of genetic polymorphisms in CRF-related genes with EtOH drinking phenotypes. The rhesus macaque *CRH* gene has been sequenced and examined for functional variants. One variant (−2232C→G) was shown to decrease DNA–protein interactions and decrease sensitivity of the *CRH* promoter to glucocorticoids in an *in vitro* assay. This variant was also associated with reduced CRF in the cerebral spinal fluid, and increased plasma ACTH, under non-stress conditions. It was also associated with increased EtOH consumption in adult macaques. The authors state that the genetic effect was specifically in macaques that were mother-reared in social groups, as opposed to macaques that were first isolate reared by human caregivers and then placed with peers from 37 days forward; however, intake data were not presented for the latter group (Barr *et al.* 2008). A single nucleotide polymorphism (SNP) within the rhesus macaque *CRH* promoter (−248C→T) was found to increase DNA–protein interactions and to increase EtOH consumption in animals that were isolate-peer reared, but not mother-reared. These monkeys also exhibited a larger stress-axis response to social separation stress (Barr *et al.* 2009). The authors suggested that effects of mutations may be specific to environmental conditions. Thus, for example, some may have effects under social drinking situations and others may affect stress-related drinking.

The electroencephalographic response to CRF was examined in P and NP rats as a marker of CRF-induced neural activation. P rats exhibited a larger response, compared with NP rats, and a lower basal concentration of CRF was found in P rats in several brain regions. These results led to the speculation that CRF receptors may be upregulated in P rats and that these differences in CRF neural regulation may contribute to differences in EtOH consumption (Ehlers *et al.* 1992). Subsequently, the finding of lower CRF in P rats was confirmed, but it was not replicated in another set of HAD and LAD lines (Hwang *et al.* 2004). Also, basal CRF levels in the CeA of sP rats were higher than in Sardinian non-preferring (sNP) rats (Richter *et al.* 2000; Zhou *et al.* 2013), a region where it had been found to be lower in P rats (Ehlers *et al.* 1992; Hwang *et al.* 2004). Furthermore, no difference was found between the high EtOH drinking C57BL/6J and EtOH avoiding DBA/2J mouse strains (Hayes *et al.* 2005). Therefore, a clear relationship between CRF level and genetically determined level of EtOH intake is not apparent. However, differences in innate anxiety level found between the P and NP (P > NP) rats, but not between HAD and LAD rats, may reflect variation in the specific genes involved in the selection traits across EtOH consumption selected lines and also support significant involvement of CRF specifically in anxiety- or stress-related drinking. Based on human SNP association analysis, Enoch *et al.* (2008) suggested that the CRF-BP gene (*CRHBP*) plays a role in stress-related EtOH use. However, a negative, rather than positive, correlation was found between level of anxiety-like behavior and CRF level in sP and sNP rats. Chen *et al.* (2010) suggested a role for *CRHR1* genetic variation in vulnerability to alcohol use disorder, and Treutlein *et al.* (2006) suggested an association of *CRHR1* polymorphisms with pattern of alcohol consumption. Additional genetic investigations will be needed to substantiate these relationships and identify gene networks that are likely to influence complex alcohol-related traits and possibly be population-specific.

A SNP in the promoter region of the CRF_1 gene (*Crhr1*) of the Marchigian–Sardinian preferring (mSP) rat may influence their heightened stress-induced EtOH drinking phenotype. This polymorphism results in upregulation of *Crhr1* in several brain regions, compared with levels seen in control Wistar rats. When these rats were treated with a CRF_1 antagonist, stress-induced reinstatement of EtOH drinking was blocked in mSP, but not in Wistar, rats (Hansson *et al.* 2006). Furthermore, chronic free-choice EtOH drinking was associated with downregulation of the CRF_1 protein in the amygdala and NAcc (Hansson *et al.* 2007). The authors suggested that heightened levels of CRF_1 drive excessive EtOH intake, which consequently reduces CRF_1 activity.

Because the gene coding for CRF_2 (*Crhr2*) maps to a genetic region associated with EtOH consumption, specifically in the inbred P and NP rats, *Crhr2* expression and sequence were examined and a receptor function assay was performed. Lower levels of *Crhr2* expression were found in P rats in some brain regions. In addition, a 7 base pair insertion polymorphism in the promoter region of the gene was found in the P rat, as well as a coding region polymorphism and an amino acid deletion in the 3′ untranslated region. The effect of the promoter insertion *in vitro* was to lower *Crhr2* expression, and CRF_2 density in the amygdala was lower in P, compared with NP rats (Yong *et al.* 2014). Whether these differences directly relate to differences in EtOH consumption between P and NP rats will require further investigation.

