

# Mechanisms and Mediators of the Relationship between Anxiety Disorders and Alcohol Use Disorders: Focus on Amygdalar NPY

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## Abstract

High rates of co morbidity for Alcohol Use Disorders (AUDs) and anxiety disorders suggest a causative relationship between these disorders, as well as overlapping neurobiological mechanisms. While it is well established that alcohol withdrawal can precipitate and exacerbate the expression of anxiety, the extent to which pre-existing anxiety disorders contribute to the development of AUDs is less clear. Anxiety relief is commonly cited as a motivation to consume alcohol and recent preclinical studies focusing on the relationship between innate anxiety phenotypes and alcohol-related behaviors support the notion that elevated anxiety may contribute to increased alcohol consumption. However, the endogenous neural mechanisms that mediate this relationship have yet to be fully defined. This review focuses on the relationship between anxiety-related responses and acute alcohol effects, including the potential role that the Neuropeptide Y (NPY) system in the amygdala plays in mediating the neurobiological intersection of anxiety-alcohol effects.

**Keywords:** Alcohol; Central amygdala; Anxiety; Neuropeptide Y

## Introduction

Alcohol Use Disorders (AUDs), including alcohol abuse and dependence, are maladaptive patterns of alcohol consumption resulting in clinically significant distress or impairment [1]. AUDs rank among the most diagnosed mental health disorders in the United States, with lifetime prevalence rates approaching 18% and the associated mental and physical health problems and disruptions in occupational and social functioning make AUDs a significant public health issue [2,3]. Much of the current research in the alcohol field has focused on understanding the behavioral and neurobiological mechanisms that underlie the transition from controlled alcohol use to alcohol abuse and dependence. Alcohol dependence (alcohol addiction) is a chronic, relapsing disorder characterized by compulsive, excessive alcohol use, and the presence of withdrawal symptoms during abstinence. Dependence is thought to evolve over time from specific neuroadaptations that arise following repeated cycles of alcohol abuse i.e. heavy alcohol consumption and intoxication, bouts of withdrawal in the absence of alcohol, and craving for alcohol during periods of abstinence. Therefore, one of the primary research goals has been to characterize the neurobiological changes that occur following chronic alcohol consumption and how these adaptations relate to the expression of alcohol withdrawal syndrome, alcohol craving and relapse to alcohol abuse.

However, to fully understand the mechanisms that underlie the development of AUDs, the acute effects of alcohol must also be considered. Acute alcohol challenge modulates many of the same neurobiological pathways that are altered following chronic alcohol consumption, indicating that these acute neurobiological changes contribute to the persistent neuroadaptations that underlie the transition to alcohol dependence. Moreover, the acute subjective effects of alcohol, particularly the mood enhancing effects, contribute to the initiation and maintenance of drinking, and both human and rodent studies indicate that elevated sensitivity to these acute effects may be a significant risk factor for increased alcohol consumption and AUDs [4-8]. Consequently, characterizing both the acute behavioral and neurobiological effects of alcohol is crucial for a comprehensive understanding of the transition from initial alcohol consumption to abuse and the individual factors that contribute to risk for alcohol abuse and dependence. This review will focus on the interplay between

anxiety and acute alcohol administration and the suggestion that alcohol-induced modulation of the amygdalar Neuropeptide Y (NPY) system during early stages of alcohol use contributes to the progression from moderate alcohol consumption to alcohol abuse and dependence.

## Alcohol and Anxiety

AUDs frequently co-occur with anxiety disorders, with 75% of individuals that abuse alcohol having a current or previous diagnosis of an anxiety disorder [9-11]. The anxiogenic effects of withdrawal from chronic alcohol consumption are well established. Increased anxiety is one of the key symptoms of the alcohol withdrawal syndrome in humans (APA 2013) and preclinical studies have demonstrated elevated measures of anxiety-like behaviors in various animal models following withdrawal from chronic alcohol exposure [12-14]. Taken together, these data suggest that for many individuals diagnosed with an AUD, the comorbid anxiety disorder is precipitated by alcohol withdrawal-induced increases in anxiety symptoms. Moreover, this elevated anxiety can persist long after the physical symptoms of alcohol withdrawal have subsided, which likely contributes to continued alcohol use in both dependent and non-dependent individuals [15]. While current evidence suggests a causal relationship between withdrawal-induced anxiety and the progression from moderate alcohol consumption to alcohol abuse and dependence, less is known about the extent to which pre-existing anxiety disorders contribute to the development of AUDs.

