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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.

Keywords

central amygdala; corticotropin releasing factor; synaptic transmission; transgenic mouse lines

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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, & Zorilla, 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 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

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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.

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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; Nie et al., 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 transmission. As the majority of these studies were performed prior to our understanding of the dissociable roles of CeL and CeM in anxiety-like behaviors, in future studies it will be important to explore these actions in a CeA-subregion specific manner.

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Ethanol modulation of central amygdala glutamate transmission

Ethanol has been shown to inhibit glutamatergic transmission in many brain regions, and this mechanism is hypothesized to be important in many of the amnestic and anxiolytic effects of ethanol (for review, see Tsai & Coyle, 1998). In the CeA, ethanol has been shown to inhibit evoked glutamatergic transmission via effects at NMDA and non-NMDA receptors in CeA (Roberto, Bajo, Crawford, Madamba, & Siggins, 2006; Roberto, Schweitzer, et al., 2004). Ethanol has also been shown to inhibit miniature excitatory synaptic transmission in this brain region (Zhu, Bie, & Pan, 2007). Withdrawal from chronic ethanol also appears to sensitize NMDA receptors to subsequent acute ethanol application (Roberto, Schweitzer, et al., 2004). It is not yet known whether ethanol produces a divergent effect on glutamate transmission in the various CeA subregions or whether subregion effects of ethanol differentially regulate ethanol-related behaviors. However, such studies may be warranted based on recent evidence of dissociable behavioral effects of CeA subregions on behavioral measures of anxiety.

It is also not clear whether ethanol utilizes CRFR signaling to modulate glutamate transmission in the CeA similar to the effects of ethanol on CeA GABAergic transmission. Conditional knockout of CRFR1 from forebrain GABAergic synapses produces no significant effects on basal or stimulated anxiety-like behaviors, suggesting that ethanol/CRF interactions with CeA GABAergic signaling may not be the sole source for the anxiolytic properties of ethanol. Conditional CRFR1 deletion from forebrain glutamatergic synapses, however, significantly decreases anxiety-like behaviors (Refojo et al., 2011). Together, these findings suggest that CRF may modulate CeA glutamatergic signaling and that this mechanism could have more functional relevance to anxiety-like behaviors, and potentially ethanol-related behaviors, than CRFR modulation of CeA GABAergic signaling. Supporting this hypothesis, CRFR modulation of CeA glutamate signaling is known to be involved in behavioral responses to acute stress (Reznikov et al., 2007), expression of anxiety-like behaviors (Kalin, Shelton, & Davidson, 2004), conditioned place aversion (Watanabe et al., 2002), as well as fear learning (Skórzewska et al., 2009) and consolidation of fear memories (Pitts, Todorovic, Blank, & Takahashi, 2009).

A limited number of studies have examined CRFR modulation of glutamatergic transmission in the CeA utilizing *in vitro* electrophysiology methods (Ji, Fu, Adwanikar, & Neugebauer, 2013; Liu et al., 2004; Pollandt et al., 2006; Silberman & Winder, 2013) with somewhat conflicting results. While the studies mentioned in the previous section show that CRF modulates evoked and spontaneous forms of CeA GABAergic transmission in a similar fashion, CRF may differentially alter evoked vs. spontaneous forms of CeA glutamate transmission. Specifically, CRF can inhibit evoked glutamatergic transmission in the CeA and can also enhance spontaneous CeA glutamate release in drug-naïve mice/rats (Liu et al., 2004; Silberman & Winder, 2013). This apparently contradictory finding may be explained by the proposed possibility that distinct pools of synaptic vesicles may be released by evoked synaptic transmission compared to those released during spontaneous transmission events at certain synapses (Ramirez, Khvotchev, Trauterman, & Kavalali, 2012). This hypothesis would further suggest that CRF might differentially modulate distinct pools of glutamate vesicles in the CeA. It is thought that spontaneous neurotransmission may play an

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important homeostatic role in established synaptic networks and may play a permissive role in synaptic plasticity by sensitizing post-synaptic neurons to activity-dependent transmission (Kavalali et al., 2011; Ramirez & Kavalali, 2011). This may indeed be the case for CeA glutamate synapses, as the ability of CRF to inhibit evoked glutamatergic transmission is lost following chronic cocaine treatment, at which point CRF elicits increases in evoked excitatory transmission (Pollandt et al., 2006). These findings suggest chronic cocaine exposure may have, through some yet unknown mechanism, first mobilized CRFR-sensitive spontaneous glutamatergic transmission in the CeA and produced plasticity of these synapses such that subsequent CRFR activation results in increased CeA excitability. Such increased CeA excitability may be linked to enhanced reward preference and incentive motivation for particular rewards (Robinson et al., 2014), which may play a role in the development of addictive behaviors. This hypothesis should be more closely examined regarding ethanol-related behaviors in future studies.