The role of CRF systems in EtOH-induced neuroadaptation

Dependence, withdrawal and relapse

One potential consequence of repeated EtOH administration is the development of dependence. Dependence can be inferred from certain symptoms that may be seen when chronic EtOH is withdrawn. Affective symptoms associated with EtOH withdrawal include increased anxiety, dysphoria and depressed mood, symptoms that have been posited to involve changes in the stress axis and central CRF-mediated process (Breese *et al.* 2005a; Ciccocioppo *et al.* 2009; Clapp *et al.* 2008; Griffin 2014; Koob 2010; Koob *et al.* 2014; Lowery & Thiele 2010; Shalev *et al.* 2010; Zorrilla *et al.* 2013). In addition, repeated bouts of EtOH exposure and withdrawal have been associated with escalation of EtOH intake (see description and history of this model in Vendruscolo & Roberts 2014) and a number of studies have explored the involvement of CRF systems in this effect, and in reinstatement of EtOH drinking and seeking, as traits relevant to relapse.

Single gene mutant mice studies

Few studies have utilized mutant mice to investigate the role of the CRF system in EtOH withdrawal-related effects. When CRF_1 KO and WT mice were made dependent on EtOH using a liquid diet, the KO mice did not exhibit withdrawal-induced increased EtOH seeking, whereas WT mice did; KO and WT mice were similar in EtOH seeking in the non-dependent state (Chu *et al.* 2007). The same paper reported that the CRF_1 antagonist, antalarmin, blocked withdrawal-induced increases in EtOH seeking in C57BL/6J background strain mice made dependent using EtOH vapor inhalation. These data support CRF_1 involvement in dependence-induced increases in EtOH seeking. We were not able to find additional studies examining effects in CRF-related KO mice.

Pharmacological studies

A number of studies have investigated the role of CRF and its related peptides in withdrawal-induced increases in EtOH drinking or self-administration using pharmacological manipulations. Data collected in C57BL/6J mice, in which a $CRF_{1/2}$ antagonist was microinjected into the CeA, showed a decrease in EtOH withdrawal-associated EtOH intake in the absence of an effect on non-dependent mice (Finn *et al.* 2007). A larger number of studies have examined the specific involvement of CRF_1 and there is general agreement that CRF_1 antagonists attenuate withdrawal-associated increases in EtOH drinking/self-administration (Chu *et al.* 2007; Funk *et al.* 2007; Gehlert *et al.* 2007; Overstreet *et al.* 2007; Roberto *et al.* 2010; Sabino *et al.* 2006). In most cases, the CRF_1 antagonist effects did not generalize to non-dependent animals; however, in one study that examined EtOH intake during operant sessions, rather than number of reinforcers, attenuating effects of R121919 were seen in both dependent and non-dependent rats (Roberto *et al.* 2010). It is worth mentioning, however, that in this study, repeated R121919 treatment was given 24 h before each operant testing session.

A few studies have examined the role of CRF_2. One study examined EtOH withdrawal-associated increased self-administration after intra-CeA infusion of Ucn_3 and attenuation was found (Funk & Koob 2007); however, EtOH self-administration in the non-dependent rats in this study was increased by intra-CeA Ucn_3 infusion. Others have found decreased EtOH intake with ICV infusion of Ucn_3 in C57BL/6J mice using DID procedures in which higher levels of EtOH intake are induced (Lowery *et al.* 2010; Sharpe & Phillips 2009). Therefore, while studies on KO mice do not appear to support a role for CRF_2, these pharmacological studies suggest that both CRF_1 and CRF_2 may influence higher levels of EtOH intake.

