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Neuropeptides in Central Amygdala: Role in Anxiety- and 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 upregulated during the transition to alcohol dependence. These and other neuropeptides may converge on inhibitory networks in the CeA to affect GABAergic transmission and inhibit or disinhibit downstream target regions of CeA projection neurons, thereby regulating the negative emotional state characteristic of withdrawal from chronic high-dose alcohol exposure as well as subsequent motivation to seek and consume alcohol.

Keywords

Amygdala; BNST; PAG; NPY; CRF; Stress; Alcohol Withdrawal; Alcohol Self-Administration

The Central Amygdala

Negative emotion circuitry

The extended amygdala is a conceptual macrostructure (Heimer and Alheid, 1991) that plays a prominent role in both fear and anxiety behaviors (Davis et al., 2010). Two major components of the extended amygdala are the central nucleus of the amygdala (CeA) and the bed nucleus of the stria terminalis (BNST; see the review by Kash in this issue). These two regions exhibit a high degree of interconnectivity and play central roles in generating

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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 and Herkenham, 1991), sends prominent glutamatergic projections to CeA and BNST (Dong et al., 2001; Krettek and Price, 1978; Pitkänen et al., 1995), and is integral in both fear conditioning (Phelps and LeDoux, 2005) and fear extinction (Quirk and Mueller, 2008) processes. The CeA is composed mostly of GABAergic projection neurons and interneurons (Sun and Cassell, 1993; Veinante and 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 sends projection neurons to the BNST (Krettek and Price, 1978; Weller and Smith, 1982), another structure dominated by inhibitory neurotransmission (Sun and Cassell, 1993; Veinante and Freund-Mercier, 1998). Importantly, 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 et al., 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 at this point regarding the precise circuitry and emotional significance of complex intra-amygdala connections (Ehrlich et al., 2009; Pape and 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 grey, 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 downregulation of dopamine signaling in the mesocorticolimbic reward system, hyperactivity of glutamate signaling, and dysregulation of brain stress systems (Koob and 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 and 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 affective aspects of 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 interact in a complex way in the extended amygdala to modulate excitatory and inhibitory transmission, and that this peptidergic modulation of neurotransmission is significantly altered in animals that have a history of exposure to high quantities of alcohol, presumably contributing to the negative emotional state often observed during absence of alcohol in those animals.

This review will focus on the roles of corticotropin-releasing factor (CRF) and neuropeptide Y (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 and Thatcher-Britton, 1985), increases in arousal (Koob et al., 1984), and decreases in feeding (Levine et al., 1983), whereas NPY promotes decreases in anxiety-like behavior (Heilig et al., 1993), decreases in arousal (Heilig and Murison, 1987), and increases in feeding (Stanley and Leibowitz, 1984). Furthermore, 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 (e.g. selective breeding), repeatedly cycled through periods of alcohol withdrawal, or innately anxious (see below). 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 excitatory and inhibitory 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 neurotransmitter and neuropeptide content. The lateral portion of the CeA contains a much higher density of neuropeptides (e.g., CRF; Cassell et al., 1999; Cassell et al., 1986; Shimada et al., 1989; Veening et al., 1984) than the medial CeA.

Animal Models of Excessive Alcohol Drinking

Animal models of alcoholism aim to mimic specific components of the human addiction phenotype rather than the disorder as a whole. Genetic animal models have been used to mimic the heritable aspects of spontaneous and persistent high and low alcohol preference in the offspring of animals that consume either high or low quantities of alcohol (Grahame et al., 1999; Murphy et al., 2002). Selective breeding procedures have produced many pairs of rat and mouse lines (from various genetically heterogeneous stocks), all of which have been selectively bred for many generations to produce high and low levels of alcohol preference. Here, data will be discussed from the alcohol-preferring (P) and –non-preferring (NP) rat lines (Indiana University), originally selected from Wistar rats. The selection criterion for this pair of lines is intake >5 g ethanol/kg body weight/day in P rats and intake <1.5 g ethanol/kg body weight/day in NP rats, with ethanol, water and food continuously available (Lumeng et al., 1977).

Another approach to animal models of excessive alcohol consumption has been the development of models of chronic high-dose alcohol exposure. These models have been utilized to produce the excessive alcohol-seeking and –taking behaviors characteristic of humans that abuse and/or are dependent on alcohol, and allow examination of the neural dysregulation that mediates these behavioral changes. One such procedure utilizes chronic alcohol vapor inhalation to produce alcohol dependence. Alcohol vapor inhalation procedures allow for precise experimenter control of the dose, duration, and pattern of alcohol exposure (Rogers et al., 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

et al., 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 and Thurman, 1980; Gilpin et al., 2009; Lieber and DeCarli, 1982). Upon termination of alcohol vapor exposure, rats can be tested for a multitude of acute withdrawal- and protracted abstinence-related behaviors (Roberts et al., 1996; Rogers et al., 1979; Valdez et al., 2003; Zhao et al., 2007). Behavioral data will be discussed from experiments that utilized each 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 and anxiety- and alcohol-related behaviors

Amygdalar CRF and 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, 1986), and hyperfunction of CRF systems in these regions produces increases in anxiety-like behavior (Lee et al., 2008; Rainnie et al., 2004; Sajdyk et al., 1999a). Conversely, intra-CeA infusion of a CRF receptor antagonist reverses alcohol withdrawal-induced increases in anxiety-like behavior (Rassnick et al., 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 is tightly linked with the production and neurotransmission of other stress-related transmitters and hormones. 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). This CRF-NE loop between CeA and LC may contribute to sensitization of stress responses following multiple stress exposures (Koob, 1999) and is likely dysregulated during the transition to alcohol dependence (Funk et al., 2006; Gilpin and Koob, 2010; Walker et al., 2008). Furthermore, glucocorticoids act at receptors in the CeA to regulate CRF synthesis following fear conditioning (Arnett et al., 2011), and glucocorticoids in BLA positively modulate β -adrenoceptor-mediated consolidation of fear memories via seemingly direct interactions with CRF₁Rs and α_1 -adrenoceptors on the post-synaptic terminal (Rooszendaal et al., 2008).

Amygdalar CRF and Alcohol-Related Behavior—In central amygdala, both stress and alcohol dependence produce increases in extracellular CRF levels (Merlo-Pich et al., 1995; Zorrilla et al., 2001). Alcohol withdrawal produces increases in CRF synthesis and release in CeA (Funk et al., 2006; Roberto et al., 2010; Sommer et al., 2008) and BNST (Olive et al., 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 repeatedly 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 CRF₁ receptors (CRF₁Rs; 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., 1993b).

Recent research has highlighted the role of CRF₁Rs in mediating the effects of limbic CRF on anxiety-like behavior and alcohol drinking. CRF₁R antagonists block the anxiogenic effects of many stressors (including alcohol withdrawal) in a variety of behavioral assays (Arborelius et al., 2000; Zorrilla et al., 2007). CRF₁R antagonists also block increases in alcohol self-administration produced by stressors and alcohol withdrawal (Funk et al., 2007; Gehlert et al., 2007; Hansson et al., 2006; Lowery et al., 2008; Marinelli et al., 2007; Richardson et al., 2008), and chronic antagonism of CRF₁Rs 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 CRF₁R 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 CRF₁R levels (Ciccocioppo et al., 2006), and also exhibit heightened sensitivity to CRF₁R antagonists following development of alcohol dependence (Gilpin et al., 2008c; Sabino et al., 2006). Similarly, CRF₁R knockout (KO) mice exhibit decreased anxiety-like behavior (Muller et al., 2003), as well as decreased alcohol drinking following withdrawal from chronic high-dose alcohol exposure (Chu et al., 2007).

