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The Role of the Central Amygdala in Alcohol Dependence

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Alcohol dependence is a chronically relapsing disorder characterized by compulsive drug-seeking and drug-taking, loss of control in limiting intake, and the emergence of a withdrawal syndrome in the absence of the drug. Accumulating evidence suggests an important role for synaptic transmission in the central nucleus of the amygdala (CeA) in mediating alcohol-related behaviors and neuroadaptive mechanisms associated with alcohol dependence. Acute alcohol facilitates γ-aminobutyric acid (GABA)ergic transmission in the CeA via both pre- and postsynaptic mechanisms, and chronic alcohol increases baseline GABAergic transmission. Acute alcohol inhibits glutamatergic transmission via effects at *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in the CeA, whereas chronic alcohol up-regulates NMDA receptor (NMDAR)-mediated transmission. Pro- (e.g., corticotropin-releasing factor [CRF]) and antistress (e.g., nociceptin/orphanin FQ, oxytocin) neuropeptides affect alcohol- and anxiety-related behaviors, and also alter the alcohol-induced effects on CeA neurotransmission. Alcohol dependence produces plasticity in these neuropeptide systems, reflecting a recruitment of those systems during the transition to alcohol dependence.

Alcohol use disorder (AUD) is a global health problem. Alcoholism (i.e., dependence on alcohol) is a complex chronically relapsing disorder characterized by persistent alcohol-seeking and alcohol-taking behaviors, loss of control in limiting intake (in terms of amount of drug per bout and number of drug-taking bouts), and the emergence of a withdrawal syndrome in the absence of the drug that includes, but is not limited to, dysphoria, sleep disturbances, disruption of autonomic processes, and increases in anxiety and irritability (ICD-10 and DSM-V). Preoccupation/anticipation, binge/intoxication, and withdrawal/negative affect are three stages conceptualized as feeding into one another, becoming more intense over time, and ultimately leading to the pathological state known as addiction/dependence (Wise and Koob 2014).

Decades of research have shaped the current view that excessive alcohol consumption is largely mediated by an organism's past experience with alcohol (e.g., intake pattern and frequency), and is driven by emotional disturbances rather than physical disturbances associated with alcohol withdrawal and abstinence

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(Koob 2003; Heilig et al. 2010). Importantly, the progression of alcohol addiction involves alterations in normal brain circuitry that result in long-lasting drug-induced neuroadaptations. The central nucleus of the amygdala (CeA) is a brain region implicated in anxiety, stress-related disorders, and the reinforcing effects of alcohol and other drugs of abuse. Despite the critical role of the CeA in such negative affective alcohol responses, the neuronal circuitry underlying these behavioral stages is still not well understood. This review will focus on neurotransmission in the central amygdala and its role in driving the negative state of the withdrawal phase of alcohol addiction. Throughout this review, acute alcohol (ethanol) exposure refers to in vitro application of alcohol onto the slice preparation, whereas chronic alcohol exposure refers to long duration (at least several weeks) of in vivo alcohol exposure.

CENTRAL AMYGDALA IS A HUB FOR NEGATIVE EMOTIONAL CIRCUITRY

Chronic consumption of large quantities of drugs, including alcohol, promotes a transition from casual drug use to drug dependence that is defined in part by down-regulation of dopamine signaling in the mesocorticolimbic reward system, hyperactivity of glutamate signaling, and dysregulation of brain stress systems (Koob and Zorrilla 2010; Koob and Volkow 2016). Chronic alcohol effects on brain stress systems can refer to either alcohol-induced changes in neuroendocrine function (i.e., hypothalamic–pituitary–adrenal [HPA] axis) (Kiefer and Wiedemann 2004; Clarke et al. 2008) or the recruitment of extrahypothalamic brain stress systems such as the amygdala (Koob and Simon 2009; Koob 2016; Koob and Mason 2016). This review details the effects of acute and chronic alcohol on synaptic transmission and plasticity in the CeA and neighboring regions, and the role of these regions in mediating alcohol-related behaviors. We will review the literature on peptidergic modulation of inhibitory and excitatory transmission in the central (and extended) amygdala, because these peptides share a common cellular target and interact with each other and alcohol and thus represent novel therapeutic targets.

Many of the long-term emotional disturbances associated with alcohol abuse and dependence are attributed to neurotransmission within a conceptual macrostructure in the basal forebrain called the "extended amygdala" (Koob 2008). The major constituents of the extended amygdala are the CeA, the shell of the nucleus accumbens (NAc), and the lateral portion of the bed nucleus of the stria terminalis (BNST) (Heimer 1991). These brain structures share similar cytomorphology, a high degree of interconnectivity, and overlapping afferents from limbic cortices, hippocampus, and basolateral amygdala (BLA). The outputs of the extended amygdala project largely to effector regions, including lateral hypothalamus and various brainstem regions, that produce behaviors related to fear and anxiety (Davis et al. 2010).

The role of the extended amygdala in fear and anxiety has been previously described in detail (Ciocchi et al. 2010; Davis et al. 2010; Tye et al. 2011; Roberto et al. 2012). The CeA and the BNST are integral in mediating fear and anxiety responses. The BLA receives significant sensory input from the thalamus and cortex, sends prominent glutamatergic projections to the CeA and BNST, and is integral in both conditioning (Phelps and LeDoux 2005) and extinction (Quirk and Mueller 2008) processes. The CeA is composed mostly of γ-aminobutyric acid (GABA)ergic projection neurons and interneurons (Sun and Cassell 1993; Veinante and Freund-Mercier 1998), and the BNST is a major target of CeA projection neurons (Krettek and Price 1978; Weller and Smith 1982; Sun and Cassell 1993; Veinante and Freund-Mercier 1998). Of major relevance for this review, connections between CeA and BNST often contain neuropeptide cotransmitters (Penzo et al. 2014; Pomrenze et al. 2015). For example, the CeA is a major source of corticotropin-releasing factor (CRF) in the BNST (Sakanaka et al. 1986). Therefore, the CeA is uniquely situated to convert sensory information into behavioral and physiological responses, and this is particularly true for stress- and alcohol-related stimuli.

Neuropeptides in the CeA are important for producing the negative affective state observed during withdrawal from drugs, including alco-

hol (Koob 2008; Gilpin and Roberto 2012). Here we will describe alcohol-induced neuroadaptations in select neuropeptidergic systems (CRF, nociceptin/orphanin FQ [nociceptin], oxytocin), largely in the context of the CeA. It is becoming increasingly evident that these peptides interact in complex ways in the CeA to modulate GABAergic inhibitory and glutamatergic excitatory transmission, and that dysregulation of these peptide systems by alcohol alters the way in which they modulate CeA activity. Conceptually, these neuropeptides have been divided into prostress peptides and antistress peptides, which, respectively, promote and rescue negative affective disturbances during drug abstinence following heavy drug use. Prostress peptides include CRF, dynorphin, orexin, and vasopressin, whereas antistress peptides include neuropeptide Y (NPY), enkephalin, oxytocin, and nociceptin; however, because of space limitations, we will discuss only a select few of these peptides.

