The Central Amygdala as an Integrative Hub for Anxiety and Alcohol Use Disorders

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ABSTRACT

The central amygdala (CeA) plays a central role in physiologic and behavioral responses to fearful stimuli, stressful stimuli, and drug-related stimuli. The CeA receives dense inputs from cortical regions, is the major output region of the amygdala, is primarily GABAergic (inhibitory), and expresses high levels of pro-stress and anti-stress peptides. The CeA is also a constituent region of a conceptual macrostructure called the extended amygdala that is recruited during the transition to alcohol dependence. We discuss neurotransmission in the CeA as a potential integrative hub between anxiety disorders and alcohol use disorder, which are commonly co-occurring in humans. Imaging studies in humans and multidisciplinary work in animals collectively suggest that CeA structure and function are altered in individuals with anxiety disorders and alcohol use disorder, the end result of which may be disinhibition of downstream “effector” regions that regulate anxiety-related and alcohol-related behaviors.

Keywords: Anxiety disorder, CRF, Extended amygdala, GABA, NPY, Posttraumatic stress disorder

Anxiety disorders and alcohol use disorders (AUD) are highly comorbid in humans. Anxiety disorders often precipitate alcohol abuse, and high anxiety is a hallmark symptom of alcohol dependence that manifests during withdrawal. Many anxiety disorders are marked by hyperactivity or hyperreactivity of the amygdala (1), as supported by neuroimaging data, although functional magnetic resonance imaging and positron emission tomography do not yet possess the resolution to differentiate amygdaloid nuclei reliably.

In healthy humans, amygdala activity is increased during fear conditioning (2,3). Patients with posttraumatic stress disorder (PTSD) exhibit higher amygdala activity at rest (4), and hyperreactivity of the amygdala to trauma-related stimuli (5) is predictive of symptom severity in patients with PTSD (6,7). Higher levels of amygdala activation are seen in generalized anxiety disorder (8,9), social phobia (10), specific phobia (11,12), and panic disorder (13,14).

Alcohol withdrawal is defined by lasting increases in anxiety (15) that contribute to relapse (16,17). Withdrawal-induced anxiety is attributable to recruitment of both neuroendocrine and extrahypothalamic stress systems in humans and animals (18,19). Individuals with alcohol dependence exhibit reduced amygdala volume, which predicts alcohol craving and relapse (20,21). Moderate-to-heavy nondependent drinkers exhibit reduced amygdala activation during a risk-taking task (22). Individuals with a family history of alcohol dependence exhibit reduced amygdala volume (23) and reduced amygdala activation in response to fearful faces (24). Patients with PTSD who abuse alcohol exhibit altered amygdala blood flow relative to normal controls (4). In a cue-reactivity functional magnetic resonance imaging task, alcohol cues activate amygdala, striatum, and cortical regions (25,26). Amygdala abnormalities may result in disinhibition of downstream brain regions that regulate physiology and behavior, as detailed subsequently.

CENTRAL AMYGDALA

The central amygdala (CeA) functions as an integrative hub that converts emotionally relevant sensory information about the external and internal environment into behavioral and physiologic responses. The CeA is part of the extended amygdala (EA), a collection of limbic forebrain structures (including the lateral division of the bed nucleus of the stria terminalis [BNST] and nucleus accumbens shell [27]) that exhibit similar cytoarchitecture, overlapping afferents and efferents, and strong interconnectivity (28,29). The EA mediates negative affective states associated with stress and AUD (30,31) and is densely populated by pro-stress and anti-stress neuropeptides (32). In this article, we discuss CeA dysregulation in anxiety disorders and AUD and the contribution of CeA peptides to these pathologies with emphasis on the pro-stress peptide corticotropin-releasing factor (CRF) and the anti-stress peptide neuropeptide Y (NPY).