CRF and 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 et al., 2005) and also by activating post-synaptic GABA_A receptors. Alcohol (1–100 mM) selectively potentiates the function of GABA_A receptors that contain particular subunit compositions, but findings have been inconsistent across laboratories examining alcohol effects in heterologous systems (for review, see Aguayo et al., 2002; Lovinger and Homanics, 2007; Lovinger and Roberto, in press). Acute alcohol increases GABAergic synaptic transmission in the central (Roberto et al., 2003) and basolateral (Zhu and Lovinger, 2006) amygdaloid nuclei. These acute effects of alcohol are rapid and reversible, and have a significant pre-synaptic component.

In the CeA, chronic alcohol exposure facilitates GABA release, mainly via actions at pre-synaptic GABAergic terminals (Roberto et al., 2004, 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.

Chronic alcohol produces neuroadaptations at the level of GABA receptors that may account for some aspects of alcohol tolerance (Siggins et al., 2005; Weiner and Valenzuela, 2006). Substantial evidence suggests that alcohol-induced behavioral and neural adaptations are attributable to changes in GABA_AR subunit assembly rather than decreases in the number of GABA_ARs (Devaud et al., 1995; Eckardt et al., 1998; Grobin et al., 1998; Kumar et al., 2004, 2009; Morrow et al., 1992). For example, α_1 and α_4 subunit expression is significantly decreased after two weeks of chronic alcohol consumption, suggesting that chronic alcohol may increase GABA_A receptor function in the amygdala by altering GABA_AR subunit expression in that region (Papadeas et al., 2001).

Pre-synaptic GABA_BRs may mediate inhibitory feedback that limits the ability of acute alcohol to facilitate GABA neurotransmission (Ariwodola and Weiner, 2004). For example, acute alcohol facilitates GABAergic transmission in hippocampus (Ariwodola and Weiner, 2004; Wan et al., 1996; Wu and Saggau, 1994) and nucleus accumbens (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 IPSPs by acute alcohol nor does it potentiate this effect (Roberto et al., 2003), suggesting this effect is brain region-specific. GABA_BRs in the CeA do undergo neuroadaptations following chronic alcohol exposure (Roberto et al., 2008). More specifically, GABA_BR function is decreased following chronic alcohol exposure (Roberto et al., 2008). Alcohol-naïve rats exhibit tonic activation of pre-synaptic GABA_BRs, effects that are either absent or greatly attenuated in the CeA of alcohol-dependent rats, suggesting that chronic alcohol dampens GABA_BR function, which may explain the increased GABAergic tone observed in the CeA of alcohol-dependent rats (Roberto et al., 2008).

CRF modulation of inhibitory transmission 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. CRF₁R antagonists also block the ability of acute alcohol to augment GABAergic transmission in CeA. In some CeA neurons from alcohol-naïve rats, CRF and acute alcohol produce additive increases in evoked IPSC amplitudes (Roberto et al., 2005). The ability of CRF and acute alcohol to augment GABAergic transmission in CeA is contingent on the integrity of protein kinase C epsilon (PKCε) intracellular signaling pathways (Bajo et al., 2008). These data are consistent with the idea that PKCδ-cells in the lateral division of the CeA are activated by aversive conditioned stimuli (Haubensak et al., 2010) and inhibit PKCδ+ cells (perhaps NPY-containing GABA neurons; Choi et al., 2010) in that nucleus, which themselves do not respond to aversive conditioned stimuli and project to the medial division of the CeA (Haubensak et al., 2010). 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 effect in the BNST (see review by Kash, this issue).

NPY and Alcohol in Central Amygdala

NPY and anxiety- and alcohol-related behaviors

Amygdalar NPY and Anxiety-Related Behavior—NPY is a 36-amino-acid peptide that has strong effects on feeding (Levine and Morley, 1984; Stanley and Leibowitz, 1984), arousal (Gilpin et al., 2004; Heilig and Murison, 1987; Naveilhan et al., 2001), and anxiety-related behaviors (Heilig et al., 1993). Neuropeptide Y (NPY) 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 (Heilig et al., 1989, 1992; Broqua et al., 1995; Britton et al., 1997; Sajdyk et al., 1999b). The robust anxiolytic effects of NPY are mediated by the central and basolateral amygdaloid nuclei (Heilig et al., 1993; Sajdyk et al., 1999b), regions that are densely populated by NPY fibers and receptors (Allen et al., 1984; de Quidt and Emson, 1986; Dumont et al. 1990; Gustafson et al. 1997; Migita et al., 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 et al., 1993; Gustafson et al., 1997; Kopp et al., 2002; Parker and 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 Y₁Rs reverses the anxiolytic effects of NPY (Sajdyk et al., 1999b).

Site-specific ablation of the Y₂R gene in CeA and BLA affects anxiety-like and depression-like behaviors in mice, presumably via alteration of GABAergic transmission (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 (Sajdyk et al., 2002a,b). Like CRF, NPY interacts with NE in the LC (Illes et al., 1993) to affect anxiety-like behavior via Y₂Rs (Kask et al., 1998). A major target for inhibitory efferent projections from CeA is periaqueductal gray (PAG) matter in the brainstem (Pitkänen, 2000), a region attributed an important role in behavioral responses to aversive stimuli in the environment (e.g., stimuli previously paired with shock; Johansen et al., 2010; Kim et al., 1993; Koch, 1999). 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 (Cicchi et al., 2010), fitting well with the hypothesis that NPY-containing GABA neurons are PKC δ + cells (Choi et al., 2010) that receive inputs from local PKC δ - cells (perhaps CRF-containing GABA neurons) in the lateral CeA and project onto efferents in the medial CeA (Haubensak et al., 2010).

Amygdalar NPY and 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 et al., 2005), perhaps via intracellular PKA pathways (Zhang and Pandey, 2003). Alcohol-withdrawn rats exhibit increases in anxiety-like behavior and decreased amygdalar NPY, perhaps via epigenetic modifications (i.e., decreases in histone acetylation; Pandey et al., 2008; Roy and 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 et al., 2008a; Thorsell et al., 2007). Similarly, increases in NPY activity in CeA reduce alcohol consumption by rats selectively bred to prefer alcohol (Gilpin et al., 2008b) and rats that are innately anxious (Primeaux et al., 2006). Withdrawal-induced decreases in CeA NPY likely contribute to increased GABAergic tone in alcohol-dependent animals, particularly since application of exogenous NPY normalizes dependence-induced increases in GABA release in CeA (Gilpin et al., 2011). Consistent with this hypothesis, it has been proposed that inhibitory neuronal populations in CeA mediate the ability of ICV NPY to block stress-induced reinstatement of alcohol-seeking behavior (Cippitelli et al., 2010).

Chronic NPY administration during prior alcohol withdrawals blocks increases in alcohol self-administration during subsequent withdrawals (Gilpin et al., 2011), a hallmark behavioral feature of the transition to alcohol dependence. The ability of chronic NPY treatment during prior withdrawals to suppress alcohol drinking during subsequent withdrawals may be due to blockade of withdrawal-induced increases in anxiety-like behavior, as has been suggested with other anti-anxiety compounds chronically administered

in a similar protocol (Breese et al., 2005), an effect likely to be mediated by amygdala. Indeed, NPY in BLA blocks CRF-induced increases in anxiety-like behavior (Sajdyk et al., 2006), and chronic NPY administration into the BLA produces long-term decreases in stress-induced anxiety-like behavior (Sajdyk et al., 2008). These findings suggest a close interaction between amygdalar CRF and NPY systems, perhaps via convergence on GABA neurons.

Both post-synaptic Y_1 Rs and pre-synaptic Y_2 Rs have been implicated in the effects of NPY on alcohol consumption. Findings in mice indicate that Y_1 Rs are responsible for mediating the suppressive effects of NPY on alcohol drinking (Eva et al., 2006; Sparta et al., 2004; Thiele et al., 2002). Likewise, acute stress and alcohol withdrawal produce increases in amygdalar Y_1 R expression in rodents (Eva et al., 2006). Seemingly contradictory to these findings (but see next section), antagonism of Y_1 Rs in the amygdala suppresses operant alcohol responding in rats (Schroeder et al., 2003).