AMYGDALAR INHIBITORY GABAergic SYSTEM AND ALCOHOL

GABA, the major inhibitory transmitter in the brain, acts on two classes of GABA receptors: $GABA_A$ (which includes $GABA_A$-rho subclass, formerly $GABA_C$) and $GABA_B$. $GABA_A$ receptors are ligand-gated ion channels, whereas $GABA_B$ receptors are G-protein-coupled receptors (GPCRs). There is considerable evidence that GABAergic transmission mediates some aspects of alcohol-drinking behavior, but there is ambiguity in the literature with respect to the directions of these effects. Early studies showed that systemic administration of $GABA_A$ agonists increased voluntary alcohol drinking, whereas $GABA_A$ antagonists and benzodiazepine inverse agonists reduced alcohol intake (Boyle et al. 1993; Rassnick et al. 1993). Infusion of both $GABA_A$ agonists and antagonists into the NAc suppressed alcohol drinking by nondependent rats (Hodge et al. 1995). Systemic administration of a $GABA_B$ agonist suppressed alcohol drinking in all rats, but alcohol-dependent rats were more sensitive to this effect, suggesting an up-regulation of $GABA_B$ function (Walker and Koob 2007). Other studies highlight a role for GABA circuitry in alcohol-drinking behavior, particularly in regions implicated in the negative reinforcing properties of the drug (i.e., extended amygdala). Hyytiä and Koob (1995) found that injection of $GABA_A$ antagonists in the three major regions of the extended amygdala suppressed alcohol drinking by nondependent rats, but this effect was most potent and selective for alcohol when infused into the CeA. Another study showed that antagonism of $GABA_A$ in the BNST reversed decreases in alcohol drinking elicited by a D_2 dopamine receptor antagonist infused into the ventral tegmental area (VTA) of alcohol-preferring (P) rats (Eiler and June 2007). Interestingly, infusion of a $GABA_A$ agonist directly into the amygdala suppresses drinking by alcohol-dependent rats without affecting intake by nondependent controls (Roberts et al. 1996). Although there are considerable methodological differences between these studies, they suggest that (1) chronic alcohol produces neuroadaptations in GABAergic neurotransmission and changes sensitivity to GABAergic compounds, (2) GABAergic neurotransmission regulates alcohol drinking, and (3) in the case of excessive alcohol consumption by alcohol-dependent rats, the CeA is a strong candidate region for localization of these effects.

ACUTE ALCOHOL AUGMENTS INHIBITORY GABAergic TRANSMISSION IN THE CeA

$GABA_A$ receptors mediate two distinct forms of inhibitory transmission: synaptic $GABA_A$ receptors mediating phasic inhibition and a tonic inhibition stemming from the activation of extrasynaptically located $GABA_A$ receptors by low concentrations of ambient GABA (Belelli et al. 2009; Hines et al. 2012). In heterologous expression systems, alcohol (1–100 mM) selectively enhances the function of distinct $GABA_A$ receptor subtypes (Harris et al. 2008; Sauguet et al. 2013; Wallner et al. 2014) but such findings have been inconsistent across laboratories (for reviews, see Aguayo et al. 2002; Lovinger and Homanics 2007).

The effects of acute alcohol on $GABA_A$ function have also been extensively studied with in

vitro brain slice preparations, an approach that allows multiple methods for detecting changes in presynaptic transmitter release (for reviews, see Criswell and Breese 2005; Siggins et al. 2005; Weiner and Valenzuela 2006; Lovinger and Roberto 2013). Alcohol increases GABAergic synaptic transmission in the CeA (Roberto et al. 2003) and BLA (Zhu and Lovinger 2006) via increased presynaptic GABA release. Specifically, alcohol augments evoked inhibitory postsynaptic currents (IPSCs), decreases paired-pulse facilitation (PPF) of evoked IPSCs, and increases the frequency of miniature IPSCs (mIPSCs) (i.e., in tetrodotoxin [TTX] to eliminate action potential firing) in most CeA neurons, suggesting that alcohol increases vesicular GABA release (Roberto et al. 2003; Varodayan et al. 2017b,c).

Although the molecular mechanism(s) for alcohol effects have yet to be fully identified, we demonstrated that activation of adenylyl cyclase (AC), protein kinase C (PKC), and/or voltage-gated calcium channels are critical to the effects of acute alcohol on CeA $GABA_A$-mediated transmission (Bajo et al. 2008; Cruz et al. 2011; Varodayan et al. 2017b,c). Specifically, in CeA slices of mice lacking PKCε or when pretreated with a PKCε antagonist, the ability of acute alcohol to augment IPSCs is impaired (Bajo et al. 2008), suggesting that PKCε facilitates alcohol-elicited vesicular GABA release. Notably, basal GABA release is greater in the CeA of PKCε knockout than wild-type mice, suggesting that in wild-type neurons, PKCε limits spontaneous GABA release. Therefore, PKCε serves at least two roles in the CeA: (1) limiting baseline GABA release, and (2) facilitating alcohol-stimulated release of GABA.

CeA neurons comprise three main cell types: (1) low-threshold bursting (LTB) characterized by one or two action potentials elicited by depolarizing current steps and after-hyperpolarization action potentials, (2) late spiking (LS) neurons exhibiting delayed action potentials elicited by depolarizing current steps, and (3) regular spiking (RS) neurons displaying consistent action potentials in response to depolarizing current steps (Dumont et al. 2002; Chieng et al. 2006). We found that acute alcohol increases spontaneous action-potential firing of LTB neurons, decreases firing of LS neurons, and has mixed effects on RS neurons, suggesting divergent cell-type-specific effects of alcohol on the excitability of rat CeA neurons. Interestingly, LTB and some RS neurons possess a persistently active tonic conductance, mediated by α1-subunit containing $GABA_A$ receptors, which is insensitive to the effects of acute ethanol. In contrast, LS and a separate group of RS neurons do not possess an ongoing tonic conductance, but do display the potential for a tonic conductance, which is mediated by δ-subunit containing $GABA_A$ receptors, and can be stimulated by acute ethanol or by elevating ambient GABA concentrations (Herman and Roberto 2016).

