Corticotropin-releasing factor (CRF) and neuropeptide Y (NPY): Effects on inhibitory transmission in central amygdala, and anxiety- & alcohol-related behaviors

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ABSTRACT

The central amygdala (CeA) is uniquely situated to function as an interface between stress- and addiction-related processes. This brain region has long been attributed an important role in aversive (e.g., fear) conditioning, as well as the negative emotional states that define alcohol dependence and withdrawal. The CeA is the major output region of the amygdala and receives complex inputs from other amygdaloid nuclei as well as regions that integrate sensory information from the external environment (e.g., thalamus, cortex). The CeA is functionally and anatomically divided into lateral and medial subdivisions that themselves are interconnected and populated by inhibitory interneurons and projections neurons. Neuropeptides are highly expressed in the CeA, particularly in the lateral subdivision, and the role of many of these peptides in regulating anxiety- and alcohol-related behaviors has been localized to the CeA. This review focuses on two of these peptides, corticotropin-releasing factor (CRF) and neuropeptide Y (NPY), that exhibit a high degree of neuroanatomical overlap (e.g., in CeA) and largely opposite behavioral profiles (e.g., in regulating anxiety- and alcohol-related behavior). CRF and NPY systems in the CeA appear to be recruited and/or up-regulated during the transition to alcohol dependence. These and other neuropeptides may converge on GABA synapses in CeA to control projection neurons and downstream effector regions, thereby translating negative affective states into anxiety-like behavior and excessive alcohol consumption.

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The central amygdala

Negative emotion circuitry

The extended amygdala is a conceptual macrostructure (Heimer & Alheid, 1991) that plays a prominent role in both fear and anxiety behaviors (Davis, Walker, Miles, & Grillon, 2010). Two major components of the extended amygdala are the central nucleus of the amygdala (CeA) and the bed nucleus of the stria terminals (BNST; see the review by Kash, 2012, in this issue). These two regions exhibit a high degree of interconnectivity and play central roles in generating negative emotional responses (i.e., fear and anxiety) to environmental stimuli. The lateral amygdaloid complex (i.e., lateral and basolateral amygdala) receives significant sensory input from thalamus as well as dense cortical inputs (McDonald, 1998; Turner & Herkenham, 1991), sends prominent glutamatergic projections to CeA and BNST (Dong, Petrovich, & Swanson, 2001; Krettek & Price, 1978; Pitkänen et al., 1995), and is integral in both fear conditioning (Phelps & LeDoux, 2005) and fear extinction (Quirk & Mueller, 2008) processes. The CeA is composed mostly of GABAergic projection neurons and interneurons (Sun & Cassell, 1993; Veinante & Freund-Mercier, 1998), and has been divided into two subdivisions, the lateral and medial CeA, based on the connectivity and functionality of these subregions. The lateral division of the CeA projects to the BNST (Krettek & Price, 1978; Weller & Smith, 1982), and reciprocal connections between CeA and BNST contain neuropeptide co-transmitters, for example, the CeA is a major source of corticotropin-releasing factor (CRF) in the BNST (Sakanaka, Shibasaki, & Lederis, 1986). At a finer level, the lateral division of the CeA sends inhibitory projections to the medial division of the CeA, although there is not complete understanding of how emotional processing maps onto complex intra-amygdala connections (Ehrlich et al., 2009; Pape & Pare, 2010). The medial division of the CeA is the major output region of the amygdala and sends inhibitory projections to various effector regions (e.g., hypothalamus, periaqueductal gray, locus coeruleus, nucleus of the solitary tract, pedunculopontine tegmental nucleus; Pitkänen, 2000). Therefore, the amygdala receives strong inputs about the external...
environment and projects lateromedially to convert sensory information into appropriate behavioral and physiological responses.

**The central amygdala and excessive alcohol drinking**

Chronic alcohol consumption over long periods of time is defined by a transition from low/moderate to high levels of alcohol consumption. This transition is defined neurally by down-regulation of dopamine signaling in the mesocorticolimbic reward system, hyperactivity of glutamate signaling, and dysregulation of brain stress systems (Koob & Volkow, 2010). Chronic alcohol effects on brain stress systems can refer to either alcohol-induced changes in neuroendocrine function (i.e., hypothalamic–pituitary–adrenal axis; Clarke et al., 2008; Kiefer & Wiedemann, 2004) or the recruitment of extra-hypothalamic (e.g., amygdalar) brain stress systems. This review will discuss the pivotal role of the CeA in mediating excessive alcohol consumption and other alcohol-related behaviors, as well as the important role of the CeA in regulating the negative emotional states often observed in excessive alcohol drinking phenotypes. In particular, genetic and environmental influences (e.g., alcohol withdrawal) may produce dysregulation of CeA (and extended amygdala circuitry at large) that resembles plasticity seen in those regions following exposure to fear- and anxiety-inducing environmental stimuli.