Current epidemiological evidence indicates that anxiety disorders are frequently present prior to the development of AUDs, as well as other substance dependence disorders, suggesting that high levels of

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anxiety may contribute to increased risk for AUDs [16-18]. It has long been proposed that elevated innate anxiety contributes to increased risk for AUDs by promoting heavy alcohol use [19-22]. However the hypothesis that high anxiety levels increase alcohol consumption remains controversial, as results from clinical studies examining the effects of stress and anxiety on alcohol craving and consumption have been variable [23-27]. Nevertheless, moderate doses of alcohol produce feelings of relaxation and reduced anxiety in humans [5] and preclinical studies have confirmed the anxiolytic effects of alcohol in a number of animal models of anxiety. In rats, low to moderate doses of alcohol (0.5-1.5 g/kg) increased open arm exploration and decreased risk assessment behaviors in the Elevated Plus Maze (EPM) [28-30], decreased defensive burying behaviors [30] and increased transitions in the light: dark box [31,32]. Moreover, rats will voluntarily consume enough alcohol in limited access paradigms to elicit these anxiolytic effects on the EPM [33,34]. Therefore, it is unsurprising that anxiety relief is often cited as a motivation to drink, especially among those who suffer from social anxiety disorder [35]. Moreover, individuals who are more anxiety sensitive (i.e. fearful of experiencing anxiety symptoms) report increased quantity and frequency of alcohol consumption, as well as coping-motivated alcohol consumption [36,37], suggesting that these anxiolytic effects can be a significant incentive to consume alcohol in some individuals. Although these studies point toward elevated anxiety increasing risk for alcohol abuse, and ultimately alcohol dependence, much of the data is correlative and the extent to which innate anxiety contributes to patterns of alcohol use has not been fully characterized.

## Animal Models

One of the difficulties in examining the impact of differences in innate anxiety on the development of AUDs in humans is assembling a cohort in which anxiety phenotypes are characterized prior to the initiation of alcohol use and then tracking the differences in alcohol consumption patterns over time. Different animal models have been used to overcome this limitation. A number of studies have examined the role of stress-induced anxiety on alcohol intake in adult animals, with an emphasis on stress-related changes to established drinking patterns; however, a full discussion of these models is beyond the scope of this review [38]. This assessment suggested that chronic stress tended to elevate drinking, especially if the stressor occurred early in development. Therefore, one specific approach has been to examine the effects of early stressors on anxiety responses and alcohol drinking by using maternal separation paradigms. Epidemiological evidence suggests that early adverse experience is a risk factor for both anxiety disorders and AUDs [39,40]. In rats, daily maternal separation sessions prior to weaning lead to decreased open arm time on the EPM in adult animals [41]. Maternal separation stress also significantly increased alcohol consumption and preference scores in adult animals [41,42], as well as causing escalating ethanol consumption over time [43]. A comparable effect of early life stress has been reported in non-human primates. Rhesus macaques that were peer-raised had higher levels of anxiety and increased alcohol consumption in adulthood as compared to maternal rearing [43]. Interestingly, elevated anxiety and alcohol consumption after peer-raising stress were associated with a functional variation in the NPY gene resulting in reduced NPY expression in adulthood, alluding to a specific role for genetic variation in NPY expression in the risk for both anxiety disorders and AUDs.

A second approach has been to examine anxiety-related responses in animals selectively bred for extremes of alcohol-related behaviors. In fact, some of the rodent lines selectively bred for differential alcohol consumption and/or preference also show differences in anxiety-like

behaviors (Table 1). Both alcohol preferring (P) rats and Sardinian alcohol preferring (sP) rats demonstrate more anxiety-like behavior on the EPM than the associated non-preferring (NP and sNP) lines [45-48]. However, not all the data point to a positive correlation between elevated alcohol consumption and increased anxiety-like behavior. Viglinskaya et al. (1995) [49] showed no significant behavioral difference between P and NP rats in the EPM. The High Alcohol Drinking (HAD) and Low Alcohol Drinking (LAD) rat lines do not show differences in anxiety-like behavior in EPM and alcohol preferring AA (Alko Alcohol) show either equal or less anxiety-like behavior than alcohol avoiding ANA (Alko Non-Alcohol) rats [49-54]. Behavioral differences among the various alcohol-preferring lines are not entirely unexpected. The background strains for the individual lines were different, and although the behavioral selection criteria for the individual lines were similar, the specific genetic differences that contribute to differential alcohol intake may be unique for each line. Therefore, the lack of consistency in anxiety-like behavior in these lines may be the result of different genetic backgrounds or selection for different neurobiological mechanisms controlling alcohol preference and intake in the individual lines.

Similarly, rat lines selectively bred for high and low anxiety-like behaviors have also been used to examine the relationship between anxiety phenotype and alcohol consumption, but again, no consistent correlation has been demonstrated (Table 2). Low anxiety (Low Anxiety-Related Behavior; LAB) animals consume more alcohol and have higher alcohol preference scores than the high anxiety (High Anxiety-Related Behavior; HAB) animals [55]. In comparisons of the Floripa L (low anxiety) and Floripa H (high anxiety) lines, only females of the H line showed elevated alcohol consumption as compared to L females and both H and L males [56,57]. Finally, high anxiety Roman high avoidance rats consume more alcohol than the associated low anxiety line (Roman low avoidance), at least during initiation of alcohol consumption [58]. Here too, neurobiological differences due to background strains and selection criteria may be influencing these disparate drinking behaviors. The HAB and LAB lines were selected based on open arm time on the EPM, while the Floripa H and L lines

Rat Model	Anxiety phenotype (EPM)	Reference
Alcohol Preferring versus Non-Preferring P Vs NP	P > NP	[46,49]
	P=NP	[50]
Sardinian Alcohol Preferring versus Non-Preferring sP Vs sNP	sP > sNP	[45,48]
High alcohol drinking versus Low alcohol drinking HAD Vs LAD	HAD=LAD	[51,54]
Alko Alcohol versus Alko Non-Alcohol AA Vs ANA	AA=ANA	[50,55]
	AA<ANA	[93,54]

**Table 1:** Comparison of innate anxiety phenotype (as measured on the EPM) in rat lines selectively bred for differential alcohol intake and alcohol preference.