The ability of alcohol to facilitate GABA transmission may be limited by GABA feedback onto presynaptic $GABA_B$ (Wan et al. 1996; Ariwodola and Weiner 2004). For example, acute alcohol facilitates GABAergic transmission in the hippocampus (Wu and Saggau 1994; Wan et al. 1996; Ariwodola and Weiner 2004) and NAc (Nie et al. 2000) only if $GABA_B$ receptors are blocked. However, in the CeA, $GABA_B$ receptor blockade is not required for the enhancement of inhibitory postsynaptic potentials (IPSPs) by acute alcohol nor does it potentiate this effect (Roberto et al. 2003). Thus, the involvement of $GABA_B$ receptors in alcohol-induced GABA release may depend on the presence of presynaptic $GABA_B$ receptors in certain brain regions (Ariwodola and Weiner 2004; Breese et al. 2005).

CHRONIC ALCOHOL EFFECTS ON GABAergic TRANSMISSION IN THE CeA

Our in vitro electrophysiological results show that chronic alcohol exposure augments CeA GABA release (Roberto et al. 2004a). Alcohol-dependent rats exhibit larger baseline-evoked CeA $GABA_A$-mediated IPSP/C amplitudes, smaller baseline PPF of evoked IPSCs, and higher baseline frequency of mIPSCs compared to alcohol-naive rats. In vivo, microdialysis studies also indicate a large increase of baseline dialysate GABA concentrations in the CeA of alcohol-dependent rats relative to naive controls, as well as lack of tolerance for acute alcohol-induced

increases in dialysate GABA levels (Roberto et al. 2004a), strongly supporting the hypothesis that both acute and chronic alcohol alter presynaptic elements of CeA GABAergic synapses. Elevated $GABA_A$ receptor-mediated signaling in the CeA following chronic alcohol exposure emerges to be a common characteristic in alcohol dependence across species including mice (Herman et al. 2016), nonhuman primates (rhesus monkeys with a history of chronic ethanol self-administration and repeated abstinence) (Jimenez et al. 2019), and P rats (Herman et al. 2013b). For the latter, elevated GABA signaling was attributed to decreased CeA GABA transporter GAT-3 levels, indicative of impaired synaptic GABA clearance. Importantly, GAT-3 expression was also selectively decreased in the CeA of alcohol-dependent patients, suggesting a potential shared molecular mechanism for elevated CeA GABA transmission across species (Augier et al. 2018).

In chronically alcohol-exposed rats, acute alcohol augments CeA IPSCs, decreases PPF of IPSCs, and increases mIPSC frequency similarly in alcohol-dependent and alcohol-naive rats, suggesting a lack of tolerance for these acute effects of alcohol (Roberto et al. 2004a; Varodayan et al. 2017b). However, chronic intermittent ethanol (CIE) exposure leads to a loss of tonic conductance in rat LTB/RS neurons, likely leading to a disinhibition of this specific neuronal population, which leads to increased intra-CeA GABA release in dependent rats as compared to naive controls. Conversely, the emergence of an ongoing tonic conductance in LS/RS neurons following chronic intermittent alcohol exposure might blunt output function from these neurons. Given that tonic inhibition—a fine-tuned inhibitory control of neuronal activity—is a critical regulator of overall network activity (Semyanov et al. 2004; Brickley and Mody 2012), and dysfunctional $GABA_A$ receptor-mediated tonic signaling is associated with a number of neurological disorders (Hines et al. 2012), the loss of selective inhibition of these neurons following chronic alcohol could provide one potential mechanism by which chronic alcohol alters CeA output and contributes to behaviors associated with alcohol dependence (Herman and Roberto 2016). In nonhuman primates, acute ethanol also significantly increased spontaneous IPSC (sIPSC) frequency in controls, but not in abstinent drinkers, suggesting a tolerance to ethanol-induced GABA release in abstinent rhesus monkeys with a history of chronic ethanol self-administration and repeated abstinence (Jimenez et al. 2019).

Future studies will determine the exact molecular mechanisms responsible for chronic alcohol-induced adaptations in CeA neurons and their behavioral implications in alcohol-dependent and/or alcohol-withdrawn organisms. These ongoing studies may elucidate the mechanism(s) underlying reductions in alcohol withdrawal hyperexcitability produced by GABA mimetic drugs (Ticku and Burch 1980; McCown et al. 1985; Breese et al. 2006; Roberto et al. 2008), and could impact treatment of pathological alcohol-drinking behaviors.

Chronic alcohol exposure produces tolerance to many behavioral effects of the drug, including the anxiolytic, sedative, ataxic, and positive reinforcing effects (Kumar et al. 2004, 2009). Chronic alcohol also produces physical and motivational dependence, and alcohol withdrawal is associated with increased neuronal excitability in several brain regions (but not CeA) (Kliethermes 2005; Weiner and Valenzuela 2006). Chronic alcohol effects may reflect, in part, compensatory adaptations to the facilitatory effects of alcohol on GABAergic synapses (Siggins et al. 2005; Weiner and Valenzuela 2006). We showed that evoked IPSCs in CeA slices from alcohol-dependent rats are significantly larger than those from naive controls (Roberto et al. 2004a). Some CeA neurons from alcohol-dependent rats also exhibit increased mIPSC amplitudes relative to naive rats, suggesting a postsynaptic effect of chronic alcohol (Roberto et al. 2004a). Substantial evidence suggests that alcohol-induced behavioral and neural adaptations are attributable to changes in $GABA_A$ subunit composition rather than changes in the number of $GABA_A$ (Morrow et al. 1992; Eckardt et al. 1998; Grobin et al. 1998; Papadeas et al. 2001; Kumar et al. 2004, 2009; Lee et al. 2014).

In alcohol-naive rats, a $GABA_B$ antagonist increased the amplitude of evoked IPSCs and

decreased PPF of IPSCs in the CeA, suggesting tonic activation of presynaptic $GABA_B$ (Roberto et al. 2008). Conversely, a $GABA_B$ agonist markedly depressed evoked IPSC amplitudes and increased PPF of IPSCs in the CeA of alcohol-naive rats, indicating decreased presynaptic GABA release. These effects of $GABA_B$ agonists and antagonists were absent or greatly attenuated in the CeA of alcohol-dependent rats, suggesting chronic alcohol-induced down-regulation of the $GABA_B$ system, which may explain the increased GABAergic tone observed in the CeA of dependent rats (Roberto et al. 2008). These alcohol-dependence-induced neuroadaptations of the $GABA_B$ system also may account for chronic alcohol-induced changes in gabapentin effects on inhibitory transmission in the CeA. Gabapentin, a structural analog of GABA (Sills 2006), increases the amplitudes of evoked IPSCs in CeA neurons from nondependent rats (an effect blocked by a $GABA_B$ antagonist), but decreases IPSC amplitudes in the CeA of alcohol-dependent rats. Notably, gabapentin infused into the CeA reverses dependence-induced increases in operant alcohol responding but tends to increase alcohol drinking by nondependent rats (Roberto et al. 2008).