**Central amygdala neuropeptides and alcohol**

Neuropeptides in the extended amygdala have been attributed a prominent role in the negative affect produced by addiction to drugs, including alcohol (Koob, 2008). More specifically, these peptides have been conceptually divided into pro-stress peptides and anti-stress peptides that respectively promote and rescue negative affective disturbances during drug abstinence following heavy drug use. Many pro- and anti-stress peptides are highly expressed in the CeA and manipulation of those systems produces profound effects on affective-like and alcohol-related behaviors, effects that are often revealed or augmented in animals that exhibit excessive alcohol drinking phenotypes. Pro-stress peptides include CRF, dynorphin, hypocretin/orexin, and vasopressin, whereas anti-stress peptides include neuropeptide Y (NPY) and nociceptin. It is becoming increasingly evident that these peptides modulate synaptic transmission in the extended amygdala, and that this modulation is significantly altered in animals with a history of chronic high-dose alcohol exposure, presumably contributing to the negative emotional state observed in the absence of alcohol in those animals.

This review will focus on the roles of CRF and NPY in excessive alcohol drinking and related behavioral dysregulation, as well as modulation by these peptides of inhibitory transmission in the CeA. It is interesting that CRF and NPY show a high degree of neuroanatomical overlap and largely opposite behavioral profiles. For example, CRF promotes increases in anxiety-like behavior (Koob & Thatcher-Britton, 1985), increases in arousal (Koob et al., 1984), and decreases in feeding (Levine, Rogers, Kneip, Grace, & Morley, 1983), whereas NPY promotes decreases in anxiety-like behavior (Heilig et al., 1993), decreases in arousal (Heilig & Murison, 1987), and increases in feeding (Stanley & Leibowitz, 1984). As discussed below, alcohol-related behaviors exhibit heightened sensitivity to manipulation of brain CRF and NPY systems in individuals that are either alcohol-dependent, genetically vulnerable to consume high quantities of alcohol drinking, repeatedly cycled through periods of alcohol withdrawal, or innately. Of particular relevance to this review, the effects of CRF and NPY on anxiety-like and alcohol-related behaviors have been localized to the amygdala and neighboring regions, and are likely attributable to their modulation of synaptic transmission in those regions, which is altered following chronic alcohol exposure. It should be noted here that just as the lateral and medial divisions of the CeA differ in afferent inputs and efferent projections (for review of amygdala anatomical organization, see Pitkänen, 2000), they also differ in terms of their neuropeptide content, in that the lateral portion of the CeA contains a much higher density of neuropeptides (e.g., CRF; Cassell, Freedman, & Shi, 1999; Cassell, Gray, & Kiss, 1986; Shimada et al., 1989; Veening et al., 1984) than the medial CeA.

**Rat models of alcohol dependence**

Animal models of alcoholism aim to mimic specific components of the human addiction phenotype rather than the disorder as a whole. Much of the data discussed in this review comes from studies that utilize chronic high-dose alcohol exposure in the form of either alcohol vapor inhalation or alcohol liquid-diet to produce alcohol dependence in rats. These models have been utilized to produce the excessive alcohol-seeking and -drinking behaviors characteristic of humans that abuse and/or are dependent on alcohol, and allow examination of the neural dysregulation that mediates these behavioral changes. Throughout this review, acute alcohol exposure refers to in vitro application of ethanol to the slice preparation, whereas chronic alcohol exposure refers to long-term (at least several weeks) in vivo alcohol exposure. Furthermore, the chronic alcohol exposure protocols described here reliably produce somatic and motivational signs of alcohol dependence (Gilpin et al., 2009), therefore, the terms chronic alcohol exposure and dependence will be used interchangeably in this review.