The finding that CRF increases spontaneous glutamate transmission in the CeA raises a novel hypothesis that ethanol, via its known interaction with CeA CRFR signaling, may simultaneously increase both GABAergic and glutamatergic signaling in this brain region. Given the apparent unique relationship between CRF and spontaneous glutamatergic transmission in the CeA and the potential role of spontaneous neurotransmission in preparing synaptic connections for plasticity to occur, it will be of great value to investigate the effects of ethanol on spontaneous glutamatergic transmission in the CeA and determine if any potential effects are regulated by CRFR signaling. Overall, these converging lines of evidence indicate that ethanol modulation of CeA CRFR/glutamate signaling may provide critical new insights into the role of CeA network activity in the development of alcoholism or in the potential advent of new treatments for this disease.

Neuronal cell types in the CeA

Our limited, but growing, understanding of CeA neurocircuitry recruitment during specific behaviors (Ciocchi et al., 2010; Tye et al., 2011) and newer work indicating novel roles of the CeA in reward-directed behaviors (Robinson et al., 2014) highlight the need for studies that can precisely measure the effects of ethanol on specific neuronal populations in the CeA. The continued advent of novel transgenic reporter mice lines in recent years for use in examining CeA neuron populations, such as the CRF1:GFP (Herman, Contet, Justice, Vale, & Roberto, 2013), CRF-*tomato* (Silberman, Matthews, & Winder, 2013), PKC-δ::GluClα-iCre (Haubensak et al., 2010), and the somatostatin-IRES-Cre (Li et al., 2013) lines, is likely to play an important role in gaining a better understanding of the general architecture of CeA neurocircuitry and how specific neuronal cell types and signaling systems may modulate CeA activity in functional responses to environmental stimuli or in the development of multiple disease states. With the exception of the CRF1:GFP mouse, these lines of mice have yet to be tested specifically in the context of ethanol research, but the available evidence suggests future studies with these mouse lines in examining the mechanism of ethanol action in the CeA and in ethanol-related behaviors may be warranted. Information on these mouse lines is briefly summarized in the following sections.

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CRF1:GFP mice

Based on the wealth of previous literature (Nie et al., 2004; Nie et al., 2009; Roberto et al., 2010; Roberto et al., 2003; Roberto, Madamba, et al., 2004), it is hypothesized that acute ethanol mobilizes CRF signaling in the CeA, activating a putative CRFR1-containing interneuron population or presynaptic GABAergic release site, to inhibit CeA outputs to modulate anxiety-like behaviors. A more recent report began to address some of these hypotheses utilizing a CRF1:GFP reporter mouse (Herman et al., 2013). The CRF1:GFP mouse was first developed to examine the distribution of CRFR1 expression (Justice, Yuan, Sawchenko, & Vale, 2008) that was not previously achievable due to a lack of highly specific antibodies (Refojo et al., 2011). Electrophysiology studies with this mouse line showed that ethanol enhances the activity of CeA neurons containing CRFR1 (Herman et al., 2013). This effect was most likely due to ethanol increasing tonic inhibition and decreasing action potential firing of neighboring, synaptically connected GABAergic neurons, resulting in a subsequent disinhibition of CRFR1+ neurons. Based on previous findings (Nie et al., 2004; Nie et al., 2009; Roberto et al., 2010; Roberto et al., 2003; Roberto, Madamba, et al., 2004), it may have been expected that ethanol might modify CRFR1+ neuron firing by enhancing the activity of neighboring neurons that would act to release CRF. Part of this discrepancy may be due to the fact that CRFR1+ neurons were predominantly located in the CeM, while the location of recordings in previous work examining ethanol/CRF interactions on GABAergic transmission were not defined in most cases. It is not yet clear what role previously described ethanol-mediated CRF release in this brain region would have on this novel circuit. It also remains to be seen how CRFR1+ neurons are modulated by chronic ethanol exposure or the roles that these neurons may play in modulating CeA neurocircuitry in regard to anxiety or ethanol-drinking behaviors. Answers to these questions will provide novel insights into overall CeA neurocircuitry design and the way this circuit is manipulated by chronic ethanol to produce pathology and dependence.

