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# Ethanol and corticotropin releasing factor receptor modulation of central amygdala neurocircuitry: An update and future directions

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ABSTRACT

The central amygdala is a critical brain region for many aspects of alcohol dependence. Much of the work examining the mechanisms by which the central amygdala mediates the development of alcohol dependence has focused on the interaction of acute and chronic ethanol with central amygdala corticotropin releasing factor signaling. This work has led to a great deal of success in furthering the general understanding of central amygdala neurocircuitry and its role in alcohol dependence. Much of this work has primarily focused on the hypothesis that ethanol utilizes endogenous corticotropin releasing factor signaling to upregulate inhibitory GABAergic transmission in the central amygdala. Work that is more recent suggests that corticotropin releasing factor also plays an important role in mediating anxiety-like behaviors via the enhancement of central amygdala glutamatergic transmission, implying that ethanol/corticotropin releasing factor interactions may modulate excitatory neurotransmission in this brain region. In addition, a number of studies utilizing optogenetic strategies or transgenic mouse lines have begun to examine specific central amygdala neurocircuit dynamics and neuronal subpopulations to better understand overall central amygdala neurocircuitry and the role of neuronal subtypes in mediating anxiety-like behaviors. This review will provide a brief update on this literature and describe some potential future directions that may be important for the development of better treatments for alcohol addiction.



## Introduction

A great deal of work has focused on the role of the central amygdala (CeA) in the development of alcoholism as it plays a role in initial ethanol preference, binge drinking, and late-stage dependence. Much of this work has shown that corticotropin releasing factor (CRF) receptor signaling in the CeA plays a critical role in many aspects of these ethanol-related behaviors (Koob, 2009; Koob & Volkow, 2010; Logrip, Koob, & Zorrilla, 2011; Lowery & Thiele, 2010; Sprow & Thiele, 2012; Zorrilla, Logrip, & Koob, 2014). Although behavioral data indicate that CRF modulation of CeA function is important for the regulation of ethanol-related behaviors and anxiety (for review, see Gilpin, Herman, & Roberto, 2014), this has to date not translated into effective clinical therapeutics based on these findings (Coric et al., 2010). We suggest that a deeper understanding of the actions of the CRF receptor system in the CeA may lead to improved treatment strategies in the future.

Heightened levels of anxiety in both clinical and preclinical literature have been associated with elevated CeA activity, while decreased activity is associated with reductions in anxiety-like behavior (Adhikari, 2014). For instance, preclinical findings suggest that activation of receptors for the inhibitory neurotransmitter GABA in CeA produces anxiolysis (Carvalho, Moreira, Zanoveli, & Brandão, 2012; Moreira, Masson, Carvalho, & Brandão, 2007) and a reduction in physiologic responses to stressors (Sullivan, Henke, Ray, Hebert, & Trimper, 1989). In support of the idea that enhanced CeA activity is associated with increases in anxiety, it is well understood that the activity of a large excitatory glutamatergic projection from the basolateral amygdala to the CeA is enhanced during fear conditioning (Duvarci & Pare, 2014), a behavioral paradigm that is related to anxiety disorders. This literature has proven to be fruitful ground for the study of ethanol dependence, as ethanol has anxiolytic properties and the GABA-mimetic profile of ethanol has been proposed to be an important mediator of many aspects of ethanol dependence (Breese et al., 2006). Other work indicates that stimulation of glutamatergic basolateral amygdala

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inputs to the CeA can increase the incentive motivation for self-administration of one particular reward over others (Robinson, Warlow, & Berridge, 2014), a behavior consistent with the onset of addiction. Thus, one might expect that increased excitatory drive to the CeA leads to increased anxiety, and would likely result in avoidance of certain external stimuli. On the other hand, one could argue – based on the self-administration studies – that increased excitatory drive to the CeA may increase approach to a particular external stimulus. This raises the question as to how activation of what appears to be the same CeA pathway may lead to multiple effects that on face value can be construed to be at opposite ends of the behavioral spectrum.

The answer to this question may be accounted for, in part, by which CeA subregion is activated by these diverse behavioral paradigms. The CeA can be divided into four major subdivisions, the lateral capsular (CeLC), the lateral (CeL), the medial (CeM), and the intermediate (CeI), subregions that maintain distinct inter-subregion connectivity (Akmaev, Kalimullina, & Sharipova, 2004; Cassell, Freedman, & Shi, 1999; Sah, Faber, Lopez De Armentia, & Power, 2003). In general, the CeL is thought to be the major input subregion and the CeM appears to be the major output subnucleus, while the other subregions can gate inter-regional activity. Since much less is known about the other two regions, we will primarily restrict our discussion to CeM and CeL. These subregions have distinct subclasses of neurons based on morphology and peptide and/or protein content. The majority of neurons in the CeA are medium spiny GABAergic neurons, but these neurons contain a wide variety of other co-transmitters, peptides, or protein markers such as enkephalin, CRF, substance P, neurotensin, somatostatin, calbindin d28k, and various protein kinase C (PKC) isoforms, which are arranged in a loosely subregion-specific organization. For instance, CRF neurons are predominantly located in CeL (Asan et al., 2005; Treweek, Jaferi, Colago, Zhou, & Pickel, 2009), while substance P neurons are more heavily expressed in the CeM (Shimada et al., 1989). Overall, studies examining the neurocircuit architecture of the CeA may be somewhat limited by the lack of strong boundary demarcations between CeA subregions, and the potential for cell types typical of one subregion to also overlap into others (For more complete reviews of CeA neuroanatomy, see Akmaev et al., 2004; Cassell et al., 1999).