Single nucleotide polymorphisms in the gene encoding Y_2 Rs are associated with alcohol dependence and alcohol withdrawal symptoms in humans (Wetherill et al., 2008). Intra-ventricular administration of the Y_2 R antagonist, BIIE0246, suppresses alcohol consumption by rats (Thorsell et al., 2002), and alcohol-dependent rats exhibit increased sensitivity to the suppressive effects of BIIE0246 on alcohol drinking (Rimondini et al., 2005). Y_2 R KO mice consume significantly less alcohol than wild-type controls (Thiele et al., 2004). Furthermore, Y_2 Rs bind ligands in an apparently irreversible and non-competitive manner, and NPY dissociates from Y_2 Rs much more slowly than from Y_1 Rs (Dautzenberg and Neysari, 2005), suggesting that Y_2 R may be the mechanism whereby NPY produces long-term suppressive effects on alcohol drinking (Gilpin et al., 2003, 2011). A major obstacle in the pharmacotherapeutic development of NPY receptor ligands has been the unavailability of ligands that cross the blood-brain barrier and effectively bind Y_1 or Y_2 receptors in brain. Recently introduced was the Y_2 R antagonist, JNJ-31020028, which can be injected systemically and binds Y_2 Rs in brain (Shoblock et al., 2009). Unfortunately, neither systemic nor intra-ventricular injection of this Y_2 R antagonist affects basal or post-stress anxiety-like behavior or alcohol consumption in a reliable way (Cippitelli et al., 2011; Shoblock et al., 2009). That said, the antagonist dose-dependently reverses stress-induced increases in corticosterone levels (Shoblock et al., 2009) and increases in anxiety-like behavior produced by hangover/withdrawal from a single bolus injection of alcohol (Cippitelli et al., 2011), suggesting that NPY systems are recruited following a challenge to the system. This idea is consistent with augmented effects of NPY in CeA on alcohol consumption in alcohol-dependent rats (Gilpin et al., 2008a), and the ability of NPY to reverse dependence-induced increases in GABA release in CeA, likely via actions at pre-synaptic Y_2 Rs (Gilpin et al., 2011).

NPY and alcohol effects on inhibitory transmission in CeA

NPY prevents and reverses acute alcohol-induced increases in evoked GABAergic transmission in CeA (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 Y_1 R and Y_2 R antagonists confirm the pre-synaptic site of action and suggest that NPY blocks alcohol effects on GABA release via activation of pre-synaptic Y_2 Rs. NPY alone does not decrease GABAergic transmission in CeA unless post-synaptic Y_1 Rs are blocked, suggesting that functional Y_1 Rs in CeA buffer the effects of NPY at pre-synaptic Y_2 Rs. 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 and Winder, 2006) and the suprachiasmatic nucleus (SCN) of the hypothalamus (Chen and van den Pol, 1996), supporting the notion that Y₂Rs function not only as autoreceptors regulating NPY release (Chen et al., 1997), but also as heteroreceptors regulating the release of other neurotransmitters (Greber et al., 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 (Kallupi, Koob and Gilpin, unpublished data). Furthermore, the ability of intra-CeA infusion of a Y₁R antagonist to reduce alcohol self-administration in rats (Schroeder et al., 2003) may occur via elimination of the Y₁R “brake” on tonic NPY action at Y₂Rs in CeA (Gilpin et al., 2011).

CeA Peptides and 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 proalcohol-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 results. 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 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 and intercalated GABA cells). 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, PAG, LC, nucleus of the solitary tract, pedunculopontine tegmental nucleus), whereas recorded decreases in GABAergic transmission reflect a net inhibition of downstream target regions. Therefore, *increases and decreases in local GABAergic transmission in CeA produce decreases and increases in inhibitory output from the CeA to downstream effector regions, and increases and decreases in anxiety-like behavior, respectively* (see also Davis et al., 2010; Paré et al., 2004; Tye et al., 2011).

The ability of CRF and NPY to affect anxiety-like behavior and alcohol drinking has been localized to the amygdala. Amygdalar CRF is increased following exposure to stress and

during withdrawal from alcohol and other drugs (Funk et al., 2006; Merlo-Pich et al., 1995; Roberto et al., 2010; Sommer et al., 2008; Zorrilla et al., 2001), and intra-CeA administration of CRF receptor antagonists decreases anxiety-like behavior and alcohol self-administration by rodents, particularly following the development of alcohol dependence (Funk et al., 2006; Rassnick et al., 1993). Amygdalar NPY, on the other hand, is decreased during alcohol withdrawal (Pandey et al., 2008; Roy and Pandey, 2002), and intra-amygdala administration of exogenous NPY decreases anxiety-like behavior (Heilig et al., 1993; Sajdyk et al., 1999b) and reduces excessive alcohol self-administration by vulnerable subpopulations of drinkers (Gilpin et al., 2008a,b; Primeaux et al., 2006). Because CRF and NPY produce opposite behavioral profiles but have very similar neuroanatomical distributions, it has long been hypothesized that these two peptides co-exist in a yin-yang type of relationship (Heilig et al., 1994). Recent electrophysiological data suggest that the interaction effects of CRF and NPY on anxiety-like behavior and alcohol self-administration are attributable to convergence on GABAergic transmission in CeA. The effects of CRF, NPY, and other peptides on GABAergic transmission in CeA is up-regulated in the alcohol-dependent organism (Gilpin et al., 2011; Roberto et al., 2006, 2010), suggesting the CeA is a prominent locus for neuroadaptation during the development of alcohol dependence. Although manipulation of many of these systems affects alcohol drinking exclusively in the alcohol-dependent organism, it is not surprising that these neuropeptides affect basal transmission in CeA of alcohol-naïve animals, particularly because they modulate anxiety-related behavior independent of alcohol exposure history. This final point also contributes to our understanding of why *these* neuropeptide systems are recruited and/or up-regulated during the transition to alcohol dependence, a dynamic disease state defined largely by a negative emotional state in the absence of the drug.

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REFERENCES

- Abu-Murad C, Thurman RG. Reversal of tolerance to ethanol associated with the induction of physical dependence on ethanol in the rat. *Adv. Exp. Med. Biol.* 1980; 132:697–704. [PubMed: 7191627]
- Aguilar-Valles A, Sánchez E, de Gortari P, Baldera I, Ramírez-Amaya V, Bermúdez-Rattoni F, Joseph-Bravo P. Analysis of the stress response in rats trained in the water-maze: differential expression of corticotropin-releasing hormone, CRHR1, glucocorticoid receptors and brain-derived neurotrophic factor in limbic regions. *Neuroendocrinol.* 2005; 82:306–319.
- Aguiayo LG, Peoples RW, Yeh HH, Yevenes GE. GABA(A) receptors as molecular sites of ethanol action. Direct or indirect actions? *Curr. Top. Med. Chem.* 2002; 2:869–885. [PubMed: 12171577]
- Allen YS, Roberts GW, Bloom SR, Crow TJ, Polak JM. Neuropeptide Y in the stria terminalis: evidence for an amygdalofugal projection. *Brain Res.* 1984; 321:357–362. [PubMed: 6548654]
- Arborelius L, Skelton KH, Thirivikraman KV, Plotsky PM, Schulz DW, Owens MJ. Chronic administration of the selective corticotropin-releasing factor 1 receptor antagonist CP-154,526: behavioral, endocrine and neurochemical effects in the rat. *J. Pharmacol. Exp. Ther.* 2000; 294:588–597. [PubMed: 10900236]
- Ariwodola OJ, Weiner JL. Ethanol potentiation of GABAergic synaptic transmission may be self-limiting: role of presynaptic GABA(B) receptors. *J. Neurosci.* 2004; 24:10679–10686. [PubMed: 15564584]
- Arnett MG, Kolber BJ, Boyle MP, Muglia LJ. Behavioral insights from mouse models of forebrain- and amygdala-specific glucocorticoid receptor genetic disruption. *Mol. Cell. Endocrinol.* 2011; 336:2–5. [PubMed: 21094675]
- Bajo M, Cruz MT, Siggins GR, Messing R, Roberto M. Protein kinase C epsilon mediation of CRF- and ethanol-induced GABA release in central amygdala. *Proc. Natl. Acad. Sci. USA.* 2008; 105:8410–8415. [PubMed: 18541912]