Chronic intermittent alcohol vapor inhalation is a dependence induction procedure that allows for precise experimenter control of the dose, duration, and pattern of alcohol exposure (Rogers, Wiener, & Bloom, 1979), and stable blood-alcohol levels can be maintained for long periods of time in the presence of normal ingestive behaviors and weight gain (Roberts, Heyser, Cole, Griffin, & Koob, 2000). A second procedure used to produce alcohol dependence in rats makes an alcohol liquid-diet available to animals where diet is the sole source of available nutrition. This procedure allows animals to ingest large quantities via the natural route of administration, but there is substantial individual variability in the dose, duration, and pattern of alcohol exposure and resultant blood-alcohol levels (BALs) across animals. Both of these procedures produce alcohol tolerance and physical dependence on alcohol (Abu-Murad & Thurman, 1980; Gilpin et al., 2009; Lieber & DeCarli, 1982). Upon termination of alcohol vapor exposure, rats can be tested for a multitude of acute withdrawal- and protracted abstinence-related behaviors (Roberts, Cole, & Koob, 1996; Rogers et al., 1979; Valdez, Zorrilla, Roberts, & Koob, 2003; Zhao, Weiss, & Zorrilla, 2007). Behavioral data will be discussed from experiments that utilized both of these procedures, as well as electrophysiological data collected from brain slices of rats following chronic exposure to alcohol vapor. More specifically, the data reviewed below focus on neurotransmission in the CeA as it is relevant to the withdrawal/negative affect phase of the alcohol addiction cycle (Koob, 2003).

**CRF and alcohol in central amygdala**

**CRF & anxiety- and alcohol-related behaviors**

**Amygdalar CRF & anxiety-related behavior**

CRF is a 41-amino acid peptide that plays a central role in arousal as well as the hormonal, sympathetic, and behavioral responses to stress. CRF and its receptors are abundantly expressed in CeA, BNST, and BLA (De Souza et al., 1984; Sakanaka et al., 1986), and
hyperfuction of CRF systems in these regions produces increases in anxiety-like behavior (Lee, Fitz, Johnson, & Shekhar, 2008; Rainnie et al., 2004; Sajdyk, Schober, Gehlert, & Shekhar, 1999). Conversely, intra-CeA infusion of a CRF receptor antagonist reverses alcohol withdrawal-induced increases in anxiety-like behavior (Rassnick, D'Amico, Riley, & Koob, 1993). Furthermore, CRF content in amygdala and BNST is highly interconnected since, for example, CeA sends dense CRF projections to the BNST (Sakanaka et al., 1986).

CRF in the extended amygdala and norepinephrine (NE) in the locus coeruleus (LC) are linked in a feed-forward loop such that CeA and BNST send CRF projections to LC and receive NE inputs from that same region (Koob, 1999). Monosynaptic GABAergic projections from CeA to LC often co-localize CRF (Reyes et al., 2011) and CRF-containing inhibitory projections out of CeA synapse directly on NE neurons in LC (Valentino & Van Bockstaele, 2008). CRF may alter activity of NE neurons in LC by modulating pre-synaptic GABA release as in the CeA (see below; Roberto et al., 2010) or by activating post-synaptic CRF-Rs as in the BNST (Kash & Winder, 2006). Although it is not clear exactly how NE inputs from LC modulate CRF neurons in the extended amygdala, NE projections from brainstem to limbic regions are linked to behavioral responses to stressors (Delfs, Zhu, Druhan, & Aston-Jones, 2000; Forray & Gylys, 2004) and there is evidence for direct interaction between these two systems in amygdala. For example, activation of β-adrenoceptors enhances excitatory transmission in BNST and this effect is dependent on CRF-R signaling (Nobis, Kash, Silberman, & Winder, 2011). Furthermore, glucocorticoids in BLA positively modulate β-adrenoceptor-mediated consolidation of fear memories via seemingly direct interactions with CRF-Rs and α1-adrenoceptors on the postsynaptic terminal (Roozendaal, Schelling, & McCaughey, 2008). It is worth mentioning that a recent study showed that deletion of CRF-Rs from forebrain GABA neurons did not affect anxiety-like behavior in mice (Refojo et al., 2011). This result is not surprising because (1) anxiety-like behavior is affected by CRF-R function in multiple brain regions (e.g., see Sahuhe et al., 2006), (2) CRF-Rs have been attributed a role in mediating anxiety-like behaviors (Valdez, Sabino, & Koob, 2004), and (3) CRF affects not only inhibitory, but also excitatory transmission in the CeA (Fu & Neugebauer, 2008; Liu et al., 2004).