ALCOHOL AND GLUTAMATERGIC TRANSMISSION IN THE AMYGDALA

Glutamate, the major excitatory neurotransmitter, has long been implicated in the reinforcing actions of alcohol. Glutamate receptors include three major classes of ionotropic (iGluRs), with varying ratios of selectivity for Na^+, K^+, and Ca^{2+}. The iGluRs include α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (AMPARs), *N*-methyl-D-aspartic acid (NMDA) receptors (NMDARs), and kainate receptors (KARs). Additionally, there are various subclasses of metabotropic GLuRs (mGluRs) that are GPCRs.

In contrast to its potentiating effects on GABA systems, alcohol generally inhibits glutamate neurotransmission in the brain (Lovinger and Roberto 2013). Dysregulation of glutamate systems may contribute to hyperexcitability and craving associated with alcohol withdrawal (Pulvirenti and Diana 2001). In the CeA of P rats, long-term consumption of high quantities of alcohol increases expression of mGluRs, NMDAR subunits, and a scaffolding protein regulating expression of these receptors in the cell membrane (Obara et al. 2009). Group II mGluRs also may block stress- and cue-induced reinstatement of alcohol-seeking behavior via neuronal activation in the CeA or BNST (Zhao et al. 2006). Excitatory transmission in the CeA may also mediate some of the aversive aspects of withdrawal from abused drugs (e.g., morphine [Watanabe et al. 2002]).

Acamprosate, approved for treatment of alcoholic patients, modulates glutamate transmission via actions at NMDARs and/or mGluRs (Berton et al. 1998; Blednov and Harris 2008; Mann et al. 2008). Notably, acamprosate dampens the increased glutamate levels in abstinent alcoholics measured by magnetic resonance spectroscopy (Umhau et al. 2010), and reduces excessive alcohol drinking in alcoholics, presumably by reducing craving and negative affect (for review, see Littleton 2007). AMPARs may be important in regulating relapse-like behaviors without playing a central role in alcohol consumption per se (Sanchis-Segura et al. 2006).

ACUTE ALCOHOL EFFECTS ON GLUTAMATERGIC TRANSMISSION IN THE CeA

We demonstrated that acute alcohol (5–66 mM) decreases excitatory postsynaptic potentials (EPSPs) and currents (EPSCs) in the CeA, and that these effects are mediated by both NMDAR and non-NMDAR mechanisms (Roberto et al. 2004b). In contrast to alcohol effects on GABA release, the majority of studies indicate that acute alcohol either has no effect on or inhibits glutamate release (for review, see Roberto et al. 2005) and inhibits NMDAR, AMPAR, and KAR function in some neuron types (for review, see Lovinger and Roberto 2013).

CHRONIC ALCOHOL EFFECTS ON EXCITATORY TRANSMISSION IN THE CeA

Chronic alcohol produces neuroadaptations in glutamatergic synaptic transmission. For

example, acute alcohol decreases NMDAR-mediated EPSPs and EPSCs in the CeA of alcohol-dependent rats more than in alcohol-naive controls. With local NMDA application, acute alcohol inhibits NMDA currents more in slices from alcohol-dependent rats, suggesting that alcohol dependence sensitizes NMDARs to alcohol (Roberto et al. 2004b, 2006). NMDARs containing the NR2B subunit are most sensitive to chronic alcohol exposure (Floyd et al. 2003; Carpenter-Hyland et al. 2004; Roberto et al. 2004b; Kash et al. 2009). Chronic alcohol increases NR2B messenger RNA (mRNA) and/or protein levels (Roberto et al. 2006; Kash et al. 2009) in the CeA and BNST, but not in other brain regions (Cebere et al. 1999; Floyd et al. 2003; Läck et al. 2005). It is not yet clear whether increased NR2B subunit expression is the major driving force behind alcohol-induced increases in NMDAR function, or what molecular mechanisms underlie these subunit changes.

Chronic alcohol has also been associated with functional up-regulation of $mGluR_{2/3}$ receptor signaling in the CeA and BNST (Kufahl et al. 2011). Furthermore, chronic ethanol self-administration (alcohol-deprivation model) also increased spontaneous EPSC (sEPSC) rise times indicative of compromised CeA glutamatergic receptor function (Suárez et al. 2019). Additionally, we found that CIE treatment did not alter evoked CeA glutamate but decreased both spontaneous vesicular glutamate (miniature EPSCs [mEPSCs]) release and postsynaptic glutamate receptor function at CeA synapses (Varodayan et al. 2017a).

Microdialysis experiments in the amygdala revealed increased glutamate release following chronic alcohol exposure (at 2–8 h withdrawal in the CeA [Roberto et al. 2004b]; at 24 h withdrawal in BLA [Läck et al. 2007]). Our laboratory found that chronic alcohol exposure unmasks the ability of acute alcohol to increase presynaptic glutamate release in the CeA (Roberto et al. 2004b), an effect that persisted 2 weeks into abstinence (Roberto et al. 2006).

Collectively, these data suggest that multiple factors contribute to increased extracellular glutamate levels and altered glutamatergic transmission following chronic alcohol exposure and withdrawal.

CENTRAL AMYGDALA NEUROPEPTIDES AND ALCOHOL DEPENDENCE

Neuropeptidergic systems play key roles in regulating anxiety-like and alcohol-drinking behaviors in subjects that are either alcohol-dependent, genetically vulnerable to developing excessive drinking (e.g., via selective breeding), and genetically predisposed to anxiety, as well as repeatedly cycled through periods of alcohol withdrawal. The CeA contains high concentrations of prostress (e.g., CRF, orexin, vasopressin, etc.) and antistress (e.g., nociceptin, oxytocin, enkephalin, NPY, etc.) neuropeptides, and many effects of these neuropeptides on anxiety- and alcohol-related behaviors have been localized to the CeA. Because of space limitations, we review here the actions of a few neuropeptides on inhibitory transmission in the CeA, with a focus on the CRF system that is heavily recruited during the transition from casual alcohol use to dependence (Koob and Zorrilla 2010).

The CeA is the major output center of the amygdala, and about 95% of CeA neurons are medium spiny GABAergic neurons (McDonald 1982). The CeA is not a homogeneous neuroanatomical structure, and can be subdivided into lateral (CeL) and medial (CeM) aspects that differ in neuropeptide content, origin of afferents, and target sites of efferent projections (for reviews, see Sun et al. 1991; Petrovich et al. 1996; Pitkänen et al. 2000; Dong et al. 2001; Ciocchi et al. 2010; Davis et al. 2010; Haubensak et al. 2010; McCullough et al. 2016). The CeL contains a much higher density of neuropeptides (e.g., dynorphin, CRF [Veening et al. 1984; Cassell et al. 1986; Shimada et al. 1989; Pomrenze et al. 2015; Wolfe et al. 2019]) than the CeM, receives input from cortex and thalamus, and sends inhibitory inputs to the CeM, thereby gating the output activity of the CeA, but also to more distant brain regions such as the periaqueductal gray and paraventricular nucleus (PVN) of the thalamus (Penzo et al. 2014). By contrast, the

CeM receives prominent inputs from other amygdaloid nuclei (especially glutamatergic afferents from BLA) and sends inhibitory projections to various effector regions (e.g., hypothalamus, periaqueductal gray, locus coeruleus, and pedunculopontine tegmental nucleus) (Krettek and Price 1978; Pitkänen and Amaral 1994). Both the CeL and CeM project to the BNST (Sun et al. 1991).