AMYGDALA CIRCUITRY

The amygdala is a collection of nuclei including the lateral amygdala (LA), the basolateral amygdala (BLA), and the CeA, which contains lateral (CeAL) and medial (CeAM) subdivisions (Figure 1A). The amygdala exhibits a lateromedial flow of information from the LA and BLA to and through the intercalated cells (ITCs) and into the CeA, which sends out information through amygdala efferents (33). The LA receives multisensory information from thalamus (34,35), integrated
sensory information from cortex (36), and noxious stimulus information from brainstem regions (37). The CeA also receives noxious stimulus information from brainstem regions (38,39). Glutamatergic neurons in the LA synapse onto glutamatergic BLA neurons and onto GABAergic medial ITCs (40) that separate the BLA from the CeA (41,42). The LA and BLA send dense glutamatergic projections to the CeA, with the LA projecting only to the CeAL and the BLA projecting to both the CeAL and the CeAM (28,43,44). Projections out of the BLA also synapse onto gamma-aminobutyric acid (GABA) ITCs, which synapse on CeA neurons (45).

The CeAL and CeAM receive GABAergic afferents from other structures (46) and contain local GABA interneurons and GABAergic projection neurons (47,48) that may inhibit each other via axon collaterals (Figure 1B) (49). The CeAL projects to the CeAM, with no reciprocal projection from the CeAM to the CeAL (50). The CeAM is the major output nucleus of the amygdala and projects to regions that produce behavioral and physiologic responses to emotionally relevant events (49–51), but more recent data suggest the CeAL also sends GABAergic projections to behavioral and physiologic effector regions (52).

Amygdala microcircuitry is critical for emotional processing, especially for interpretation of emotionally relevant stimuli or the attachment of emotional relevance to otherwise neutral stimuli (i.e., learning). Amygdala microcircuitry receives and integrates complex multimodal information to produce behavioral responses. Amygdala dysfunction is implicated in both anxiety disorders (53) and substance abuse (50).

**CeA AS A HUB FOR ANXIETY AND ALCOHOL CIRCUITS**

**Origins of Amygdala Afferents**

Afferents from thalamus and cortex synapse in the LA and ITCs, which each project to the CeA (Figure 1A). Medial prefrontal cortex (PFC) inputs to amygdala have well-defined contributions to pathologic behavioral states in humans and animals. Medial PFC pyramidal neurons send excitatory projections to the amygdala and are controlled by a complex network of GABA interneurons (54,55). Human and animal studies suggest that alcohol and stress affect medial PFC function and medial PFC–amygdala functional connectivity.

The CeA integrates cortical and sensory inputs with innervation from “downstream” brainstem regions (56), including 1) the ventral tegmental area (VTA), important for reward and synthesis of forebrain dopamine; 2) the locus coeruleus (LC) and nucleus tractus solitarius, important for stress response, autonomic function, and synthesis of brain norepinephrine; and 3) the periaqueductal gray (PAG), critical for pain processing. The CeA also receives input from the BNST (57,58), important for anxiety regulation, and is sensitized by glucocorticoid feedback following hypothalamic–pituitary–adrenal (HPA) axis activation (57,58), in contrast to glucocorticoid-mediated negative feedback in the paraventricular hypothalamus (PVN).

**Effector Regions Targeted by CeA Efferents**

The CeA integrates cortical, brainstem, and intra-amygdala afferents to coordinate behavioral and physiologic responses via projections to downstream “effector” regions (Figure 1A). The target of specific CeAM projections determines the behavioral consequences of changes in amygdala activity, but evidence also exists for a subpopulation of CeAL neurons (i.e., oxytocin receptor–expressing neurons) with terminals in the CeAM and ventral forebrain that dictate whether fear coping behaviors are passive (e.g., freezing) or active (e.g., exploratory/risk assessment) (59). Whether CeAM projection neurons exhibit mutually exclusive or overlapping targets and activation profiles is not fully understood. Basal amygdala projection neurons display anatomic and functional specificity in fear expression versus extinction conditions (60), raising the possibility that CeAM populations are likewise differentially activated by specific stimulus conditions.

**PAG.** The PAG is important for descending behavioral and physiologic responses to fearful and painful stimuli (61). The
CeA sends dense and organized GABAergic projections to PAG (62) that colocalize CRF and substance P (63) and gate the antinociceptive pain response mediated by opioids in PAG (64,65). The amygdala and PAG are activated by unconditioned aversive stimuli, and this response is dampened by signals predictive of those stimuli (66).