- Breese GR, Overstreet DH, Knapp DJ, Navarro M. Prior multiple ethanol withdrawals enhance stress-induced anxiety-like behavior: inhibition by CRF1- and benzodiazepine-receptor antagonists and a 5-HT1a-receptor agonist. *Neuropsychopharmacol.* 2005; 30:1662–1669.
- Britton KT, Southerland S, Van Uden E, Kirby D, Rivier J, Koob G. Anxiolytic activity of NPY receptor agonists in the conflict test. *Psychopharmacol.* 1997; 132:6–13.
- Broqua P, Wettstein JG, Rocher MN, Gauthier-Martin B, Junien JL. Behavioral effects of neuropeptide Y receptor agonists in the elevated plus-maze and fear-potentiated startle procedures. *Behav. Pharmacol.* 1995; 6:215–222. [PubMed: 11224329]
- Cassell MD, Freedman LJ, Shi C. The intrinsic organization of the central extended amygdala. *Ann. N. Y. Acad. Sci.* 1999; 877:217–241. [PubMed: 10415652]
- Cassell MD, Gray TS, Kiss JZ. Neuronal architecture in the rat central nucleus of the amygdala: a cytological, hodological, and immunocytochemical study. *J. Comp. Neurol.* 1986; 246(4):478–499. [PubMed: 2422231]
- Chen G, van den Pol AN. Multiple NPY receptors coexist in pre- and postsynaptic sites: inhibition of GABA release in isolated self-innervating SCN neurons. *J. Neurosci.* 1996; 16:7711–7724. [PubMed: 8922427]
- Chen X, DiMaggio DA, Han SP, Westfall TC. Auto receptor-induced inhibition of neuropeptide Y release from PC-12 cells is mediated by Y2 receptors. *J. Am. Physiol.* 1997; 273:H1737–H1744.
- Choi DS, Nam HW, Lesscher HMB, McMahon T, Amitai N, Chou WH, Messing RO. Regulation of neuropeptide signaling by PKC isozymes in the amygdala. *Alcohol. Clin. Exp. Res.* 2010; 34(s3):55A.
- Chu K, Koob GF, Cole M, Zorrilla EP, Roberts AJ. Dependence-induced increases in ethanol self-administration in mice are blocked by the CRF1 receptor antagonist antalarmin and by CRF1 receptor knockout. *Pharmacol. Biochem. Behav.* 2007; 86(4):813–821. [PubMed: 17482248]
- Ciccocioppo R, Economidou D, Cippitelli A, Cucculelli M, Ubaldi M, Soverchia L, Lourdusamy A, Massi M. Genetically selected Marchigian Sardinian alcohol-preferring (msP) rats: an animal model to study the neurobiology of alcoholism. *Addiction Biol.* 2006; 11:339–355.
- Ciocchi S, Herry C, Grenier F, Wolff SBE, Letzkus JJ, Vlachos I, Ehrlich I, Sprengel R, Deisseroth K, Stadler MB, Müller C, Lüthi A. Encoding of conditioned fear in central amygdala inhibitory circuits. *Nature.* 2010; 468:277–282. [PubMed: 21068837]
- Cippitelli A, Damadzic R, Hansson AC, Singley E, Sommer WH, Eskay R, et al. Neuropeptide Y (NPY) suppresses yohimbine-induced reinstatement of alcohol seeking. *Psychopharmacol.* 2010; 208:417–426.
- Cippitelli A, Rezvani A, Robinson JE, Eisenberg L, Levin ED, Bonaventure P. The novel, selective, brain-penetrant neuropeptide Y Y2 receptor antagonist, JNJ-31020028, tested in animal models of alcohol consumption, relapse, and anxiety. *Alcohol.* 2011; 45:567–576. [PubMed: 21145691]
- Clarke TK, Treutlein J, Zimmermann US, Kiefer F, Skowronek MH, Rietschel M, Mann K, Schumann G. HPA-axis activity in alcoholism: examples for a gene-environment interaction. *Addict. Biol.* 2008; 13(1):1–14. [PubMed: 17910738]
- Dautzenberg FM, Neysari S. Irreversible binding kinetics of neuropeptide Y ligands to Y2 but not to Y1 and Y5 receptors. *Pharmacol.* 2005; 75:21–29.
- Davis M, Walker DL, Miles L, Grillon C. Phasic vs sustained fear in rats and humans: role of the extended amygdala in fear vs anxiety. *Neuropsychopharmacology.* 2010; 35(1):105–135. [PubMed: 19693004]
- De Quidt ME, Emson PC. Distribution of Neuropeptide Y-like immunoreactivity in the rat central nervous system-II. Immunohistochemical analysis. *Neurosci.* 1986; 18:545–618.
- De Souza EB, Perrin MH, Insel TR, Rivier J, Vale WW, Kuhar MJ. Corticotropin-releasing factor receptors in rat forebrain: autoradiographic identification. *Science.* 1984; 224:1449–1451. [PubMed: 6328656]
- Devaud LL, Morrow AL, Criswell HE, Breese GR, Duncan GE, Simson PE, Knapp DJ, McCown TJ. Regional differences in the effects of chronic ethanol administration on [3H]zolpidem binding in rat brain. *Alcohol. Clin. Exp. Res.* 1995; 19:910–914. [PubMed: 7485838]
- Dong H-W, Petrovich GD, Swanson LW. Topography of projections from amygdala to bed nuclei of the stria terminalis. *Brain Res. Rev.* 2001; 38:192–246. [PubMed: 11750933]