CRF-*tomato* mice

Of the other transgenic reporter lines mentioned above, the CRF-*tomato* mouse line may prove especially useful based on the key role of CRF signaling in ethanol modulation of CeA neurocircuitry. This line is a cross between two other mouse lines: the CRF-IRES-Cre line [strain B6(Cg)-Crhtm1(cre)Zjh/J (Taniguchi et al., 2011)] – originally used to characterize interneurons in the cerebral cortex – and the ROSA26-Ai9 line [strain B6.Cg-Gt(ROSA)26S or <tm14(CAG-tdTomato)Hze>/J (Madisen et al., 2010)] – originally developed as a high-throughput Cre-reporting characterization system for the whole brain. Currently, use of the CRF-*tomato* line to examine CeA CRF neurons has been limited to a basic characterization of neuronal properties in response to current injections (Silberman et al., 2013). Even from such limited studies, a number of interesting findings have arisen. Based on their firing properties, CeA CRF neurons appear to be quite homogeneous in nature compared to CRF neurons in the BNST, and have the characteristic firing properties typically associated with projection-type neurons (Silberman et al., 2013). It will be important in future studies to use this mouse line to determine if ethanol-mediated increases in CRFR1 signaling and concomitant increases in GABAergic (and potentially

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glutamatergic) transmission in the CeA arises from increased activity of local CRF neuron populations or from increased CRF release from sources outside the CeA (Uryu, Okumura, Shibasaki, & Sakanaka, 1992). Furthermore, this mouse line could also be used for studies in which CRF neurons may be manipulated by optogenetic and chemogenetic approaches to examine the role of these specific neurons in ethanol-related behaviors.

PKC-δ::GluClα-iCre mice

PKC-δ::GluClα-iCre mice were generated to specifically target and analyze the activity of PKCδ+ neurons in the CeA. PKCδ+ neurons account for about 50% of GABAergic neurons in the CeL, and anterograde tracing shows that these neurons project to the CeM, where they provide feed-forward inhibition of CeM output neurons. These neurons are also part of a reciprocal local inhibitory circuit with PKCδ− neurons, a group of CeL neurons that also project to the CeM (Haubensak et al., 2010). Furthermore, PKCδ+ neurons are functionally similar to CeL off units, neuronal ensembles previously described to become inhibited *in vivo* following exposure to a conditioned stimulus during fear learning (Ciocchi et al., 2010). Therefore, it seems likely that PKCδ+ neurons play an important role in the tonic inhibition of CeM output neurons, and become inactivated by external stimuli of sufficient strength to elicit freezing behaviors via disinhibition of CeM projection neurons. It is possible, therefore, that ethanol may produce anxiolysis, at least in part, via activation of CeA PKCδ+ neurons and enhanced inhibition of CeM outputs even in the presence of anxiogenic stimuli. PKCδ+ neurons also appear to have minimal overlap with neurons containing CRF mRNA in the CeL (Haubensak et al., 2010). Although it remains to be seen what role, if any, PKCδ + neurons play in the ethanol-related behaviors, acute ethanol has been shown to alter phosphorylation of CeA PKC (Wilkie et al., 2007), and PKC signaling is critical for ethanol/CRF modulation of GABAergic signaling in the CeA (Bajo, Cruz, Siggins, Messing, & Roberto, 2008), suggesting that PKCδ+ neurons may be downstream of CRF-producing neurons in the CeL, and that PKCδ− neurons examined in the above-described study are also not likely to have been CRF-containing neurons. It will be important to integrate these findings with the previous research indicating that ethanol can enhance GABAergic, and potentially glutamatergic, transmission in this brain region via a CRF-dependent mechanism. Overall, though, these findings may suggest that PKC signaling in general, and potentially PKCδ+ neurons in particular, may be important factors in regulating CeA-mediated ethanol-related behaviors dependent on CRF signaling.