Lateral Hypothalamus. The lateral hypothalamus (LHA) mediates autonomic responses to fearful stimuli (61) and houses dopamine fibers that project from the ventral tegmental area to forebrain and mediate brain reward function (67). The CeA sends dense GABAergic projections to the LHA (28,68). Electrical kindling of the CeA increases the sensitivity of the LHA to drug-induced facilitation of brain reward function (69), whereas the CeA lesion reduces dopamine activity in the LHA (70).

PVN. The PVN regulates the neuroendocrine stress response via CRF projections to the pituitary that promote adrenocorticotropin hormone and cortisol/corticosterone production and release. The CeAM, but not the CeAS, sends monosynaptic (71) and disynaptic (72) projections to the PVN, which may function as a relay station to brainstem nuclei (73). Electrical CeA stimulation activates the HPA stress axis (74), and the CeA mediates proinflammatory cytokine-induced activation of the HPA axis (75).

LC. The LC produces norepinephrine and regulates autonomic responses to stress (76). GABAergic projections from the CeA to the LC (77) often colocalize the proressor peptides, CRF and dynorphin (78), and synapse onto norepinephrine neurons in LC (79), creating a feed-forward loop that is activated during stress and alcohol withdrawal (80,81). These CeA neurons also express glucocorticoid receptors, suggesting regulation by neuroendocrine feedback from the HPA axis (82).

Dorsal Vagal Complex. The dorsal vagal complex comprises the nucleus tractus solitarius and the dorsal motor nucleus of the vagus and is important for autonomic regulation. The CeAM sends GABAergic projections to the nucleus tractus solitarius and dorsal motor nucleus of the vagus (83,84), which mediate autonomic (i.e., parasympathetic) responses to aversive stimuli (85) and contribute to chronic stress-induced hypertension (86).

CeA IN ANXIETY AND ALCOHOL EFFECTS

CeA Neurotransmission in Regulation of Anxiety Responses

Amygdala activation mediates emotional responses to fearful or anxiety-provoking stimuli in healthy humans (87), and this response is specific to stimuli with a negative valence, even when the valence is not consciously registered (88). Humans with an anxiety disorder (e.g., PTSD) often exhibit hyperactive amygdala responses to these types of stimuli (89). Similarly, chronically stressed rats exhibit hyperexcitability of the LA (90), and CeA lesion blocks chronic stress-induced increases in anxiety-like behavior (91).

Within the amygdala, optical stimulation and inhibition of BLA-to-CeA projection neurons bidirectionally modulates anxiety-like behavior in rodents (53). This finding may explain the strong correlation between BLA and CeA activation, as measured by extracellular signal-regulated kinase phosphorylation, observed in previously stressed animals exposed to a stress reminder (92). Human and animal studies also suggest that individuals that exhibit high reactivity (i.e., poor coping) to traumatic stress exhibit heightened functional connectivity between PFC and amygdala nuclei (89,92).

CeA Neurotransmission in Fear Conditioning

Rodent fear conditioning experiments have significantly contributed to our understanding of the circuitry mediating anxiety disorders. Plasticity in the LA has a central role in fear conditioning (37), but the CeA also has roles in acquisition, expression, generalization, consolidation, and extinction of conditioned fear (93–97). Plasticity of the CeA contributes to acquisition of conditioned fear, and CeAM output neurons are excited by fear stimuli in a manner that decays with extinction and that is sensitive to the activity of somatostatin-positive CeAS neurons (97–99).

Fear extinction relies heavily on descending projections from infralimbic cortex to amygdala. Intercalated GABA cells are critical for mediating fear extinction via projections to the CeA (100,101). For example, CeAM neurons of fear-extinguished animals exhibit greater synaptic inhibition by ITCs, likely as a result of increased excitatory drive from the BLA onto ITCs, an effect that is contingent on infralimbic cortex activity during extinction (102). The net result of fear extinction is reduced inhibitory output from the CeAM to brainstem effector regions, an effect caused by more inhibitory ITC input onto CeAM neurons, less inhibitory ITC input onto CeA GABA neurons that project to CeAM, or both (40,103).