Even with this knowledge, one of the major limitations in moving preclinical findings into novel treatments for alcoholism is that our understanding of CeA neurocircuitry is not complete. In contrast to the above discussion indicating that increased excitation of the CeA would be expected to increase anxiety-like behaviors, recent work indicates that selective activation of CeA subregions may produce effects opposite to those predicted for anxiety-like behaviors (Ciocchi et al., 2010; Haubensak et al., 2010; Tye et al., 2011). For instance, Ciocchi et al. (2010) show that inactivation of the CeL can elicit freezing behaviors (one measure of anxiety-like behavior) and disrupt the acquisition of fear conditioning. Optogenetic stimulation of the CeM, on the other hand, produces robust freezing while inactivation of the CeM produces deficits in the retrieval/expression of conditioned freezing behaviors 24 h after fear conditioning. Overall, these findings suggest that the CeM is necessary and sufficient for freezing behaviors, that the CeL is critical for acquisition of fear conditioning, and that the CeM is under tonic inhibitory control by some neurons in the CeL (Ciocchi et al., 2010).

Supporting the hypothesis that the CeM is under inhibitory control of the CeL, Tye et al. (2011) indicate that selective stimulation of basolateral amygdala glutamatergic terminals in the CeL can elicit decreases in anxiety-like behavior through activation of CeL-mediated feed-forward inhibition of CeM output neurons. When BLA projections to CeL neurons were selectively inhibited, mice showed anxiogenic behavior in the open-field test, which is likely due to losses of tonic inhibitory tone from CeL neurons to CeM output neurons (Tye et al., 2011). Other work to be discussed in this review suggests that specific neuronal populations within these CeA subregions may also play divergent roles in anxiety-like behaviors. These findings highlight the need for more specific interrogation of select neuronal populations and subregions in properly evaluating the mechanism by which ethanol modulates CeA neurocircuitry, and to produce more effective pharmacotherapeutic treatments for alcoholism in the future. This review will focus on the role of ethanol and CRF receptor system interactions in modulating CeA neurotransmission and will attempt to lay out some novel avenues for research based on the recent work examining the behavioral roles of distinct CeA subregions and neuronal subpopulations.

## Ethanol modulation of central amygdala GABAergic signaling

It is now well understood that ethanol interactions with CeA neurocircuitry likely play critical roles in mediating the acute anxiolytic effects of this drug. Given that ethanol has strong anxiolytic properties similar to $GABA_A$ receptor agonists (for review, see Breese et al., 2006) and can increase GABAergic transmission in multiple brain regions (Jia, Chandra, Homanics, & Harrison, 2008; Mameli, Botta, Zamudio, Zucca, & Valenzuela, 2008; Silberman, Shi, Brunso-Bechtold, & Weiner, 2008; Theile, Morikawa, Gonzales, & Morrisett, 2008; Werner et al., 2006), it was reasoned that ethanol may alter CeA GABAergic signaling as an underlying mechanism of action for ethanol-induced reductions in anxiety-like behaviors. Indeed, acute ethanol can increase presynaptic GABA release and enhance postsynaptic GABA receptor function in the CeA (Roberto, Madamba, Moore, Tallent, & Siggins, 2003), while chronic ethanol exposure can promote increased basal GABA release in the CeA without tolerance to the acute presynaptic effects of ethanol at these synapses (Roberto, Madamba, Stouffer, Parsons, & Siggins, 2004). Furthermore, intra-CeA microinjection of gabapentin can reduce elevated operant ethanol responding in ethanol-dependent rats (Roberto et al., 2008) and intra-CeA microinjection of a mixed benzodiazepine agonist/antagonist can inhibit ethanol-maintained responses in ethanol-preferring rat lines (Foster et al., 2003).

As noted in the introduction, CRF receptor (CRFR) signaling in the CeA plays a critical role in many aspects of ethanol-related behaviors and responses. For instance, CeA CRFR activity is thought to be recruited in animal models of binge-like ethanol drinking (Lowery-Gionta et al., 2012), is critical for increased anxiety and decreased social interactions during ethanol withdrawal (Rassnick, Heinrichs, Britton, & Koob, 1993; Wills, Knapp, Overstreet, & Breese, 2010), and is important for increased ethanol drinking during withdrawal (Finn et al., 2007; Funk, O'Dell, Crawford, & Koob, 2006). In addition, numerous studies demonstrate that CRFR agonists can enhance GABAergic transmission in the CeA, and that this mechanism of action plays an important role in the acute and chronic effects of ethanol on CeA circuitry (Nie et al., 2004, 2009; Roberto et al., 2010). Specifically, these studies indicate that the ability of acute ethanol to increase GABA release in the CeA is completely blocked by pretreating CeA slices with a CRFR1 antagonist, suggesting that ethanol utilizes endogenous CRFR signaling to modulate GABAergic transmission in the CeA. Overall, these findings suggest that ethanol causes a release of CRF in the CeA, presumably from local CRF-producing neurons, which in turn acts to promote activity of CRFR1-containing CeA neurons to induce GABA release onto CeA output neurons.

These studies provide an important body of literature demonstrating CRFR-dependent actions of ethanol on CeA GABA