In mice made dependent using EtOH liquid diet, the non-selective CRF receptor antagonist α-helical $CRF_{(9-41)}$, given ICV, blocked the anxiogenic-like effects of EtOH withdrawal on the elevated plus maze, but did not alter other withdrawal symptoms, including tail stiffness, tremor or ventromedial distal flexion (Baldwin *et al.* 1991). The attenuating effect of CRF receptor antagonism on the anxiogenic-like response has been replicated (Valdez *et al.* 2003). Further, when CRF was microinjected into several brain regions, but not others (see Table 2), dose-dependent sensitization of an EtOH withdrawal-induced decrease in social interaction was seen (Huang *et al.* 2010), which has been posited to be an anxiety-like behavior (File 1980). Examination of the brain regions that supported these effects suggests that the extended amygdala is involved in withdrawal-associated anxiogenic behaviors. Several additional studies have used the social interaction test, as a behavioral index of anxiety-related behavior and have found that CRF_1 receptor-selective antagonists given during EtOH withdrawal can blunt anxiety-like behavior (Breese *et al.* 2004, 2005b; Knapp *et al.* 2004; Overstreet *et al.* 2007; Sommer *et al.* 2008; Wills *et al.* 2009). Furthermore, the CRF_1-selective antagonist, SSR125543, blocked CRF- and stressor-sensitized withdrawal-induced anxiety-like behavior (Breese *et al.* 2005b; Huang *et al.* 2010; Knapp *et al.* 2011a). However, the endogenous CRF_2-selective agonist, Ucn_3, given ICV or into several brain regions, did not affect EtOH withdrawal-associated anxiogenic effects (Huang *et al.* 2010), nor did the CRF_2 antagonist antisauvagine-30 (Overstreet *et al.* 2004). Taken together, these results suggest that CRF_1 plays a role in withdrawal-induced anxiogenic behaviors.

The role of CRF signaling has also been extensively studied in the context of behaviors thought to model relapse; in particular, reinstatement of EtOH seeking/drinking behavior in rodents. Corticotropin-releasing factor signaling involvement in escalation of use after periods of deprivation has also been examined. Most commonly, reinstatement studies have used operant methods in which animals are trained to perform an operant response to gain access to a reservoir or sipper containing EtOH, and then, once stable responding is achieved, extinction procedures are used that lead to low levels of the behavior that previously resulted in EtOH access. Post-extinction, active drug taking or seeking behavior (responding in the absence of drug delivery) can be re-established by drug priming, presentation of cues that were previously associated with drug availability or application of a stressor. In the case of EtOH, CRF signaling appears to play an important role in those mechanisms that particularly mediate stress-induced reinstatement, but not in those that facilitate drug prime or cue/context-induced reinstatement. For example, CRF_1 antagonists selectively reduce footshock-induced reinstatement of responding for EtOH (Le *et al.* 2000; Liu & Weiss 2002), an effect that appears to be especially prominent in EtOH-dependent or genetically selected EtOH preferring rats and is mediated by extra-hypothalamic mechanisms (Gehlert *et al.* 2007; Le *et al.* 2000; Liu & Weiss 2002). In addition, CRF signaling via CRF_1 modulates pharmacologically-induced stress effects on EtOH reinstatement; thus, stress-axis activation induced by yohimbine, an α2 adrenoreceptor antagonist that activates the ascending noradrenaline system and increases anxiety-like responses, reinstates responding for EtOH. This reinstatement is prevented by CRF and CRF_1 antagonism (Le *et al.* 2000, 2002; Marinelli *et al.* 2007), which appears to be mediated by CRF receptors in the median raphe nucleus (Le *et al.* 2013). On the other hand, the NAcc appears to be an important brain structure involved in the role of CRF in stress-induced escalation of EtOH intake during periods of deprivation. For example, when EtOH-preferring P rats that have a history of EtOH drinking are re-introduced to EtOH,

intake can be increased as a consequence of exposure to restraint stress administered during a period of EtOH deprivation; this effect was prevented by intra-NAcc injection of a CRF_1 antagonist (Knapp *et al.* 2011a). Furthermore, increased EtOH intake can be induced by intra-NAcc administration of CRF during the deprivation period (Knapp *et al.* 2011a). For a review of additional research examining stress-induced reinstatement of drug seeking and the role of CRF (among other neuropeptides), see Shalev *et al.* (2010).