CeA Neurotransmission in Acute Alcohol Effects

Compared with the literature on fear and anxiety, less is known about the molecular identity and projection pattern of specific CeA circuits mediating alcohol effects. Acute alcohol application increases GABAergic transmission in the BLA via increased presynaptic GABA release (104,105), which has implications for downstream CeA neurons via dense excitatory projections to CeA (45). An emerging story has been the potentially overlapping role of corticoamygdalar projections in conditioning and extinction processes related to cues and contexts associated with both fear and alcohol and drugs. Specifically, prelimbic projections to the nucleus accumbens core and BLA facilitate expression of cocaine-seeking behavior and fear, respectively, whereas infralimbic projections to the nucleus accumbens shell and CeA (via ITCs) facilitate extinction of cocaine-seeking behavior and fear, respectively (106). More recent data suggest that prelimbic and infralimbic cortices regulate extinction and reinstatement of alcohol-seeking behavior (107), and it is possible that these effects are mediated by projections to amygdala.

Two types of inhibition are displayed by CeA neurons: phasic, which involves inhibitory postsynaptic currents that reflect “point-to-point” transmission, and tonic, which involves persistent inhibitory currents resulting from ambient GABA acting at highly sensitized GABA receptors (108,109). Tonic inhibition regulates neural network activity (110) and is modulated by both acute and chronic alcohol exposure (111,112). Acute alcohol application dose dependently and reversibly
increases phasic GABA release in the CeA (113,114), independent of GABA<sub>α</sub> receptor blockade (113). Acute alcohol application also increases phasic and tonic inhibition in a population of CeA neurons that synapse onto CeA<sub>M</sub> output neurons, resulting in disinhibition of CeA output to the BNST (115).

**CeA Neuroadaptations in Response to Chronic Alcohol**

Offspring of alcohol-dependent humans exhibit reduced amygdala volume and reduced amygdala functional magnetic resonance imaging activation in response to fearful faces (24,116). Moderate-to-heavy drinking humans exhibit reduced amygdala activation during impulse control tasks (22). Alcohol-dependent humans who have endured more detoxifications and exhibit more loss of control over drinking also exhibit increased PFC-amygdala connectivity during attentional and executive function tasks (117).

Much of what is known about alcohol-induced neuroadaptations in CeA comes from studies on animals with chronic exposure to intermittent bouts of alcohol with repeated withdrawal periods. This protocol accelerates the emergence of somatic, affective, and motivational indices of alcohol dependence (118,119). Relative to stress models, these dependence models may be most appropriately compared with findings from chronic stress studies. Alcohol dependence has been conceptualized in terms of a stress kindling process, in which CeA neuroadaptations play a central role (120).

Many studies on chronic alcohol effects on CeA neurotransmission use a chronic intermittent ethanol vapor inhalation model in rodents (121). Chronic intermittent ethanol augments spontaneous and evoked CeA GABA transmission via presynaptic and postsynaptic mechanisms (104,114,122). Alcohol-dependent rats exhibit increased GABA release in CeA during withdrawal but do not exhibit tolerance to acute alcohol effects on CeA GABAergic transmission (114). Reductions in basal presynaptic GABA<sub>α</sub> receptor activity during withdrawal may account for increased baseline CeA GABAergic transmission in alcohol-dependent rats (123). Gabapentin, a structural analogue of GABA, facilitates evoked GABAergic transmission in alcohol-naïve rats, an effect that is blocked by a GABA<sub>α</sub> receptor antagonist. Conversely, gabapentin decreases evoked CeA GABA transmission during alcohol withdrawal, suggesting that alcohol dependence–induced GABA<sub>α</sub> receptor neuroadaptations may account for the differential behavioral effects of systemic and intra-CeA gabapentin in dependent versus nondependent animals (123).