Amygdalar CRF & alcohol-related behavior

In central amygdala, both stress and alcohol dependence produce increases in extracellular CRF levels (Merlo-Pich et al., 1995; Zorrilla, Valdez, & Weiss, 2001). Alcohol withdrawal produces increases in CRF synthesis and release in CeA (Funk, O'Dell, Crawford, & Koob, 2006; Roberto et al., 2010; Sommer et al., 2008) and BNST (Olive, Koenig, Nannini, & Hodge, 2002), the latter of which is normalized by alcohol consumption. Alcohol dependence produces increases in alcohol drinking during acute withdrawal and protracted abstinence, as well as increased sensitivity to stress-induced anxiety during protracted abstinence from chronic alcohol, and both of these behaviors are blocked by systemic administration of CRF receptor antagonists (Valdez et al., 2002, 2003). CRF-Rs have been administered into the CeA, BLA, and dorsal (but not ventral) BNST "kindles" or exaggerates the increases in anxiety-like behavior produced by alcohol withdrawal, and this effect is due to CRF action at CRF-R receptors (CRF-Rs; Huang et al., 2010). Conversely, antagonism of CRF receptors in the CeA attenuates the increase in anxiety-like behavior observed in rats withdrawing from chronic high-dose alcohol exposure (Rassnick et al., 1993).

Recent research has highlighted the role of CRF-Rs in mediating the effects of limbic CRF on anxiety-like behavior and alcohol drinking. CRF-R antagonists block the anxiogenic effects of many stressors [including alcohol withdrawal] in a variety of behavioral assays (Arborelius et al., 2000; Zorrilla, Zhao, & Koob, 2007). CRF-R antagonists also block increases in alcohol self-administration produced by stressors and alcohol withdrawal (Funk, Zorrilla, Lee, Rice, & Koob, 2007; Gehlert et al., 2007; Hansson et al., 2006; Lowery, Sparrow, Breeze, Knapp, & Thiele, 2008; Marinielli et al., 2007; Richardson et al., 2008), and chronic antagonism of CRF-Rs abolishes dependence-induced escalation of alcohol drinking in rats chronically exposed to high doses of alcohol (Roberto et al., 2010). Likewise, stressors and alcohol withdrawal produce increases in CRF-R synthesis and expression in limbic brain regions (Aguilar-Valles et al., 2005; Sommer et al., 2008). Rats selectively bred for high alcohol preference exhibit increased anxiety-like behavior and CRF-R levels (Ciccocioppo et al., 2006), and also exhibit heightened sensitivity to CRF-R antagonists following development of alcohol dependence (Gilpin, Richardson, & Koob, 2008; Sabino et al., 2006). Similarly, CRF-R knockout (KO) mice exhibit decreased anxiety-like behavior (Muller et al., 2005), as well as decreased alcohol drinking following withdrawal from chronic high-dose alcohol exposure (Chu, Koob, Cole, Zorrilla, & Roberts, 2007).

**CRF & alcohol effects on inhibitory transmission in CeA**

**Alcohol effects on inhibitory transmission in CeA**

In general, acute alcohol enhances synaptic inhibition by increasing GABA release from pre-synaptic terminals (reviewed in Siggins, Roberto, & Nie, 2005) and also by activating postsynaptic GABA<sub>A</sub> receptors. Alcohol (5–100 mM) selectively potentiates the function of GABA<sub>A</sub> receptors that contain particular subunit compositions (see Aguayo, Peoples, Yeh, & Yevenes, 2002; Lovinger & Homanics, 2007; Sundstrom-Poromaa et al., 2002; Wallner, Hanchar, & Olsen, 2003), and some of the behavioral and neural adaptations produced by alcohol may be attributable to changes in GABA<sub>A</sub>R subunit assembly (Devaud et al., 1995; Eckardt et al., 1998; Grobin, Matthews, Devaud, & Morrow, 1998; Kumar, Fleming, & Morrow, 2004; Kumar et al., 2009; Morrow, Herbert, & Montpied, 1992). Acute alcohol increases GABAergic synaptic transmission in the central (Roberto, Madamba, Moore, Tallent, & Siggins, 2003) and basolateral (Zhu & Lovinger, 2006) amygdaloid nuclei, an effect that is rapid, reversible, and has a significant pre-synaptic component.