CRF AND ALCOHOL-RELATED BEHAVIOR

CRF plays a central role in arousal and hormonal, sympathetic, and behavioral responses to stress (Roberto et al. 2017). Dysregulation of the CRF system is implicated in numerous psychiatric disorders including anxiety, depression, addiction, posttraumatic stress disorder, and eating disorders (Menzaghi et al. 1994; Koob 2003, 2008; Valdez et al. 2003; Chu et al. 2007; Funk et al. 2007; Ciocchi et al. 2010; Tye et al. 2011; Bruijnzeel et al. 2012; Iemolo et al. 2013; Ji et al. 2013; Baiamonte et al. 2014). The CeA, BNST, and BLA contain abundant CRF neurons and their cognate CRF type 1 and 2 (CRF_1 and CRF_2) receptors (De Souza et al. 1984; Sakanaka et al. 1986; Roberto et al. 2017).

Pharmacological and transgenic studies support that brain and pituitary CRF_1 receptors mediate endocrine, behavioral, and autonomic responses to stress (Koob and Heinrichs 1999; Heinrichs and Koob 2004; Zorrilla and Koob 2010; Zorrilla et al. 2013), and CRF_2 receptor activation has been associated with decreased feeding (Spina et al. 1996; Pelleymounter et al. 2000; Fekete and Zorrilla 2007; Gilpin 2012; Roberto et al. 2017).

Hyperfunction of CRF systems in the CeA, BLA, and BNST produce increases in anxiety-like behavior (Sajdyk et al. 1999; Rainnie et al. 2004; Lee et al. 2008). Extracellular CRF levels in the CeA are elevated following exposure to stress and development of alcohol dependence (Merlo Pich et al. 1995; Zorrilla et al. 2001), and alcohol withdrawal increases CRF synthesis and release in the CeA (Funk et al. 2006; Sommer et al. 2008; Roberto et al. 2010). Likewise, alcohol withdrawal increases extracellular CRF in the BNST (Olive et al. 2002), and these increases are normalized by alcohol consumption. In contrast, the effects of binge drinking on CeA CRF is dependent on the age at the time of alcohol exposure and may affect subsequent alcohol-related behaviors (Gilpin 2012). Following 2 weeks of binge-like alcohol intake, adolescent rats display decreases in CRF cell number in the CeA and no changes in BNST (Karanikas et al. 2013). In adult mice, one and six cycles of the drinking in the dark paradigm (DID) increases CeA CRF immunoreactivity, suggesting that the CRF system is recruited during early binge-like drinking episodes (Lowery-Gionta et al. 2012). Notably, the synaptic effects of CRF on CeA GABAergic transmission are reduced after repeated bouts of binge-like drinking (Lowery-Gionta et al. 2012).

CRF_1 and CRF_2 antagonists suppress dependence-induced increases in alcohol drinking during acute withdrawal and protracted abstinence (Valdez et al. 2002) and reverse increases in stress-induced anxiety during protracted abstinence (Valdez et al. 2003). CRF repeatedly administered into the CeA, BLA, or dorsal BNST exaggerates alcohol withdrawal-induced increases in anxiety-like behavior via CRF_1s (Huang et al. 2010). Conversely, antagonism of CRF receptors (CRFRs) in the CeA blunts the increases in anxiety-like behavior in rats during withdrawal from chronic high-dose alcohol exposure (Rassnick et al. 1993).

Many findings in animal models implicate CRF_1 receptors specifically in these "dark side" actions of CRF (or other endogenous CRF_1 agonists) to produce a negative emotional state. Specifically, CRF_1 antagonists block the anxiogenic effects of many stressors (Arborelius et al. 2000; Zorrilla et al. 2014). CRF_1 antagonists also block increases in alcohol self-administration elicited by stressors and withdrawal (Hansson et al. 2006; Funk et al. 2007; Gehlert et al. 2007; Marinelli et al. 2007; Lowery et al. 2008). Chronic treatment with a CRF_1 antagonist abolishes dependence-induced escalation of drinking in rats chronically exposed to high doses of alcohol (Roberto et al. 2010). Likewise: (1) stressors and alcohol withdrawal increase CRF_1 expression in limbic brain regions (Aguilar-Valles et al. 2005;

Sommer et al. 2008; Eisenhardt et al. 2015); (2) rats bred for high alcohol preference show increased anxiety-like behavior and CRF_1 levels (Ciccocioppo et al. 2006); and (3) CRF_1 knockout mice exhibit decreased anxiety-like behavior (Müller et al. 2003) and resistance to the ability of repeated forced swim stress to increase deprivation-induced ethyl alcohol (EtOH) intake (Pastor et al. 2011). In addition, conditional brain-specific *Crhr1* knockout mice (Molander et al. 2012) show reduced EtOH intake during withdrawal compared to their wild-type littermates. Notably, CRF_1 antagonists also block the decreased brain reward function observed in animal models of withdrawal from nicotine, alcohol, or opioids, evident as increased current thresholds for intracranial self-stimulation behavior (Bruijnzeel et al. 2007, 2009, 2010).

CRF-binding protein (CRF-BP) binds CRF with equal or greater affinity than do CRFRs, limiting its bioavailability and serving as an endogenous negative regulator of CRF signaling (Potter et al. 1992; Behan et al. 1995; Ketchesin et al. 2016), and/or alternatively to chaperone/complex with the peptide to provide a different signaling method. The distribution of the CRF-BP overlaps partly with that of CRF, notably in the cortex and amygdala (Potter et al. 1992; Behan et al. 1995; Ketchesin et al. 2016), where terminals containing CRF-BP colocalize with CRF-positive cell bodies supporting their regulatory interaction (Westphal and Seasholtz 2006). Recently, the CRF-BP has been considered a potential target for its role in AUD (Haass-Koffler et al. 2016; Ketchesin et al. 2016), and its role in the escalation of alcohol drinking may involve its interaction with CRF_2 (Albrechet-Souza et al. 2015; Quadros et al. 2016). In addition, gene variant studies in humans have revealed that the *CRHBP* rs1875999 locus is associated with risk for both cocaine and heroin addiction in African-Americans (Levran et al. 2014a,b). Single-nucleotide polymorphisms (SNPs) in the *CRHBP* (10 kD) fragment, rs10055255, rs10062367, and rs7728378 were each associated with increased risk of alcohol drinking and/or anxiety in patients with AUD (Haass-Koffler et al. 2016).