- Dumont Y, Fournier A, St-Pierre S, Quirion R. Comparative characterization and autoradiographic distribution of neuropeptide Y receptor subtypes in the rat brain. *J. Neurosci.* 1993; 13:73–86. [PubMed: 8423484]
- Dumont Y, Fournier A, St-Pierre S, Schwartz TW, Quirion R. Differential distribution of Y1 and Y2 receptors in the rat brain. *Eur. J. Pharmacol.* 1990; 191:501–503. [PubMed: 1964914]
- Eckardt MJ, File SE, Gessa GL, Grant KA, Guerri C, Hoffman PL, Kalant H, Koob GF, Li T-K, Tabakoff B. Effects of moderate alcohol consumption on the central nervous system. *Alcohol. Clin. Exp. Res.* 1998; 22:998–1040. [PubMed: 9726269]
- Ehrlich I, Humeau Y, Grenier F, Ciochi S, Herry C, Lüthi A. Amygdala inhibitory circuits and the control of fear memory. *Neuron.* 2009; 62:757–771. [PubMed: 19555645]
- Eva C, Oberto A, Mele P, Serra M, Biggio G. Role of brain neuroactive steroids in the functional interplay between the GABAA and the NPY-Y1 receptor mediated signals in the amygdala. *Pharmacol. Biochem. Behav.* 2006; 84:568–580. [PubMed: 16824587]
- Funk CK, O'Dell LE, Crawford EF, Koob GF. Corticotropin-releasing factor within the central nucleus of the amygdala mediates enhanced ethanol self-administration in withdrawn, ethanol-dependent rats. *J. Neurosci.* 2006; 26:11324–11332. [PubMed: 17079660]
- Funk CK, Zorrilla EP, Lee M-J, Rice KC, Koob GF. Corticotropin-releasing factor-1 antagonists selectively reduce ethanol self-administration in ethanol-dependent rats. *Biol. Psychiatry.* 2007; 61:78–86. [PubMed: 16876134]
- Gehlert DR, Cippitelli A, Thorsell A, Lê AD, Hipskind PA, Hamdouchi C, et al. 3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-8-(1-ethylpropyl)-2,6-dimethyl-imidazo[1,2-b]pyridazine: a novel brain-penetrant, orally available corticotropin-releasing factor receptor 1 antagonist with efficacy in animal models of alcoholism. *J. Neurosci.* 2007; 27(10):2718–2726. [PubMed: 17344409]
- Gilpin NW, Koob GF. Effects of β -adrenoceptor antagonists on alcohol drinking by alcohol-dependent rats. *Psychopharmacol.* 2010; 212:431–439.
- Gilpin NW, Misra K, Herman MA, Cruz MT, Koob GF, Roberto M. Neuropeptide Y opposes alcohol effects on gamma-aminobutyric acid release in amygdala and blocks the transition to alcohol dependence. *Biol. Psychiatry.* 2011; 69:1091–1099. [PubMed: 21459365]
- Gilpin NW, Misra K, Koob GF. Neuropeptide Y in the central nucleus of the amygdala suppresses dependence-induced decreases in alcohol drinking. *Pharmacol. Biochem. Behav.* 2008; 90:475–480. [PubMed: 18501411]
- Gilpin NW, Richardson HN, Koob GF. Effects of CRF1-receptor and opioid-receptor antagonists on dependence-induced increases in alcohol drinking by alcohol-preferring (P) rats. *Alcohol. Clin. Exp. Res.* 2008; 32:1535–1542. [PubMed: 18631323]
- Gilpin NW, Smith AD, Cole M, Weiss F, Koob GF, Richardson HN. Operant behavior and alcohol levels in blood and brain of alcohol-dependent rats. *Alcohol. Clin. Exp. Res.* 2009; 33:2113–2123. [PubMed: 19740131]
- Gilpin NW, Stewart RB, Badia-Elder NE. Neuropeptide Y administration into the amygdala suppresses ethanol drinking in alcohol-preferring (P) rats following multiple deprivations. *Pharmacol. Biochem. Behav.* 2008; 90:470–474. [PubMed: 18499241]
- Gilpin NW, Stewart RB, Elder RL, Kho Y, Murphy JM, Li TK, Badia-Elder NE. Sedative and motor-impairing effects of neuropeptide Y and ethanol in selectively bred P and NP rats. *Pharmacol. Biochem. Behav.* 2004; 78:65–73. [PubMed: 15159135]
- Gilpin NW, Stewart RB, Murphy JM, Li T-K, Badia-Elder NE. Neuropeptide Y reduces oral ethanol intake in alcohol-preferring (P) rats following a period of imposed ethanol abstinence. *Alcohol. Clin. Exp. Res.* 2003; 27:787–794. [PubMed: 12766623]
- Grahame NJ, Li TK, Lumeng L. Selective breeding for high and low alcohol preference in mice. *Behav. Genetics.* 1999; 29:47–57.
- Greber S, Schwarzer C, Sperk G. Neuropeptide Y inhibits potassium-stimulated glutamate release through Y2= receptors in rat hippocampal slices in vitro. *Br. J. Pharmacol.* 1994; 113:737–740. [PubMed: 7858862]
- Grobin AC, Matthews DB, Devaud LL, Morrow AL. The role of GABA(A) receptors in the acute and chronic effects of ethanol. *Psychopharmacology (Berl).* 1998; 139:2–19. [PubMed: 9768538]

- Gustafson EL, Smith KE, Durkin MM, Walker MW, Gerald C, Weinshank R, Branchek TA. Distribution of the neuropeptide Y Y2 receptor mRNA in rat central nervous system. *Mol. Brain Res.* 1997; 46:223–235. [PubMed: 9191097]
- Hansson AC, Cippitelli A, Sommer WH, Fedeli A, Bjork K, Soverchia L, Terasmaa A, Massi M, Heilig M, Ciccocioppo R. Variation at the rat *Crhr1* locus and sensitivity to relapse into alcohol seeking induced by environmental stress. *Proc. Nat. Acad. Sci. USA.* 2006; 103:15236–15241. [PubMed: 17015825]
- Haubensak W, Kunwar PS, Cai H, Ciocchi S, Wall NR, Ponnusamy R, Biag J, Dong HW, Deisseroth K, Callaway EM, Fanselow MS, Lüthi A, Anderson DJ. Genetic dissection of an amygdala microcircuit that gates conditioned fear. *Nature.* 2010; 468:270–276. [PubMed: 21068836]
- Heilig M, Koob GF, Ekman R, Britton KT. Corticotropin-releasing factor and neuropeptide Y: role in emotional integration. *Trends Neurosci.* 1994; 17:80–85. [PubMed: 7512773]
- Heilig M, McLeod S, Brot M, Heinrichs SC, Menzaghi F, Koob GF, Britton KT. Anxiolytic-like action of neuropeptide Y: mediation by Y1 receptors in amygdala, and dissociation from food intake effects. *Neuropsychopharmacol.* 1993; 8:357–363.
- Heilig M, McLeod S, Koob GK, Britton KT. Anxiolytic-like effect of neuropeptide Y (NPY), but not other peptides in an operant conflict test. *Regul. Pept.* 1992; 41:61–69. [PubMed: 1360689]
- Heilig M, Murison R. Intracerebroventricular neuropeptide Y suppresses open field and home cage activity in the rat. *Regul. Pept.* 1987; 19:221–231. [PubMed: 3432602]
- Heilig M, Söderpalm B, Engel JA, Widerlöv E. Centrally administered neuropeptide Y (NPY) produces anxiolytic-like effects in animal anxiety models. *Psychopharmacol.* 1989; 98:524–529.
- Heimer, L.; Alheid, G. Piecing together the puzzle of basal forebrain anatomy. In: Napier, TC.; Kalivas, PW.; Hanin, I., editors. *The Basal Forebrain: Anatomy to Function* (series title: *Advances in Experimental Medicine and Biology*, vol 295). New York: Plenum Press; 1991. p. 1-42.
- Huang MM, Overstreet DH, Knapp DJ, Angel R, Wills TA, Navarro M, Rivier J, Vale W, Breese GR. Corticotropin-releasing factor (CRF) sensitization of ethanol withdrawal-induced anxiety-like behavior is brain site specific and mediated by CRF-1 receptors: relation to stress-induced sensitization. *J. Pharmacol. Exp. Ther.* 2010; 332(1):298–307. [PubMed: 19843974]
- Illes P, Finta EP, Nieber K. Neuropeptide Y potentiates via Y2-receptors the inhibitory effect of noradrenaline in rat locus coeruleus neurones. *Naunyn Schmiedeberg's Arch. Pharmacol.* 1993; 348:546–548. [PubMed: 8114954]
- Johansen JP, Tarpley JW, LeDoux JE, Blair HT. Neural substrates for expectation-modulated fear learning in the amygdala and periaqueductal gray. *Nature Neurosci.* 2010; 13:979–986. [PubMed: 20601946]
- Kash TL, Winder DG. Neuropeptide Y and corticotropin-releasing factor bi-directionally modulate inhibitory synaptic transmission in the bed nucleus of the stria terminalis. *Neuropharmacology.* 2006; 51:1013–1022. [PubMed: 16904135]
- Kask A, Rägo L, Harro J. Anxiolytic-like effect of neuropeptide Y (NPY) and NPY 13–36 microinjected into vicinity of locus coeruleus in rats. *Brain Res.* 1998; 788:345–348. [PubMed: 9555090]
- Kiefer F, Wiedemann K. Neuroendocrine pathways of addictive behaviour. *Addict Biol.* 2004; 9:205–212. [PubMed: 15511714]
- Kim JJ, Rison RA, Fanselow MS. Effects of amygdala, hippocampus, and periaqueductal gray lesions on short- and long-term contextual fear. *Behav. Neurosci.* 1993; 107:1093–1098. [PubMed: 8136063]
- Koch M. The neurobiology of startle. *Prog. Neurobiol.* 1999; 59:107–128. [PubMed: 10463792]
- Koob GF. Corticotropin-releasing factor, norepinephrine, and stress. *Biol. Psychiatry.* 1999; 46:1167–1180. [PubMed: 10560023]
- Koob GF. Alcoholism: allostasis and beyond. *Alcohol. Clin. Exp. Res.* 2003; 27:232–243. [PubMed: 12605072]
- Koob. A role for brain stress systems in addiction. *Neuron.* 2008; 59:11–34. [PubMed: 18614026]
- Koob GF, Swerdlow N, Seeligson M, Eaves M, Sutton R, Rivier J, Vale W. Effects of alpha-flupenthixol and naloxone on CRF-induced locomotor activation. *Neuroendocrinol.* 1984; 39:459–464.