In the CeA, chronic alcohol exposure facilitates GABA release, mainly via actions at pre-synaptic GABA<sub>A</sub>eric terminals (Roberto, Madamba, Stouffer, Parsons, & Siggins, 2004; Roberto et al., 2010). Interestingly, acute alcohol enhances pre- and post-synaptic components of GABAergic transmission in CeA similarly in alcohol-dependent and alcohol-naïve rats, suggesting a lack of tolerance for the acute effects of alcohol in this brain region (Roberto et al., 2004). Microdialysis studies have confirmed large increases in baseline dialysate GABA concentrations in the CeA of alcohol-dependent rats relative to alcohol-naïve controls, as well as lack of tolerance for acute alcohol-induced increases in dialysate GABA levels in alcohol-dependent rats (Roberto et al., 2004), although it is unclear whether this reflects changes in release or uptake or both.

Pre-synaptic GABA<sub>A</sub>Rs may mediate inhibitory feedback that limits the ability of acute alcohol to facilitate GABA neurotransmission. For example, the ability of acute alcohol to facilitate GABAergic transmission in hippocampus is enhanced when GABA<sub>A</sub> receptors are blocked (Arwiodola & Weiner, 2004; Wan, Berton, Madamba, Francesconi, & Siggins, 1996). In the CeA, GABA<sub>B</sub>R receptor blockade is not required for enhancement of iPSPs by acute alcohol nor does it potentiate this effect (Roberto et al., 2003). GABA<sub>B</sub>R function in the CeA is decreased following chronic alcohol exposure (Roberto et al., 2008). Pre-synaptic GABA<sub>B</sub>Rs are activated under basal conditions in the CeA of alcohol-naïve rats, but this activity is absent or greatly attenuated in the CeA of alcohol-dependent rats (Roberto et al., 2008). The removal of this GABA<sub>B</sub>R...
“brake” by chronic alcohol may explain observed increases in GABAergic transmission in the CeA of alcohol-dependent rats.

**CRF and CRF1R mRNA levels in CeA**

CRF produces robust increases in GABAergic transmission in CeA of mice and rats (Nie et al., 2004; Roberto et al., 2010). Pre-synaptic GABA release is increased by CRF and decreased by antagonism of CRF1Rs, the latter of which reflects a tonic facilitation of GABA release by CRF in the CeA (see Fig. 1). CRF1R antagonists also block the ability of acute alcohol to augment GABAergic transmission in CeA. In some CeA neurons from alcohol-naive rats, CRF and acute alcohol produce additive increases in evoked IPSC amplitudes (Roberto, Madamba, Nie, & Siggins, 2005). The ability of CRF and acute alcohol to augment GABAergic transmission in CeA is contingent on the integrity of protein kinase C epsilon intracellular signaling pathways (Bajo, Cruz, Siggins, Messing, & Roberto, 2008). Alcohol-dependent rats exhibit heightened sensitivity to the effects of CRF and CRF1R antagonists on GABA release in CeA, suggesting an upregulation of the CRF–CRF1R system (Roberto et al., 2010). These electrophysiological findings are further corroborated by increased CRF and CRF1R mRNA levels in the CeA of alcohol-dependent rats, as well as reversal of alcohol dependence-induced elevations in amygdalar GABA dialysate by a CRF1R antagonist (Roberto et al., 2010). CRF effects on synaptic transmission in CeA are in many ways paralleled by its effects in the BNST (see review by Kash, 2012, in this issue).