Psychomotor sensitization

The body of data examining the role of CRF-related systems in behavioral sensitization to EtOH is small. However, data from both single gene KO mice and pharmacology have consistently indicated that CRF and CRF_1, but not CRF_2 and Ucn_1, play important roles in the neuroadaptations that underlie the development and expression of psychomotor sensitization to EtOH (Fee *et al.* 2007; Pastor *et al.* 2008, 2012; Phillips *et al.* 1997). Repeated restraint stress was previously shown to produce psychomotor sensitization to EtOH through a mechanism that involves CORT and GR (Roberts *et al.* 1995). More recent results from our laboratory and other research groups have shown a key role of CRF and CRF_1 in EtOH-induced psychomotor sensitization, even in the absence of an externally applied stressor (Fee *et al.* 2007; Pastor *et al.* 2008, 2012). Absent EtOH sensitization in CRF_1 mice was also associated with a blunted endocrine response (Pastor *et al.* 2008), suggesting an involvement of the HPA axis. Repeated injections of CORT sensitizes the locomotor-stimulant response to EtOH; however, the doses of systemic CORT necessary to induce sensitization resulted in plasma CORT levels notably higher than those produced by a sensitizing EtOH treatment (Pastor *et al.* 2012). Participation of hypothalamic CRF and CORT, therefore, appears to be necessary, but not sufficient, to explain the role of CRF/CRF_1 in the acquisition of sensitization to EtOH. In addition, the CORT synthesis inhibitor metyrapone prevents the development, but not the expression, of EtOH sensitization (Roberts *et al.* 1995). Furthermore, our data are in agreement with previous findings showing that, although EtOH- or stress-induced changes in CORT can be necessary to mediate acquisition of EtOH sensitization, no direct temporal correlation between plasma CORT levels and behavior has been seen (Pastor *et al.* 2012; Roberts *et al.* 1995). In summary, a CRF-dependent mechanism, via CRF_1, involving the HPA axis has been proposed for acquisition of sensitization, whereas an extra-hypothalamic CRF/CRF_1 mechanism has been suggested for expression of EtOH sensitization (Pastor *et al.* 2008, 2012).

Concluding remarks and future perspectives: from preclinical to clinical

The preclinical investigation of CRF receptors and ligands in stress vulnerability, EtOH dependence and relapse is among the most active research areas focused on the pharmacology and genetics of EtOH-induced behavior. This review has summarized evidence for CRF-related biological determinants that mediate stress- and EtOH-induced behavioral changes. Robust scientific evidence suggests that CRF and CRF_1 play seminal roles in stress-induced changes in EtOH consumption, binge-like EtOH intake, post-dependent heightened drinking, genetic predisposition, negative emotionality and anxiety and stress- and EtOH-induced behavioral sensitization. The field would benefit from additional research aimed at identifying the specific molecular determinants of EtOH-induced CRF_1 activation and CRF_1-mediated neuroplasticity that contributes to changes in EtOH responses. In an elegant study combining pharmacological and KO approaches, Bajo *et al.* (2008) showed that EtOH induces the release of GABA in the CeA via a mechanism that depends on a CRF_1-initiated mechanism, which requires participation of protein kinase C (PKC) epsilon. Mutant mice lacking PKC epsilon showed a stress- and EtOH-induced phenotypic profile comparable to that found in CRF_1 KO mice (Table 1), characterized by reduced anxiety-like behavior and EtOH consumption (Hodge *et al.* 1999, 2002; Olive *et al.* 2000). Additional research exploring whether this mechanism is also involved in CRF_1-induced effects in other brain regions would further define the relevant brain circuitry. As recently reviewed by Haass-Koffler and Bartlett (2012), CRF plays an important role in facilitating acquisition and maintenance of plasticity in the VTA and amygdala, particularly via enhanced glutamatergic activation and decreased GABA-mediated inhibition. Further research exploring the mechanisms supporting CRF_1-mediated plasticity would also be extremely relevant in this field.

Given its clinical relevance and the notion that CRF-mediated neuroplasticity in the mesocorticolimbic neuronal network may contribute to stress vulnerability, loss of control over EtOH consumption and relapse, an increased and particular focus should be placed on exploring strategies to block experiencing the effects of such neuroadaptations. This is sometimes referred to as the *expression* of the neuroadaptive effect. Blocking or reducing the expression of such neuroplastic changes could include not only pharmacological strategies, but also behavioral strategies. Solinas *et al.* (2008) reported that environmental enrichment can reduce some of the neurochemical and behavioral effects of repeated administrations of cocaine, and others have indicated that this type of manipulation can reduce stress levels *per se* and also reduce elevated stress hormones associated with morphine or amphetamine administration (Ravenelle *et al.* 2013; Xu *et al.* 2014). Investigating whether environmental enrichment, such as increased physical exercise (Segat *et al.* 2014), might alter behavioral and neurochemical indicators of CRF-mediated neuroplasticity associated with a history of EtOH administration may be a valuable future line of research. Clearly, preventing all exposure to EtOH is almost impossible, so focusing on the *acquisition* of neuroadaptations may be less fruitful from the perspective of treatment; however, it should be mentioned that recent findings for cocaine suggest that loss of environmental enrichment could increase vulnerability to drug use (Nader *et al.* 2012), and thus increase the probability of drug-induced neuroplasticity.