CRF EFFECTS ON SYNAPTIC TRANSMISSION IN THE CeA

CRF robustly increases GABAergic transmission in the CeA of rats (Roberto et al. 2010) and mice (Nie et al. 2004, 2009) mainly via activation of CRF_1s. Notably, CRF_1 antagonists decrease presynaptic GABA release, suggesting tonic facilitation of GABA release by endogenous CRF. CRF_1 antagonists and CRF_1 knockouts also block the alcohol-induced augmentation of GABAergic transmission in the CeA. Both CRF- and alcohol-induced facilitation of GABAergic transmission in the CeA require the PKCε signaling pathway (Bajo et al. 2008). Alcohol-dependent rats show increased sensitivity to the effects of CRF and CRF_1 antagonists on GABA release in the CeA, suggesting up-regulation of the CRF-CRF_1 system. These findings are further supported by increased CRF and CRF_1 mRNA levels seen in the CeA of alcohol-dependent rats, and by reversal of dependence-induced elevations in amygdalar GABA dialysate by a CRF_1 antagonist (Roberto et al. 2010). CRF also increases GABAergic transmission in the BNST, likely via actions at postsynaptic CRF_1s (Kash and Winder 2006; Francesconi et al. 2009).

Given the critical role of the CRF/CRF_1 system and the cellular heterogeneity in the CeA, we used a bacterial artificial chromosome (BAC) transgenic mouse line expressing the green fluorescent protein (GFP) under the *Crhr1* promoter (CRF_1:GFP) to readily identify neurons expressing CRF_1 (CRF_1^+) (Justice et al. 2008; Herman et al. 2013a, 2016) to unveil unique molecular, morphological, and functional properties that distinguish CeA CRF_1^+ neurons from their CRF_1 nonexpressing (CRF_1^-) neighbors. We found that CRF_1^+ neurons are mainly located in the CeM and exhibit an ongoing tonic GABAergic conductance driven by action potential-dependent GABA release. In contrast, CRF_1^- neurons do not display tonic inhibition (Herman et al. 2013a). We also reported functional adaptations to CIE exposure on phasic and tonic inhibition and cell firing in CRF_1^+ and CRF_1^- CeA neurons (Herman et al. 2016). Notably, ongoing tonic conductance in CRF_1^+

neurons in control mice was lost following CIE. In contrast, LS CRF_1^- neurons showed a tonic conductance following CIE that was not observed in controls. We observed a loss of tonic currents and a significantly higher basal firing rate in CRF_1^+ CeA neurons projecting to the BNST of CIE versus control mice.

Overall, our studies have yielded significant insight into cell-type-specific effects of acute and chronic alcohol in local and downstream CeA circuits revealing that CIE alters inhibitory control of CeA CRF_1 output neurons. More recently, we also assessed expression of subpopulation markers and neuropeptides, dendritic spine density and morphology, and glutamatergic transmission in the CeA CRF_1^+ versus CRF_1^- neurons (Wolfe et al. 2019). In brief, CeA CRF_1^+ neurons are GABAergic, but do not segregate with calbindin, calretinin, or PKCδ. Coexpression analysis using in situ hybridization revealed *Crhr1* had the highest coexpression with *Penk* and *Sst* and the least with *Npy*. Additionally, CeA CRF_1^+ neurons do not display differences in mature spines and, accordingly, no difference in basal CeA glutamate transmission. Application of CRF increases overall glutamate release onto both CRF_1^+ and CRF_1^- neurons but increases postsynaptic glutamate receptor functions selectively in CRF_1^+ neurons (Wolfe et al. 2019).

We also studied CIE-induced changes in the modulation of rat glutamatergic synapses by CRF (Varodayan et al. 2017a). We found that CRF (25–200 nM) also decreased CeA EPSPs evoked locally or by BLA stimulation, indicating that CRF similarly decreases glutamatergic responses. In contrast to the evoked data but in agreement with our recent work, CRF increased mEPSC frequency similarly in naive and CIE neurons, suggesting increased vesicular glutamate release (Varodayan et al. 2017a). Antagonism of CRF_1/CRF_2 and CRF_1 alone decreased mEPSC frequency in naive CeA neurons, whereas antagonism of CRF_2 increased it. Our studies also revealed that CRF_2 receptors under basal conditions tonically inhibit glutamate release, as their blockade increased vesicular glutamate release. In contrast, CRF_1 receptors tonically enhance glutamate release, in which CRF_1 blockade decreased glutamate release. Accordingly, CRF did not significantly affect glutamate release in the presence of CRFR antagonists. In contrast, in the presence of the antagonist astressin-2B, CRF further enhanced glutamate release, suggesting that CRF_2 receptors tonically inhibit glutamate, and CRF_2 blockade resulted in a larger CRF-induced facilitation of glutamate release (Varodayan et al. 2017a). Thus, the CRF-induced facilitation of glutamate release is mediated by CRF_1 receptors, but the mechanisms are complex and may involve both CRF_1 and CRF_2 receptors with opposite receptor subtype effects on glutamate release (Varodayan et al. 2017a).

NOCICEPTIN/ORPHANIN FQ AND ALCOHOL-RELATED BEHAVIOR

The neuropeptide nociceptin/orphanin FQ (N/OFQ) (Meunier et al. 1995; Reinscheid et al. 1995; Meunier 1997) and its cognate receptor nociceptin opioid peptide (NOP) are widely expressed in the brain (Neal et al. 1999). Nociceptin is an opioid-like peptide that acts at NOP receptors, although it does not bind to opioid receptors and opioids do not bind to NOP receptors. Nociceptin is abundantly expressed in the CeA and BNST (Neal et al. 1999) and is described as a functional CRF antagonist (Ciccocioppo et al. 2003). Earlier studies showed that nociceptin and other NOP agonists have an anxiolytic-like profile in animal studies (Jenck et al. 1997, 2000), and nociceptin knockout mice show increased anxiety-like behavior (Koster et al. 1999) and are more sensitive to social stress (Ouagazzal et al. 2003). Numerous studies also reported that NOP activation significantly decreased drinking, alcohol-induced conditioned place preference, and decreased reinstatement evoked by stress or cues (Ciccocioppo et al. 1999, 2004, 2014a; Martin-Fardon et al. 2000; Kuzmin et al. 2003, 2007; Witkin et al. 2014; Toll et al. 2016).

Generally, rats bred for high alcohol intake show increased sensitivity to the suppressive effects of nociceptin on drinking and related behaviors (Economidou et al. 2008). Human alcoholics express decreased levels of mRNA for

NOP in the CeA (Kuzmin et al. 2007), suggesting that the CeA is a critical site for a role of nociceptin in alcoholism.