**NPY and alcohol in central amygdala**

**NPY & anxiety- and alcohol-related behaviors**

**Amygdalar NPY & anxiety-related behavior**

NPY is a 36-amino-acid peptide that decreases anxiety-like behavior in rats in a multitude of behavioral assays, including the elevated plus-maze, social interaction test, fear-potentiated startle, and operant conflict tests (Britton et al., 1997; Broqua, Wettstein, Rocher, Gauthier-Martin, & Junien, 1995; Heilig, McLeod, Koob, & Britton, 1992; Heilig, Süderpalm, Engel, & Widerlöv, 1989; Sadjký, Vandergriff, & Gehlert, 1999). The robust anxiolytic effects of NPY are mediated by the central and basolateral amygdaloid nuclei (Heilig et al., 1993; Sadjký, Vandergriff et al., 1999), regions that are densely populated by NPY fibers and receptors (Allen, Roberts, Bloom, Crow, & Polak, 1984; De Quidt & Emson, 1986; Dumont, Fournier, St-Pierre, Schwartz, & Quirion, 1990; Gustafson et al., 1997; Mígita, Loewy, Ramabhadran, Krause, & Waters, 2001). NPY acts post-synaptically at Y1 receptors (Y1Rs) and pre-synaptically at Y2 receptors (Y2Rs), both of which are abundantly expressed in amygdala (Dumont, Fournier, St-Pierre, & Quirion, 1993; Gustafson et al., 1997; Kopp et al., 2002; Parker & Herzog, 1999). Because of their respective locations in the synapse, it is interesting that pharmacological agonists of both Y1Rs and Y2Rs produce decreases in anxiety-like behavior when administered into CeA, although Y1R agonists produce this effect at lower doses (Heilig et al., 1993). Likewise, in the BLA, antagonism of Y2Rs reverses the anxiolytic effects of NPY (Sadjký, Vandergriff et al., 1999). Site-specific ablation of the Y2R gene in CeA and BLA affects anxiety-like and depression-like behaviors in mice, an effect that may be attributable to alterations in GABAergic transmission and/or NPY release (Tasan et al., 2010). Pharmacological studies of the role of Y2Rs in the BLA in regulating emotionality are less clear since intra-BLA infusion of Y2R agonists can produce increases or decreases in anxiety-like behavior depending on the compound and dose (Sadjký, Schober, & Gehlert, 2002; Sadjký, Schober, Smiley, & Gehlert, 2002). Like CRF, NPY interacts with NE in the LC (Illes, Finta, & Nieber, 1993) to affect anxiety-like behavior via Y2Rs (Kask, Rågo, & Harro, 1998). The apparently complex interplay between pre- and post-synaptic NPY receptors in the CeA may be attributable in part to the notion that inhibitory neurons in the lateral division of the CeA synapse on each other and also on inhibitory neurons in the medial division of the CeA that project out of the medial CeA to brainstem effector regions (Ciochci et al., 2010).

**Amygdalar NPY & alcohol-related behavior**

A wealth of evidence implicates NPY in alcohol-related behaviors, particularly in subpopulations of rats that are vulnerable to excessive alcohol consumption. Rats selectively bred for high alcohol preference have low levels of NPY mRNA and NPY in CeA that are restored by voluntary alcohol consumption (Pandey, Zhang, Roy, & Xu, 2005), perhaps via intracellular mRNA pathways (Zhang & Pandey, 2003). Alcohol-withdrawn rats exhibit increases in anxiety-like behavior and decreased amygdalar NPY, perhaps via decreases in histone acetylation (Pandey, Ugale, Zhang, Tang, & Prakash, 2008; Roy & Pandey, 2002), suggesting that rescue of impaired histone acetylation in amygdala might block withdrawal-related increases in alcohol consumption and anxiety-like behavior via restoration of NPY levels. Activation of NPY systems in the CeA suppresses alcohol self-administration in alcohol-dependent rats at doses that do not affect alcohol self-administration in non-dependent rats (Gilpin, Misra, & Koob, 2008; Thorsell et al., 2007). Similarly, increases in NPY activity in CeA reduce alcohol consumption by rats selectively bred to prefer alcohol (Gilpin, Stewart, & Badia-Elder, 2008) and rats that are innately anxious.