**CeA STRESS PEPTIDES IN ANXIETY AND ALCOHOL DEPENDENCE**

**Role of CeA Prostress and Antistress Peptides in Anxiety**

Humans with anxiety disorders exhibit altered levels of prostress and antistress peptides in the central nervous system and the periphery. We discuss a few examples in the context of human PTSD, an anxiety disorder that can manifest after an acute traumatic stress event and that is highly comorbid with AUD (124). Positive coping and stress resilience in veterans with PTSD are each predicted by higher plasma levels of the anxiolytic NPY (125). There is also an association of PTSD with polymorphisms and methylation levels for the genes encoding the anxiogenic pituitary adenylate cyclase activating polypeptide and its receptor PAC1, and PAC1 messenger RNA is upregulated in the amygdala of fear-conditioned mice (126).

As illustrated in Figure 2, CRF and NPY colocalize in the CeA, where CRF promotes anxiety-like behavior (127), and NPY reduces anxiety-like behavior (128). The acoustic startle reactivity (ASR) test can be used to assess control by specific EA regions (CeA, BNST) over generalized anxiety-like or stimulus-specific fear behaviors. In rats, NPY dampens basal ASR and fear-potentiated startle and facilitates extinction of fear-potentiated startle, effects likely mediated by the CeA (129); CRF increases ASR (130) and mediates stress-induced enhancement of ASR via corticotropin-releasing factor receptor 1 (CRF1) in the BNST (131). Acute restraint or footshock stress increases CRF mRNA in rat CeA (132,133), and CeA CRF is critical for consolidation of fear memories (134). Intra-amygdala injection of a CRF agonist produces an aversive state resembling that elicited by an environmental stressor,
and both effects are blocked by intra-amygdala injection of NPY (135). After exposure to predator stress, rats with maximally dysregulated behavior (e.g., hyperarousal and high anxiety–like behavior) exhibit reduced NPY in the amygdala (136), and treatment with either NPY or a CRF1 antagonist reduces behavioral dysregulation in rodents following exposure to predator stress (136,137).

**CeA Prostress and Antistress Peptides in Alcohol Dependence**

Alcohol withdrawal is defined by a negative emotional state mediated partly by the recruitment of prostress and antistress peptides in the EA (30). In alcohol-dependent rodents, CRF and NPY in the CeA play critical roles in mediating negative affect and excessive alcohol drinking (Figure 2). Both CRF and NPY are likely produced locally in the CeA (138,139) or imported to the CeA from distal projection neurons, but it is unclear which peptide pools are dysregulated by alcohol dependence (or stress) to produce heightened anxiety-like behavior and escalated alcohol drinking.

Via activation of presynaptic CRF1, CRF increases GABA release in the CeA of rats (122) and mice (140). These effects are exaggerated during withdrawal (122) along with concomitant increases in CRF and CRF1 mRNA levels and increases in CRF release in the CeA of alcohol-dependent rats (122,141). A CRF1 antagonist reverses withdrawal-induced increases in drinking in alcohol-dependent rats and mice (142,143) via effects in the CeA (144), and chronic systemic administration of a CRF1 antagonist prevents escalation of alcohol drinking during the transition to dependence (122). Binge-like drinking increases CRF immunoreactivity in the CeA of mice (145), and CRF1 antagonists reduce binge-like drinking without affecting non-binge-like alcohol drinking (146–148). The ability of CRF to increase CeA GABAergic transmission is blunted in mice with binge-like drinking (145), in contrast to the sensitized CRF effects observed in CeA of alcohol-dependent rats (122). Binge-like alcohol drinking may abolish CRF effects on GABAergic transmission in the CeA via internalization of CRF1 in response to elevated CRF levels in binge alcohol drinkers (145), as seen in the dorsal raphe after stress (149).