Subsequent studies reported similar effects using NOP antagonists (Witkin et al. 2016; Ciccocioppo et al. 2019). For instance, the NOP antagonist LY2940094 decreased voluntary ethanol intake in alcohol-preferring and -nonpreferring rats (Rorick-Kehn et al. 2016). Moreover, NOP knockout rats self-administer significantly less alcohol than their wild-type counterpart, suggesting a facilitatory role of NOP receptors (Kallupi et al. 2017). Recent clinical data showed the translational potential of NOP modulation as LY2940094 was effective in reducing drinking in humans (Post et al. 2016). There is an ongoing debate on the mechanisms underlying similar effects using both NOP agonists and antagonists in decreasing the motivation for alcohol. One possible explanation is that NOP agonists may act through mechanisms involving desensitization of the system (Ciccocioppo et al. 2019).

NOCICEPTIN AND SYNAPTIC TRANSMISSION IN THE CeA

At the cellular level, nociceptin dose-dependently and reversibly reduced evoked and spontaneous $GABA_A$ receptor-mediated IPSCs in CeA (Roberto and Siggins 2006) via decreasing presynaptic GABA release. Notably, nociceptin both prevented (when applied before alcohol) and totally reversed (applied during alcohol) acute alcohol-induced increases in evoked IPSC amplitudes and mIPSC frequencies and decreases in PPF, thus preempting the usual alcohol-induced increase in GABA release in CeA. Further, the ability of nociceptin to decrease GABAergic transmission in CeA is augmented following alcohol dependence, suggesting that the nociceptin system in the CeA adapts during chronic alcohol exposure (Roberto and Siggins 2006).

We also found that the maximally effective and reversible concentration of nociceptin completely blocked the CRF-induced increase of IPSCs (Roberto et al. 2010), suggesting that nociceptin antagonized the effect of CRF (Cruz et al. 2012). We also reversed the sequence of drug application and applied CRF first followed by nociceptin-CRF coapplication. Nociceptin decreases the amplitude of IPSPs and completely blocks the CRF-induced increase in GABAergic transmission. Moreover, the NOP receptor antagonist $[Nphe^1]$nociceptin(1-13)NH_2 blocked the nociceptin-induced diminution of IPSCs but not the CRF-induced augmentation of IPSCs, indicating that nociceptin modulates both ethanol and CRF effects through the NOP receptors. Nociceptin also blocked CRF-induced increases in GABAergic responses in CeA from ethanol-dependent rats (Cruz et al. 2012).

Using a multidisciplinary approach, we also explored the relationship between the nociceptin and CRF systems in response to acute restraint stress (Ciccocioppo et al. 2014a). We found a selective up-regulation of the nociceptin and down-regulation of the CRF_1 receptor transcripts in the CeA and BLA after restraint. We evaluated the anxiety-like response in rats subjected to restraint stress and nonrestrained rats after nociceptin microinjection into the CeA (Ciccocioppo et al. 2014a). Notably, intra-CeA injections of nociceptin significantly and selectively reduced anxiety-like behavior in restrained rats in the elevated plus maze. Finally, we electrophysiologically explored functional interactions between CRF and nociceptin systems in the CeA. Acute application of CRF significantly increased GABAergic responses, and this enhancement was blocked by nociceptin (Ciccocioppo et al. 2014a). Importantly, in restraint stressed rats, baseline CeA GABAergic responses were elevated and nociceptin exerted a larger inhibition of GABA responses compared with nonrestrained rats. Application of the nociceptin antagonist revealed a functional recruitment of the nociceptin receptor after acute stress. These combined results demonstrate that acute stress and alcohol dependence increase nociceptin system function in the CeA and provide translational support for nociceptin as a "drugable" candidate system for medication development for the treatment of alcoholism (Ciccocioppo et al. 2014a).

As a long-term goal in the context of these basic neurobiological mechanism studies, we seek to identify novel molecules, such as nociceptin agonists, which moderate alcoholism risk by acting at the cellular level in brain regions such as the amygdala that are associated with ethanol dependence. In collaboration with Mitsubishi Pharma (Osaka, Japan), we tested a novel nociceptin receptor agonist (MT-7716) in our in vitro CeA brain slice preparation (Kallupi et al. 2014) and in behavioral studies (Ciccocioppo et al. 2014b).

Previously, we reported that in the rat CeA, acute and chronic ethanol exposures significantly decrease glutamate transmission by both pre- and postsynaptic actions (Roberto et al. 2004b).

Nociceptin diminished basal-evoked compound glutamatergic EPSPs and sEPSCs and mEPSCs by mainly decreasing glutamate release in the CeA of naive rats (Kallupi et al. 2014). Notably, nociceptin blocked the inhibition induced by acute ethanol and ethanol blocked the nociceptin-induced inhibition of evoked glutamatergic responses in CeA neurons of naive rats. In neurons from ethanol-dependent rats, the nociceptin-induced inhibition of glutamatergic responses was not significantly different from that in naive rats. Application of [Nphe^1] Nociceptin(1-13)NH_2 revealed tonic inhibitory activity of NOP on evoked CeA glutamatergic transmission only in alcohol-dependent rats. The antagonist also blocked nociceptin-induced decreases in glutamatergic responses but did not affect ethanol-induced decreases in evoked EPSP amplitude. Taken together, these studies implicate a potential role for the nociceptin system in regulating glutamatergic transmission and a complex interaction with ethanol at CeA glutamatergic synapses (Kallupi et al. 2014).