Somatostatin-IRES-Cre mice

Similar to the CRF-IRES-Cre line, the somatostatin-IRES-Cre line was originally developed to examine GABAergic neuronal populations in the cerebral cortex (Taniguchi et al., 2011). For the purposes of examining the electrophysiological properties of SOM+ neurons in the CeL, the somatostatin-IRES-Cre line was crossed with the Ai14 reporter line (Li et al., 2013). SOM+ neurons appear to be a heterogeneous group of cells, although they are clearly distinct from PKCδ+ neurons, in that there is little overlap of these two markers. Most SOM + neurons do not project to the CeM, which also suggests that SOM+ neurons were not investigated in the previous work with PKCδ− neurons. Studies with SOM+ reporter mice found that while synaptic strength of excitatory synapses originating from the basolateral

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amygdala is stronger in SOM− neurons compared to SOM+ neurons in naïve mice, this relationship is reversed following fear conditioning, such that excitatory synaptic strength is increased in SOM+ neurons following fear conditioning, while excitatory synapses onto SOM− neurons are weakened (Li et al., 2013). Since converging lines of evidence suggest that activity of the basolateral amygdala -> CeA pathway may be similarly enhanced during ethanol withdrawal as it is during fear learning (Johansen, Cain, Ostroff, & LeDoux, 2011; McCool, Christian, Diaz, & Läck, 2010), it is intriguing to speculate that changes in synaptic strength of BLA projections to SOM+ CeA neurons seen in fear learning may also occur following chronic ethanol exposure. Overall, work with PKCδ+ and SOM+ reporter lines suggests that activation of SOM+ neurons inhibits PKCδ+ (SOM−) neurons, thus resulting in disinhibition of the CeM and promoting fear expression and freezing behaviors. In addition to local circuit activity, the CeL also sends long-range projections to other brain regions, and greater than 90% of CeL neurons that send long-range projections to either the periaqueductal gray or the paraventricular thalamus are SOM+ neurons (Penzo, Robert, & Li, 2014). This work also indicates that SOM+ CeL long-range projections may not mediate fast inhibitory synaptic transmission in their target areas and instead may regulate their target brain regions through release of currently unknown co-transmitters or peptides. The CeA is known to send a CRF+ projection to the bed nucleus of the stria terminalis, and this projection is activated during stress-induced reinstatement of drug-seeking behaviors (Erb, Salmaso, Rodaros, & Stewart, 2001). Thus, it will be important in future studies to determine if SOM+ neurons represent overlapping populations with CRF+ neurons in the CeL, to help determine how some long-range CeL projections modulate activity in their target regions and how these neurons may be modulated by acute and chronic ethanol exposure.

Conclusions

Although CRF receptor signaling in the CeA is now well established as an important mediator in the development of alcoholism, the underlying mechanism by which ethanol and CRF receptor signaling interact to modulate CeA neurocircuitry is still not well understood. Ethanol has been shown to enhance GABAergic transmission via modulation of CeA CRF receptor signaling, but recent evidence advances the hypothesis that ethanol may also utilize CRF signaling to enhance CeA glutamatergic signaling. It is not clear how ethanol utilization of CRF receptor signaling to enhance both GABAergic and glutamatergic signaling concurrently in the same brain region may alter ethanol-directed behaviors, or if the relative strengths of these effects are equal in naïve and ethanol-dependent mice. It is also not clear how acute or chronic ethanol may modulate the activity of specific neurons within this circuitry and if distinct classes of CeA neurons are more or less sensitive to perturbation by ethanol. Although clearly speculative in nature at this time, since recent findings clearly indicate that CeA neuronal populations play diverse roles in mediating anxiety-like behaviors, it is likely that examining how ethanol modulates these newly defined CeA microcircuits will lead to better overall understanding of the mechanisms leading to the development of alcoholism. Answers to these questions will hopefully lead to a better understanding of the neuropathology leading to alcoholism and aid in the development of novel treatment strategies for the alleviation of this disease.

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Highlights

- CeA circuitry is critical to alcoholism but the mechanisms are unclear
- CRFRs mediate ethanol effects on CeA GABA and potentially glutamate transmission
- Direct studies on ethanol effects on CeA CRF and non-CRF neurons are now emerging
- Here, we review some novel directions for CeA neurocircuit studies in alcoholism
- Such new studies will be critical in development of novel treatments for alcoholism