- Koob GF, Thatcher-Britton K. Stimulant and anxiogenic effects of corticotropin releasing factor. *Prog. Clin. Biol. Res.* 1985; 192:499–506. [PubMed: 3878524]
- Koob GF, Volkow ND. Neurocircuitry of addiction. *Neuropsychopharmacology.* 2010; 35(1):217–238. [PubMed: 19710631]
- Kopp J, Xu Z-Q, Zhang X, Pedrazzini T, Herzog H, Kresse A, Wong H, Walsh JH, Hökfelt T. Expression of the neuropeptide Y Y1 receptor in the CNS of rat and of wild-type and Y1 receptor knock-out mice. Focus on immunohistochemical localization. *Neuroscience.* 2002; 111:443–532. [PubMed: 12031341]
- Krettek JE, Price JL. Amygdaloid projections to subcortical structures within the basal forebrain and brainstem in the rat and cat. *J. Comp. Neurol.* 1978; 178(2):225–254. [PubMed: 627625]
- Kumar S, Fleming RL, Morrow AL. Ethanol regulation of gamma-aminobutyric acid A receptors: genomic and nongenomic mechanisms. *Pharmacol. Ther.* 2004; 101:211–226. [PubMed: 15031000]
- Kumar S, Porcu P, Werner DF, Matthews DB, Diaz-Granados JL, Helfand RS, Morrow AL. The role of GABA(A) receptors in the acute and chronic effects of ethanol: a decade of progress. *Psychopharmacology (Berl).* 2009; 205:529–564. [PubMed: 19455309]
- Lee Y, Fitz S, Johnson PL, Shekhar A. Repeated stimulation of CRF receptors in the BNST of rats selectively induces social but not panic-like anxiety. *Neuropsychopharmacol.* 2008; 33:2586–2594.
- Levine AS, Morley JE. Neuropeptide Y: a potent inducer of consummatory behavior in rats. *Peptides.* 1984; 5:1025–1029. [PubMed: 6549409]
- Levine AS, Rogers B, Kneip J, Grace M, Morley JE. Effect of centrally administered corticotropin releasing factor (CRF) on multiple feeding paradigms. *Neuropharmacol.* 1983; 22(3):337–339.
- Lieber CS, DeCarli LM. The feeding of alcohol in liquid diets: two decades of applications and 1982 update. *Alcohol. Clin. Exp. Res.* 1982; 6:523–531. [PubMed: 6758624]
- Lovinger DM, Homanics GE. Tonic for what ails us? high-affinity GABA-A receptors and alcohol. *Alcohol.* 2007; 41:139–143. [PubMed: 17521844]
- Lovinger, DM.; Roberto, M. Synaptic Effects Induced by Alcohol. In: Spanagel, R.; Sommer, WH., editors. “Behavioural Neurobiology of Alcohol Addiction”, *Current Topics in Behavioral Neurosciences.* Berlin Heidelberg: Springer-Verlag; (in press)
- Lowery EG, Sparrow AM, Breese GR, Knapp DJ, Thiele TE. The CRF-1 receptor antagonist, CP-154,526, attenuates stress-induced increases in ethanol consumption by BALB/cJ mice. *Alcohol. Clin. Exp. Res.* 2008; 32(2):240–248. [PubMed: 18162074]
- Lumeng, L.; Hawkins, DT.; Li, TK. New strains of rats with alcohol preference and nonpreference. In: Thurman, RG., editor. *Alcohol and Aldehyde Metabolizing Systems.* Vol. vol. 3. New York: Academic Press; 1977. p. 537–544.
- Marinelli PW, Funk D, Juzysch W, Harding S, Rice KC, Shaham Y, Le AD. The CRF1 receptor antagonist antalarmin attenuates yohimbine-induced increases in operant alcohol self-administration and reinstatement of alcohol seeking in rats. *Psychopharmacology.* 2007; 195:345–355. [PubMed: 17705061]
- McDonald AJ. Cortical pathways to the mammalian amygdala. *Prog. Neurobiol.* 1998; 55:257–332. [PubMed: 9643556]
- Merlo-Pich E, Lorang M, Yeganeh M, Rodriguez de Fonseca F, Raber J, Koob G, Weiss F. Increase of extracellular corticotrophin-releasing factor-like immunoreactivity levels in the amygdala of awake rats during restraint stress and ethanol withdrawal as measured by microdialysis. *J. Neurosci.* 1995; 15:5439–5447. [PubMed: 7643193]
- Migita K, Loewy AD, Ramabhadran TV, Krause JE, Waters SM. Immunohistochemical localization of the neuropeptide Y Y1 receptor in rat central nervous system. *Brain Res.* 2001; 889:23–37. [PubMed: 11166683]
- Morrow AL, Herbert JS, Montpiéd P. Differential effects of chronic ethanol administration on GABA(A) receptor alpha1 and alpha6 subunit mRNA levels in rat cerebellum. *Mol. Cell Neurosci.* 1992; 3:251–258. [PubMed: 19912867]