In view of some recent findings (reviewed by Zorrilla *et al.* 2014), additional preclinical research is needed on genetic factors that contribute to differential effectiveness of CRF_1 antagonists (Heilig *et al.* 2010; Sinha 2008). Further, additional explorations are needed to substantiate data suggesting that gene polymorphisms may play a role in risk for EtOH use. For example, *Crh* and *Crhr1* polymorphisms have been associated with increased active responses to stress in animals selectively bred for high preference for EtOH (Ayanwuyi *et al.* 2013; Cippitelli *et al.* 2014) and with increased EtOH consumption in monkeys exposed to early life stress (Barr *et al.* 2009). Polymorphisms in human CRH_1 and *CRHBP* have also been associated with different aspects of alcohol use and dependence (Chen *et al.* 2010; Enoch *et al.* 2008; Treutlein *et al.* 2006).

Finally, based on promising results for CRF_1 antagonist effects on EtOH consumption in animal models, there has been considerable interest in the potential for these drugs as pharmacotherapeutics for alcohol use disorders. Zorrilla *et al.* (2013) suggest that such drugs have promise, in part, because their anxiolytic-like actions do not appear to be susceptible to tolerance (Zorrilla & Koob 2004), they do not appear to have sedative effects or adverse effects on motor coordination nor adversely affect attention or learning (Hogan *et al.* 2005; Zorrilla & Koob 2004; Zorrilla *et al.* 2002), and they may have little addiction liability (Broadbear *et al.* 2002; Sahuque *et al.* 2006; Stinus *et al.* 2005). Clinical trials began about 10 years ago (December 2004) with several CRF_1 antagonists. Traits being examined have included major depression, irritable bowel syndrome, social anxiety disorder and post-traumatic stress disorder. None appear to have completed a Phase III trial. Development of one antagonist was discontinued due to instances of elevated liver enzymes, others because of lack of efficacy in double-blind, placebo-controlled trials for major depression (Koob & Zorrilla 2012; Zorrilla & Koob 2010). Koob and Zorrilla (2012) have provided the revisionist view that CRF_1 antagonists may be most efficacious for psychiatric disorders in which stress is a more dynamic than chronic factor, including addiction. Perhaps, an alternative to consider is a drug(s) that has indirect effects on the CRF system. For example, in one study, the reduction in EtOH intake by the opioid receptor antagonist, naltrexone, was associated with blockade of CRF expression in the PVN induced by EtOH drinking (Oliva & Manzanares 2007). In another study, the effect of combined naltrexone and the CRF_1 antagonist, CP154526, was examined on intermittent access EtOH drinking in C57BL/6J mice, when infused into the DR. Each drug was effective, at least transiently, when given alone, but an increased effect was not seen when the drugs were given together (Hwa *et al.* 2014). However, this study used an intermittent access EtOH protocol that did not specifically include evaluation of the contribution of cue/context effects, which could be important. Previous data indicate that opioid antagonists not only reduce EtOH intake (Méndez & Morales-Mulia 2008), but also reduce cue-dependent reinstatement of EtOH seeking (Liu & Weiss 2002). Context-dependent EtOH reinstatement has also been seen to be mediated by opioid receptors, in particular, BLA opioid receptors (Burattini *et al.* 2006; Marinelli *et al.* 2010). These pre-clinical data are particularly relevant, as human data indicate that opioid antagonism increases duration of abstinence periods (Maisel *et al.* 2013; O'Malley et al. 2007), which might be an indicator of opioid-mediated attenuation of the relapse-triggering strength of context and other conditioned stimuli. A combined strategy that reduces vulnerability to both stress-induced and conditioned stimuli-induced relapse could be important to consider. Collectively, we agree with many other investigators that the CRF system plays a remarkably important role in the etiology and maintenance of addiction, and particularly in the effects of excessive use. The need for continued research directed at identifying ways to reverse or inhibit the effect of changes in this system on active and relapsing use is supported by the existing findings.

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