The complex alcohol effects on local CeA microcircuitry are illustrated in the differential ethanol sensitivity of CRF1-positive CeA neurons possessing an ethanol-insensitive ongoing tonic conductance and CRF1-negative CeA neurons possessing a tonic conductance that is enhanced by acute alcohol application (115). Acute alcohol decreases firing of CRF1-negative neurons but increases firing of CRF1-positive neurons, suggesting a local CeA 

In the CeA, NPY decreases GABAergic transmission and prevents and reverses acute alcohol–induced facilitation of evoked GABAergic transmission in the CeA of alcohol-naive and alcohol-dependent rats (151). Pharmacologic experiments suggest that NPY exerts NPY Y1 receptor–mediated postsynaptic effects on basal inhibitory transmission in the CeA, whereas NPY blocks alcohol effects on GABA release in CeA via NPY Y2 receptor–mediated presynaptic effects (151). Alcohol dependence produces neuroadaptations in CeA NPY systems, as evidenced by lower NPY levels in the CeA of alcohol-dependent rats during withdrawal (152) and higher NPY Y1 receptor levels in the CeA of chronic alcohol–drinking mice 48 hours after abstinence (153). In the CeA of binge-like alcohol–drinking mice, NPY reduces GABAergic transmission, but this is not the case in alcohol-naive mice (154). Chronic ventricular infusion of NPY during withdrawals early in the transition to dependence prevents excessive alcohol drinking during subsequent withdrawals (151), and NPY infused into the CeA reduces excessive drinking by alcohol-dependent rats (155), suggesting that NPY may blunt excessive drinking by alcohol-dependent rats via modulation of CeA GABAergic neurotransmission.

During stress and alcohol dependence, CRF and NPY in the CeA are recruited and exert opposite but convergent effects on anxiety-like behavior and escalated alcohol drinking, likely via modulation of CeA GABAergic transmission. Withdrawal-induced increases in anxiety-like behavior (156), alcohol drinking (143), and sensitization of anxiety-like behavior over repeated withdrawals (157) are mediated by CRF1. In contrast, intra-CeA NPY reduces withdrawal-induced increases in anxiety-like behavior (151,155). Withdrawal-induced increases in anxiety-like behavior are attenuated by NPY Y2 receptor antagonism in alcohol-dependent rats, but escalated alcohol drinking is not, suggesting that anxiolytic effects occur via NPY Y2 receptor autoreceptor modulation of NPY release, whereas effects on alcohol drinking occur via NPY Y2 receptor heteroreceptor modulation of GABA release (158). Although NPY antagonizes the behavioral effects of CRF in the amygdala, the cellular interactions of NPY and CRF in the CeA remain uncharacterized.

**CeA Prostress and Antistress Peptides in Stress-Alcohol Interactions**

Although humans report drinking alcohol to reduce anxiety (159), animal research has produced a complicated picture of stress effects on alcohol drinking. Studies report stress-induced increases, decreases, and null effects on alcohol drinking according to type and modality of stressor, intensity and frequency of stressor, time between stress and alcohol access, species and strain of animal tested, and other factors (160). One common procedure uses stress to reinstate previously extinguished alcohol-seeking behavior in a session where operant responses do not produce alcohol deliveries. Until recently, studies explicitly investigating the interaction between a PTSD-like state (which takes into account individual differences in stress reactivity) and alcohol self-administration were lacking (161). The stress-enhanced fear-learning model of PTSD was used more recently to show that a single traumatic stress increases acquisition and maintenance of voluntary alcohol consumption in previously alcohol-naive rats but does not alter drinking by rats previously trained to drink (162). Another study found that rats with high reactivity to predator odor stress exhibit escalated and compulsive-like alcohol drinking (163).
Conclusions

Anxiety disorders and AUD are highly comorbid in humans. A preexisting anxiety disorder can precipitate alcohol abuse, and high anxiety is a hallmark symptom of alcohol dependence that manifests during withdrawal. Both anxiety disorders and AUD in humans are defined by altered amygdala structure and function, the end result of which may be disinhibition of downstream “effector” regions that regulate anxiety-related and alcohol-related behaviors. Because the CeA is ascribed an important role in the aversive states and behavioral dysregulation associated with stress and alcohol dependence, it is critical to understand the overlapping and/or compounding effects of anxiety disorders and AUD on amygdala function. New research techniques that combine traditional cellular, pharmacologic, and anatomic approaches with sophisticated new genetic technologies will facilitate our understanding of how the amygdala is recruited in anxiety disorders and AUD and the tailoring of future treatment strategies.

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Article Information

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