- Muller M, Zimmermann S, Sillaber I, Hagemeyer TP, Deussing JM, Timpl P, et al. Limbic corticotrophin-releasing hormone receptor 1 mediates anxiety-related behavior and hormonal adaptation to stress. *Nat. Neurosci.* 2003; 6:1100–1107. [PubMed: 12973355]
- Murphy JM, Stewart RB, Bell RL, Badia-Elder NE, Carr LG, McBride WJ, Lumeng L, Li T-K. Phenotypic and genotypic characterization of the Indiana University rat lines selectively bred for high and low alcohol preference. *Behav. Gen.* 2002; 32:363–387.
- Naveilhan P, Canals JM, Valjakka A, Vartiainen J, Arenas E, Ernfors P. Neuropeptide Y alters sedation through a hypothalamic Y1-mediated mechanism. *Eur. J. Neurosci.* 2001; 13:2241–2246. [PubMed: 11454027]
- Nie Z, Madamba SG, Siggins GR. Ethanol enhances gamma-aminobutyric acid responses in a subpopulation of nucleus accumbens neurons: role of metabotropic glutamate receptors. *J. Pharmacol. Exp. Ther.* 2000; 293:654–661. [PubMed: 10773041]
- Olive MF, Koenig HN, Nannini MA, Hodge CW. Elevated extracellular CRF levels in the bed nucleus of the stria terminalis during ethanol withdrawal and reduction by subsequent ethanol intake. *Pharmacol. Biochem. Behav.* 2002; 72:213–220. [PubMed: 11900791]
- Pandey SC, Ugale R, Zhang H, Tang L, Prakash A. Brain chromatin remodeling: a novel mechanism of alcoholism. *J. Neurosci.* 2008; 28:3729–3737. [PubMed: 18385331]
- Pandey SC, Zhang H, Roy A, Xu T. Deficits in amygdaloid cAMP-responsive element-binding protein signaling play a role in genetic predisposition to anxiety and alcoholism. *J. Clin. Invest.* 2005; 115:2762–2773. [PubMed: 16200210]
- Papadeas S, Grobin AC, Morrow AL. Chronic ethanol consumption differentially alters GABA(A) receptor alpha1 and alpha4 subunit peptide expression and GABA(A) receptor-mediated $^{36}\text{Cl}^-$ uptake in mesocorticolimbic regions of rat brain. *Alcohol. Clin. Exp. Res.* 2001; 25:1270–1275. [PubMed: 11584145]
- Pape H-C, Pare D. Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. *Physiol. Rev.* 2010; 90:419–463. [PubMed: 20393190]
- Paré D, Quirk GJ, Ledoux JE. New vistas on amygdala networks in conditioned fear. *J. Neurophysiol.* 2004; 92:1–9. [PubMed: 15212433]
- Parker RMC, Herzog H. Regional distribution of Y-receptor subtype mRNAs in rat brain. *Eur. J. Neurosci.* 1999; 11:1431–1448. [PubMed: 10103138]
- Phelps EA, LeDoux JE. Contributions of the amygdala to emotion processing: from animal models to human behavior. *Neuron.* 2005; 48(2):175–187. [PubMed: 16242399]
- Pitkänen A. Connectivity of the rat amygdaloid complex. In: Aggleton, JP., editor. *The Amygdala*. Oxford University Press; 2000.
- Pitkänen A, Stefanacci L, Farb CR, Go G-G, Ledoux JE, Amaral DG. Intrinsic connections of the rat amygdaloid complex: projections originating in the lateral nucleus. *J. Comp. Neurol.* 1995; 356:288–310. [PubMed: 7629320]
- Primeaux SD, Wilson SP, Bray GA, York DA, Wilson MA. Overexpression of neuropeptide Y in the central nucleus of the amygdala decreases ethanol self administration in “anxious” rats. *Alcohol. Clin. Exp. Res.* 2006; 30:791–801. [PubMed: 16634847]
- Quirk GJ, Mueller D. Neural mechanisms of extinction learning and retrieval. *Neuropsychopharmacology.* 2008; 33(1):56–72. [PubMed: 17882236]
- Rainnie DG, Bergeron R, Sajdyk TJ, Patil M, Gehlert DR, Shekhar A. Corticotrophin releasing factor-induced synaptic plasticity in the amygdala translates stress into emotional disorders. *J. Neurosci.* 2004; 24:3471–3479. [PubMed: 15071094]
- Rassnick S, D'Amico E, Riley E, Koob GF. GABA antagonist and benzodiazepine partial inverse agonist reduce motivated responding for ethanol. *Alcohol. Clin. Exp. Res.* 1993; 17(1):124–130. [PubMed: 8383923]
- Richardson HN, Fekete EM, Zhao Y, Funk CK, Zorrilla EP, Koob GF. A novel small molecule antagonist of the corticotrophin-releasing factor type 1 receptor (CRF1) is a potent anxiolytic and reduces excessive alcohol intake in dependent male rats. *Pharmacol. Biochem. Behav.* 2008; 88:497–510. [PubMed: 18031798]

- Rimondini R, Thorsell A, Heilig M. Suppression of ethanol self-administration by the neuropeptide Y (NPY) Y2 receptor antagonist BIIIE0246: evidence for sensitization in rats with a history of dependence. *Neurosci. Lett.* 2005; 375:129–133. [PubMed: 15670655]
- Roberto M, Cruz MT, Gilpin NW, Sabino V, Schweitzer P, Bajo M, et al. Corticotropin releasing factor-induced amygdala gamma-aminobutyric acid release plays a key role in alcohol dependence. *Biol. Psychiatry.* 2010; 67:831–839. [PubMed: 20060104]
- Roberto M, Gilpin NW, O'Dell LE, Cruz MT, Morse AC, Siggins GR, Koob GF. Cellular and behavioral interactions of gabapentin with alcohol dependence. *J. Neurosci.* 2008; 28:5762–5771. [PubMed: 18509038]
- Roberto M, Madamba SG, Moore SD, Tallent MK, Siggins GR. Ethanol increases GABAergic transmission at both pre- and postsynaptic sites in rat central amygdala neurons. *Proc. Natl. Acad. Sci. USA.* 2003; 100:2053–2058. [PubMed: 12566570]
- Roberto M, Madamba SG, Nie Z, Siggins GR. Ethanol-CRF interactions at GABAergic synapses in rat central amygdala. *Alcohol. Clin. Exp. Res.* 2005; (Suppl. 29):26.
- Roberto M, Madamba SG, Stouffer DG, Parsons LH, Siggins GR. Increased GABA release in the central amygdala of ethanol-dependent rats. *J. Neurosci.* 2004; 24:10159–10166. [PubMed: 15537886]
- Roberts AJ, Cole M, Koob GF. Intra-amygdala muscimol decreases operant ethanol self-administration in dependent rats. *Alcohol. Clin. Exp. Res.* 1996; 20:1289–1298. [PubMed: 8904984]
- Roberts AJ, Heyser CJ, Cole M, Griffin P, Koob GF. Excessive ethanol drinking following a history of dependence: animal model of allostasis. *Neuropsychopharmacol.* 2000; 22:581–594.
- Rogers J, Wiener SG, Bloom FE. Long-term ethanol administration methods for rats: advantages of inhalation over intubation or liquid diets. *Behav. Neural Biol.* 1979; 27:466–486. [PubMed: 575037]
- Roozendaal B, Schelling G, McGaugh JL. Corticotropin-releasing factor in the basolateral amygdala enhances memory consolidation via an interaction with the β -adrenoceptor–cAMP pathway: dependence on glucocorticoid receptor activation. *J. Neurosci.* 2008; 28:6642–6651. [PubMed: 18579737]
- Roy A, Pandey SC. The decreased cellular expression of neuropeptide Y protein in rat brain structures during ethanol withdrawal after chronic ethanol exposure. *Alcohol. Clin. Exp. Res.* 2002; 26:796–803. [PubMed: 12068247]
- Sabino V, Cottone P, Koob GF, Steardo L, Lee MJ, Rice KC, Zorrilla EP. Dissociation between opioid and CRF1 antagonist sensitive drinking in Sardinian alcohol-preferring rats. *Psychopharmacol.* 2006; 189:175–186.
- Sajdyk TJ, Fitz SD, Shekhar A. The role of neuropeptide Y in the amygdala on corticotropin-releasing factor receptor-mediated behavioral stress responses in the rat. *Stress.* 2006; 9:21–28. [PubMed: 16753930]
- Sajdyk TJ, Johnson PL, Leitermann RJ, Fitz SD, Dietrich A, Morin M, Gehlert DR, Urban JH, Shekhar A. Neuropeptide Y in the amygdala induces long-term resilience to stress-induced reductions in social responses but not hypothalamic–adrenal–pituitary axis activity or hyperthermia. *J. Neurosci.* 2008; 28:893–903. [PubMed: 18216197]
- Sajdyk TJ, Schober DA, Gehlert DR. Neuropeptide Y receptor subtypes in the basolateral nucleus of the amygdala modulate anxiogenic responses in rats. *Neuropharmacol.* 2002a; 43:1165–1172.
- Sajdyk TJ, Schober DA, Gehlert DR, Shekhar A. Role of corticotropin-releasing factor and urocortin within the basolateral amygdala of rats in anxiety and panic responses. *Behav. Brain Res.* 1999a; 100:207–215. [PubMed: 10212068]
- Sajdyk TJ, Schober DA, Smiley DL, Gehlert DR. Neuropeptide Y-Y2 receptors mediate anxiety in the amygdala. *Pharmacol. Biochem. Behav.* 2002b; 71:419–423. [PubMed: 11830176]
- Sajdyk TJ, Vandergriff MG, Gehlert DR. Amygdalar neuropeptide Y Y1 receptors mediate the anxiolytic-like actions of neuropeptide Y in the social interaction test. *Eur. J. Pharmacol.* 1999b; 368:143–147. [PubMed: 10193650]