**GABAergic Synapse in Medial CeA**

Fig. 1. Effects of acute alcohol, CRF, and NPY on GABAergic synaptic transmission in the medial CeA. Schematic diagram of a medial CeA GABAergic synapse, including pre-synaptic CRF1Rs and Y2Rs, as well as post-synaptic GABAergic receptors. In the medial CeA synapse of an alcohol-naive rat, the predominant pre-synaptic effect of acute alcohol is potentiation of GABA release, likely via effects on neuromodulators such as CRF. CRF itself augments GABA release via pre-synaptic CRF1R, CRF1R antagonists also block the ability of acute alcohol to augment GABAergic transmission in CeA. In some CeA neurons from alcohol-naive rats, CRF and acute alcohol produce additive increases in evoked IPSC amplitudes (Roberto, Madamba, Nie, & Siggins, 2005). The ability of CRF and acute alcohol to augment GABAergic transmission in CeA is contingent on the integrity of protein kinase C epsilon intracellular signaling pathways (Bajo, Cruz, Siggins, Messing, & Roberto, 2008). Alcohol-dependent rats exhibit heightened sensitivity to the effects of CRF and CRF1R antagonists on GABA release in CeA, suggesting an upregulation of the CRF–CRF1R system (Roberto et al., 2010). These electrophysiological findings are further corroborated by increased CRF and CRF1R mRNA levels in the CeA of alcohol-dependent rats, as well as reversal of alcohol dependence-induced elevations in amygdalar GABA dialysate by a CRF1R antagonist (Roberto et al., 2010). CRF effects on synaptic transmission in CeA are in many ways paralleled by its effects in the BNST (see review by Kash, 2012, in this issue).
levels (Shoblock et al., 2009), as well as increases in anxiety-like dependent reverses stress-induced increases in corticosterone NPY systems, perhaps via convergence on GABA synapses. fi stress-induced anxiety-like behavior (Sajdyk et al., 2008). These NPY administration into the BLA produces long-term decreases in anxiety-like behavior (Sajdyk, Fitz, & Shekhar, 2006), and chronic & Navarro, 2005). NPY in BLA blocks CRF-induced increases in anxiety-like behavior (Sajdyk et al., 2008). These findings suggest a close interaction between amygdalar CRF and NPY systems, perhaps via convergence on GABA synapses.

Both post-synaptic Y1Rs and pre-synaptic Y2Rs have been implicated in the effects of NPY on alcohol consumption. Findings in mice indicate that Y1Rs are responsible for mediating the suppressive effects of NPY on alcohol drinking (Eva, Oberto, Mele, Serra, & Biggio, 2006; Sparta et al., 2004; Thiele, Koh, & Pedrazzini, 2002). Likewise, acute stress and alcohol withdrawal produce increases in amygdalar Y1R expression in rodents (Eva et al., 2006). Seemingly contradictory to these findings (but see next section), antagonism of Y1Rs in the amygdala suppresses operant alcohol responding in rats (Schroeder, Olive, Koenig, & Hodge, 2003).

Single nucleotide polymorphisms in the gene encoding Y2Rs are associated with alcohol dependence and alcohol withdrawal symptoms in humans (Wetherill et al., 2008). Intra-ventricular administration of the Y2R antagonist, BII0246, suppresses alcohol consumption by rats (Thorsell, Rimondini, & Heilig, 2002), and alcohol-dependent rats exhibit increased sensitivity to the suppressive effects of BII0246 on alcohol drinking during protracted abstinence (Rimondini, Thorsell, & Heilig, 2005). Y2R KO mice consume significantly less alcohol than wild-type controls (Thiele, Nolte, & Emrphors, 2004). A systemic Y1R antagonist (NJU-31020028) that is able to cross the blood–brain barrier dose-dependently reverses stress-induced increases in corticosterone levels (Shoblock et al., 2009), as well as increases in anxiety-like behavior produced by hangover/withdrawal from a single bolus injection of alcohol (Cippitelli et al., 2011). Intra-ventricular NPY administration suppresses alcohol self-administration in some cases for up to 24—72 h (Gilpin, Stewart, Murphy, Li, & Badia-Elder, 2003; Gipin et al., 2011), which may be due to the fact that Y2Rs bind ligands in an apparently irreversible and non-competitive manner, and NPY dissociates from Y2Rs much more slowly than from Y1Rs (Dautzenberg & Neysart, 2005). As discussed in more detail below, the ability of NPY in CeA to suppress alcohol self-administration may be due to Y2R-mediated alterations in GABA release (Gilpin et al., 2011).

NPY effects on inhibitory transmission in CeA

NPY prevents and reverses acute alcohol-induced increases in evoked GABAergic transmission in CeA (see Fig. 1; Gilpin et al., 2011). NPY blockade of alcohol-induced decreases in PPF ratio and increases in mIPSC frequency in CeA suggests that NPY decreases pre-synaptic GABA release. Pharmacological probes with Y1R and Y2R antagonists confirm the pre-synaptic site of action and suggest that NPY blocks alcohol effects on GABA release via activation of pre-synaptic Y2Rs. NPY alone does not decrease GABAergic transmission in CeA unless post-synaptic Y1Rs are blocked, suggesting that functional Y1Rs in CeA buffer the effects of NPY at pre-synaptic Y2Rs. NPY also normalizes alcohol dependence-induced increases in GABA release in CeA, suggesting that chronic alcohol exposure produces neuroadaptations in NPY systems that affect inhibitory transmission in CeA (Gilpin et al., 2011).