OXYTOCIN AND ALCOHOL DRINKING

The neuropeptide oxytocin is involved in many biological processes and has a purported antistress role in the brain (Lang et al. 1983; Landgraf and Neumann 2004; Bosch et al. 2005; Neumann and Slattery 2016). In response to stress, oxytocin is released both into the bloodstream via the posterior pituitary and to many extrahypothalamic brain regions including the extended amgydala, where it decreases anxiety and stress (Kasting 1988; Ebner et al. 2005; Knobloch et al. 2012; Campbell-Smith et al. 2015; Grinevich et al. 2016). Oxytocin has also been suggested as a putative treatment for addiction (Kovács et al. 1998). Exogenous oxytocin administered to animal models has been found to reduce consumption, withdrawal symptomology, and relapse associated with many drugs of abuse, including stimulants, opioids, and alcohol (Lee and Davis 1997; Kovács et al. 1998; Carson et al. 2010; Bowen et al. 2011; Bahi 2015; Baracz et al. 2016; Bowen and Neumann 2017; Cox et al. 2017; Kohtz et al. 2018). In particular, oxytocin may be an effective treatment for alcohol addiction as intranasal oxytocin administration has been found to reduce alcohol craving, consumption, and withdrawal symptoms in alcohol-dependent patients, as well as reduce neural activity in response to alcohol-related cues in heavy drinkers (Pedersen et al. 2013; Mitchell et al. 2016; Hansson et al. 2018). However, the precise mechanism by which oxytocin decreases alcohol intake and withdrawal symptoms is not yet known. In rodent models, administration of oxytocin both peripherally and directly into the central nervous system decreases alcohol consumption in nondependent animals (MacFadyen et al. 2016; King et al. 2017), and as the oxytocin receptor (OXTR) is expressed throughout the brain and peripheral systems it has not been established where oxytocin acts to affect addiction behavior. Notably, oxytocin reaches the brain following both intraperitoneal and intranasal administration in rodents (Neumann et al. 2013; Tanaka et al. 2018) and intravenous and intranasal administration in macaques (Lee et al. 2018). Additionally, intracerebroventricular oxytocin reduced cue reactivity in dependent but not nondependent rats (Hansson et al. 2018), suggesting a centrally mediated effect on alcohol-dependent individuals. In collaboration with researchers at the National Institute on Alcohol Abuse and Alcoholism and the National Institute on Drug Abuse, we found that intracerebro-

ventricular oxytocin or the non–blood barrier penetrant OXTR agonist PF-06655075 blocked the enhanced motivation and escalated alcohol drinking seen in dependent rats. In addition, peripheral administration of PF-06655075 did not decrease drinking and systemic administration of the peripherally restricted OXTR antagonist L-371,257 did not block the effects of centrally administered oxytocin (Tunstall et al. 2019). These results suggest oxytocin acts in the brain to decrease excessive alcohol drinking seen with alcohol dependence.

OXYTOCIN AND SYNAPTIC TRANSMISSION IN THE CeA

Oxytocin is primarily expressed in neurons of the PVN and supraoptic nuclei (SON) of the hypothalamus, and projections from these neurons are found in the CeA, where OXTRs are expressed on GABAergic neurons (Huber et al. 2005; Viviani et al. 2011; Stoop 2012). Some of these OXTR-expressing GABAergic neurons in the CeL project to the CeM and inhibit GABAergic output of the CeM (Huber et al. 2005). As oxytocin modulates GABAergic signaling in the CeA, and the importance of CeA GABAergic signaling in alcohol dependence, we examined the effects of oxytocin and alcohol on synaptic transmission in the CeA (Tunstall et al. 2019).

Oxytocin inhibited evoked IPSPs in nondependent rats, and increased PPF suggesting an effect of decreasing presynaptic GABA release. In dependent rats, oxytocin had no effect on evoked IPSPs, but blocked potentiation of IPSPs by acute alcohol. Oxytocin decreased network-dependent sIPSC amplitudes in both nondependent and dependent rats, indicating some postsynaptic effects of oxytocin, but again, oxytocin blocked the acute alcohol-induced increase in presynaptic GABA release (increased sIPSC frequency) in dependent rats only (Tunstall et al. 2019). The effects of oxytocin to block alcohol-induced increases in dependent rats is not only similar between these two network-dependent forms of GABAergic transmission, but is also in agreement with oxytocin suppressing escalated alcohol intake in dependent rats. These results support the idea that oxytocin blocks alcohol-dependence-induced escalation in alcohol intake through effects on GABA transmission in the CeA (Tunstall et al. 2019).

DISINHIBITION MODEL OF CeA OUTPUT

As noted above, most neurons in the CeA are GABAergic inhibitory projection neurons or interneurons that cotransmit GABA and one or more neuropeptides. Peptides that promote anxiety-like behavior and alcohol self-administration (e.g., CRF) generally increase, whereas peptides that reduce anxiety-like behavior and alcohol self-administration (e.g., nociceptin, oxytocin) decrease GABAergic transmission in the CeA. In our slice preparation, we stimulate and record locally in the medial portion of the CeA, and recordings of GABAergic transmission reflect the activity of inhibitory interneurons or projection neurons (via collaterals) within the CeA. Therefore, increases in GABAergic transmission within the CeA following application of acute alcohol or CRF will inhibit the activity of GABAergic neurons projecting out of the CeA. Conversely, decreases in GABAergic transmission in CeA neurons (e.g., following nociceptin or oxytocin application) will reduce inhibition of GABAergic neurons projecting out of the CeA, thereby facilitating release of GABA in downstream targets (e.g., BNST, periaqueductal gray). Thus, increases or decreases in inhibitory output from the CeA to downstream effector regions may decrease or increase anxiety-like behavior, respectively (Paré et al. 2004; Davis et al. 2010; Tye et al. 2011). Furthermore, alcohol markedly affects excitatory transmission in the CeA, particularly via NMDARs (Roberto et al. 2004b, 2006; Kallupi et al. 2014), lending at least partial buffering of alcohol effects on inhibitory transmission. Finally, alcohol may alter the release of local opioids (Lam et al. 2008; Gilpin et al. 2014), endocannabinoids (Roberto et al. 2010; Kirson et al. 2018), and/or NPY (Gilpin et al. 2008a,b; Gilpin and Roberto 2012) in CeA that in turn may increase GABA-mediated inhibition of downstream target areas. Other neuropeptides (e.g., substance P, galanin) are also likely to regulate the synaptic transmission within the CeA.

CONCLUSIONS

The data summarized here support the idea that the CeA is a critical locus of neuroadaptation during the transition to alcohol dependence. Alcohol has strong and persistent effects, particularly on inhibitory transmission, in the CeA of alcohol-dependent animals. Neuropeptides present at high levels in the CeA profoundly alter inhibitory transmission, and potentially also excitatory transmission. The ability of these neuropeptides to affect neurotransmission in the CeA, either alone or in combination with alcohol, is often dysregulated in alcohol dependence. Although manipulation of many of these peptides affects alcohol drinking in alcohol-dependent but not nondependent animals, it is unsurprising that these neuropeptides affect basal neurotransmission in the CeA of alcohol-naive animals, especially because these peptides, when microinjected into the CeA, modulate anxiety-related behavior independent of alcohol exposure history. This point also enhances our understanding of why these neuropeptide systems are recruited and/or up-regulated during the transition to alcohol dependence, a dynamic disease defined largely by a negative emotional state in the absence of the drug. Finally, our electrophysiological data suggest that synaptic transmission and the special neuronal circuitry in the CeA may be an important point of convergence for the neuroadaptations that occur during the transition to alcohol dependence. Our understanding of this pivotal system as a "bellwether" target for therapeutic testing for anxiety and AUDs may be reliably predicted by drug effects on synaptic transmission in the CeA. Thus, we predict that most drugs that decrease GABAergic transmission in CeA neurons will be logical candidates for treatment of anxiety and/or AUDs.

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