- Sakanaka M, Shibasaki T, Lederis K. Distribution and efferent projections of corticotropin-releasing factor-like immunoreactivity in the rat amygdaloid complex. *Brain Res.* 1986; 382:213–238. [PubMed: 2428439]
- Shimada S, Inagaki S, Kubota Y, Ogawa N, Shibasaki T, Takagi H. Coexistence of peptides (corticotropin releasing factor/neurotensin and substance P/somatostatin) in the bed nucleus of the stria terminalis and central amygdaloid nucleus of the rat. *Neuroscience.* 1989; 30(2):377–383. [PubMed: 2473417]
- Schroeder JP, Olive F, Koenig H, Hodge CW. Intra-amygdala infusion of the NPY Y1 receptor antagonist BIBP 3226 attenuates operant ethanol self-administration. *Alcohol. Clin. Exp. Res.* 2003; 27:1884–1891. [PubMed: 14691375]
- Shoblock JR, Welty N, Nepomuceno D, Lord B, Alusio L, Fraser I, et al. In vitro and in vivo characterization of JNJ-31020028(N-(4-{4-[2-(diethylamino)-2-oxo-1-phenylethyl]piperazin-1-yl}-3-fluorophenyl)-2-pyridin-3-ylbenzamide), a selective brain penetrant small molecule antagonist of the neuropeptide Y Y₂ receptor. *Psychopharmacol.* 2009; 208:265–277.
- Siggins GR, Roberto M, Nie Z. The tipsy terminal: presynaptic effects of ethanol. *Pharmacol. Ther.* 2005; 107:80–98. [PubMed: 15963352]
- Sommer WH, Rimondini R, Hansson AC, Hipskind PA, Gehlert DR, Barr CS, Heilig MA. Upregulation of voluntary alcohol intake, behavioral sensitivity to stress, and amygdala Crhr1 expression following a history of dependence. *Biol. Psychiatry.* 2008; 63:139–145. [PubMed: 17585886]
- Sparta DR, Fee JR, Hayes DM, Knapp DJ, MacNeil DJ, Thiele TE. Peripheral and central administration of a selective neuropeptide Y Y1 receptor antagonist suppresses ethanol intake by C57BL/6J mice. *Alcohol. Clin. Exp. Res.* 2004; 28:1324–1330. [PubMed: 15365302]
- Stanley BG, Leibowitz SF. Neuropeptide Y: stimulation of feeding and drinking by injection into the paraventricular nucleus. 1984
- Sun N, Cassell MD. Intrinsic GABAergic neurons in the rat central extended amygdala. *J. Comp. Neurol.* 1993; 15:381–404. [PubMed: 8385679]
- Tasan RO, Nguyen NK, Weger S, Sartori SB, Singewald N, Heilbronn R, Herzog H, Sperk G. The central and basolateral amygdala are critical sites of neuropeptide Y/Y2 receptor-mediated regulation of anxiety and depression. *J. Neurosci.* 2010; 30:6282–6290. [PubMed: 20445054]
- Thiele TE, Koh MT, Pedrazzini T. Voluntary alcohol consumption is controlled via the neuropeptide Y Y1 receptor. *J. Neurosci.* 2002; 22
- Thiele TE, Naveilhan P, Ernfor P. Assessment of ethanol consumption and water drinking by NPY Y2 receptor knockout mice. *Peptides.* 2004; 25:975–983. [PubMed: 15203244]
- Thorsell A, Rimondini R, Heilig M. Blockade of central neuropeptide Y (NPY) Y2 receptors reduces ethanol self-administration in rats. *Neurosci. Lett.* 2002; 332:1–4. [PubMed: 12377370]
- Turner BH, Herkenham M. Thalamoamygdaloid projections in the rat: a test of the amygdala's role in sensory processing. *J. Comp. Neurol.* 1991; 313:295–325. [PubMed: 1765584]
- Tye KM, Prakash R, Kim SY, Fenno LE, Grosenick L, Zarabi H, Thompson KR, Gradinaru V, Ramakrishnan C, Deisseroth K. Amygdala circuitry mediating reversible and bidirectional control of anxiety. *Nature.* 2011; 471:358–362. [PubMed: 21389985]
- Valdez GR, Roberts AJ, Chan K, Davis H, Brennan M, Zorrilla EP, Koob GF. Increased ethanol self-administration and anxiety-like behavior during acute withdrawal and protracted abstinence: regulation by corticotropin-releasing factor. *Alcohol. Clin. Exp. Res.* 2002; 26:1494–1501. [PubMed: 12394282]
- Valdez GR, Zorrilla EP, Roberts AJ, Koob GF. Antagonism of corticotropin-releasing factor attenuates the enhanced responsiveness to stress observed during protracted ethanol abstinence. *Alcohol.* 2003; 29:55–60. [PubMed: 12782246]
- Veening JG, Swanson LW, Sawchenko PE. The organization of projections from the central nucleus of the amygdala to brainstem sites involved in central autonomic regulation: a combined retrograde transport-immunohistochemical study. *Brain Res.* 1984; 303(2):337–357. [PubMed: 6204716]
- Veinante P, Freund-Mercier MJ. Intrinsic and extrinsic connections of the rat central extended amygdala: an in vivo electrophysiological study of the central amygdaloid nucleus. *Brain Res.* 1998; 794(2):188–198. [PubMed: 9622626]

- Walker BM, Rasmussen DD, Raskind MA, Koob GF. α 1-noradrenergic receptor antagonism blocks dependence-induced increases in responding for ethanol. *Alcohol*. 2008; 42:91–97. [PubMed: 18358987]
- Wan FJ, Berton F, Madamba SG, Francesconi W, Siggins GR. Low ethanol concentrations enhance GABAergic inhibitory postsynaptic potentials in hippocampal pyramidal neurons only after block of GABAB receptors. *Proc. Natl. Acad. Sci. USA*. 1996; 93:5049–5054. [PubMed: 8643527]
- Weiner JL, Valenzuela CF. Ethanol modulation of GABAergic transmission: The view from the slice. *Pharmacol. Ther.* 2006; 111:533–554. [PubMed: 16427127]
- Weller KL, Smith DA. Afferent connections to the bed nucleus of the stria terminalis. *Brain Res.* 1982; 232(2):255–270. [PubMed: 7188024]
- Wetherill L, Schuckit MA, Hesselbrock V, Xuei X, Liang T, Dick DM, et al. Neuropeptide Y receptor genes are associated with alcohol dependence, alcohol withdrawal phenotypes, and cocaine dependence. *Alcohol. Clin. Exp. Res.* 2008; 32:2031–2040. [PubMed: 18828811]
- Wu LG, Saggau P. Presynaptic calcium is increased during normal synaptic transmission and paired-pulse facilitation, but not in long-term potentiation in area CA1 of hippocampus. *J. Neurosci.* 1994; 14:645–654. [PubMed: 7905515]
- Zhang H, Pandey SC. Effects of PKA modulation on the expression of neuropeptide Y in rat amygdaloid structures during ethanol withdrawal. *Peptides*. 2003; 24:1397–1402. [PubMed: 14706555]
- Zhao Y, Weiss F, Zorrilla EP. Remission and resurgence of anxiety-like behavior across protracted withdrawal stages in ethanol-dependent rats. *Alcohol. Clin. Exp. Res.* 2007; 31:1505–1515. [PubMed: 17760785]
- Zhu PJ, Lovinger DM. Ethanol potentiates GABAergic synaptic transmission in a postsynaptic neuron/synaptic bouton preparation from basolateral amygdala. *J. Neurophysiol.* 2006; 96:433–441. [PubMed: 16624993]
- Zorrilla EP, Valdez GR, Weiss F. Changes in levels of regional CRF-like-immunoreactivity and plasma corticosterone during protracted drug withdrawal in dependent rats. *Psychopharmacol.* 2001; 158:374–381.
- Zorrilla, EP.; Zhao, Y.; Koob, GF. Anti-CRF. In: Fink, G., editor. *Encyclopedia of Stress*. Vol. Vol 1 A–E. Amsterdam: Elsevier; 2007. p. 206-214.

GABAergic Synapse in Medial CeA

Figure 1.

Figure 2.