The above described NPY modulation of GABAergic transmission in CeA is consistent with findings that NPY modulates GABA release via activation of pre-synaptic Y2Rs in both the BNST (Kash & Winder, 2006) and the suprachiasmatic nucleus (SCN) of the hypothalamus (Chen & van den Pol, 1996), supporting the notion that Y2Rs function not only as autoreceptors regulating NPY release (Chen, DiMaggio, Han, & Westfall, 1997), but also as heteroreceptors regulating the release of other neurotransmitters (Greber, Schwarzer, & Sperk, 1994). This dual role of pre-synaptic Y2Rs (regulating NPY and GABA release) may explain apparent discrepancies in the literature regarding the effects of NPY and Y2R compounds on anxiety-like behavior in alcohol-naïve animals (perhaps via Y2R regulation of NPY release) and alcohol-related behaviors in alcohol-dependent animals (perhaps via Y2R regulation of GABA release). This hypothesis is supported by recent data from our lab showing that infusion of a Y2R antagonist into CeA selectively increases alcohol drinking in alcohol-dependent rats (with no effect in non-dependent rats), but decreases anxiety-like behavior in both alcohol-naïve and alcohol-dependent rats (Kallupi, Koo, & Gilpin, unpublished data). Furthermore, the ability of intra-CeA infusion of a Y2R antagonist to reduce alcohol self-administration in rats (Schroeder, Olive, Koenig, & Hodge, 2003) may occur via elimination of the Y1R “brake” on tonic NPY action at Y2Rs in CeA (Gilpin et al., 2011).

CeA peptides & excessive alcohol drinking

Most neurons in the CeA are inhibitory projection neurons or interneurons that co-transmit GABA and one of several neuropeptides. Although it is counterintuitive that pro-anxiety pro-alcohol drinking peptides (e.g., CRF) increase GABAergic transmission in CeA, whereas anti-anxiety anti-alcohol drinking peptides (e.g., NPY) decrease GABAergic transmission in the same region, a closer look at the complexity of CeA neural connectivity helps to explain these contradictions (see Fig. 2). The amygdala is organized in such a way that more lateral nuclei (i.e., lateral and basolateral amygdala) send heavy projections to more medial aspects of the amygdala (i.e., central amygdala). Both the lateral and medial subdivisions of the CeA receive inputs from lateral amygdaloid nuclei, either directly or indirectly via intercalated GABA cells. Within the CeA, there is also a lateral-to-medial flow of information and the medial subdivision of the CeA sends GABAergic efferents to effector regions, especially in the brainstem. Medial CeA projection neurons receive excitatory inputs from BLA as well as inhibitory inputs from lateral CeA and intercalated GABA cells. In many of the slice electrophysiology experiments described above, inhibitory transmission was pharmacologically isolated and post-synaptic potentials were evoked by local electrical stimulation in the medial CeA. In many of these experiments, it is not possible to discern whether IPSPs reflect GABAergic transmission from local interneurons in CeA or inhibitory afferents from other nearby regions (e.g., BNST). Regardless of the source of input, recorded increases in evoked GABAergic transmission from CeA afferents and interneurons (e.g., following application of acute alcohol or CRF) would inhibit the activity of GABAergic neurons projecting out of CeA. Conversely, observed decreases in GABAergic transmission from CeA afferents and interneurons (e.g., following application of NPY) reduce inhibition of GABAergic neurons projecting out of CeA, thereby facilitating the
release of GABA onto downstream targets. As such, recorded increases in GABAergic transmission reflect a disinhibition of downstream target regions (e.g., hypothalamus, PAC, LC, nucleus of the solitary tract, pedunculopontine tegmental nucleus), whereas recorded decreases in GABAergic transmission in medial CeA reflects an inhibition of GABAergic neurons projecting out of CeA. Conversely, recorded decreases in GABAergic transmission in medial CeA (e.g., following application of NPY) reflect a disinhibition of GABAergic neurons projecting out of CeA, thereby facilitating the release of GABA onto downstream targets. In this way, recorded increases in GABAergic transmission reflect a disinhibition of downstream target regions, whereas recorded decreases in GABAergic transmission reflect a net inhibition of downstream target regions.

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