Rat Model	Alcohol Intake	Reference
High anxiety-related behavior versus Low anxiety-related behavior (HAB Vs LAB)	HAB < LAB (4-bottle choice)	[56]
Floripa H (high anxiety) versus Floripa L (low anxiety) Females Males	Floripa L < Floripa H	[57]
	Floripa L=Floripa H (2-bottle choice)	[58]
Roman High Avoidance versus Low Avoidance	High > Low (2-bottle choice)	[59]

**Table 2:** Comparison of alcohol intake in rat lines selectively bred for differential anxiety-like behavior.

were selected based on center locomotion in the open field test and the Roman high and low avoidance lines were selected based on shuttle box behaviors [59].

Another approach to modeling the effects of pre-existing anxiety responses on alcohol consumption has been to compare alcohol intake either between or within non-selected strains that show differential basal anxiety-like behavior. These models are thought to better represent the genetic diversity seen in the human population and may provide a means for examining the more subtle individual differences in behavior, neurobiology, and perhaps genetics that underlie the development of AUDs in those with anxiety disorders. Langen and Fink (2004) [60] compared alcohol consumption in three rat strains that differed significantly in open arm time on the EPM. The strain with the lowest open arm time consumed less alcohol during two-bottle choice access (12 weeks) and during progressive ratio testing than the less anxious strains with greater open arm time. Interestingly, alcohol consumption during the initiation phase, consisting of two weeks of *ad lib* sweetened alcohol, was not different between the three strains and it was not until after sucrose was removed from the alcohol solution that the differences in intake were found. This is of note as it is thought that the neurobiological mechanisms that control feeding behaviors also control some aspects of alcohol consumption. Comparisons in alcohol consumption have also been made between Spontaneous Hypertensive Rats (SHR) and Lewis rats. Based on behavioral measures from both the EPM and open field test, SHR rats exhibit significantly less anxiety-like behavior than Lewis rats [61,62]. Under short-term two bottle continuous access conditions, characterized as acquisition of alcohol intake, the low anxiety SHR rats consumed more alcohol and have higher alcohol preference scores than Lewis rats [63]. Sensitivity to the anxiolytic effects of alcohol was also compared in these two lines. Although alcohol increased open arm time on the EPM in both lines, only the SHR rats showed increase center time and locomotion following alcohol administration in the open field test. Therefore, while sensitivity to alcohol's anxiolytic effects may indeed be important for promoting or maintaining alcohol consumption, these results are dependent on the anxiety test used.

In contrast, another approach using outbred rodent strains generally show a positive correlation between high anxiety-like behavior and elevated alcohol intake, although this is dependent on the model of ethanol consumption. Work from our laboratory and others has shown that outbred Long-Evans rats show highly variable anxiety-like behavior on the EPM [32,64-67]. In this rat model, anxiety phenotype is directly related to alcohol consumption and preference in chronic two bottle choice paradigms. Rats characterized as having a high anxiety phenotype consumed more alcohol during limited (1 hour/day for 6 weeks) two bottle access [64] and had higher alcohol preference scores during continuous (24 hours/day for 2 weeks) two bottle access, as compared to low anxiety animals [66]. Wistar rats and Tuck-Ordinary mice show similar relationships. High anxiety animals (based on plus maze behavior) showed higher alcohol intake and alcohol preference than low anxiety animals in two bottle choice paradigms [68,69], suggesting that within these rodent strains higher basal anxiety may contribute to higher alcohol consumption. However, the relationship between anxiety-like behaviors and alcohol consumption may be dependent on the drinking paradigm used. In an acute voluntary alcohol consumption paradigm based on the murine drinking in the dark (DID) model [70], Long-Evans rats characterized as having a high anxiety phenotype consumed significantly less alcohol than low anxiety animals [67]. These distinct patterns of consumption may reflect differences in how innate anxiety influences the various

stages of the transition from controlled alcohol use to alcohol abuse and dependence. The four day limited access drinking paradigm based on the DID model more closely replicates initiation to drinking, as opposed to the long-term two bottle choice paradigms that model more experienced drinking behaviors. Further, since such paradigms of chronic alcohol consumption are potent stressors, this may suggest differential sensitivity to stress-induced alcohol consumption [38]. Finally, as with the between strain studies [60], the continuous access models often involve a sucrose fading procedure that is not needed in the DID paradigms. Thus, although these data indicate that individual differences in anxiety measures are associated with differences in alcohol preference and consumption patterns, further study is necessary to clarify how individual differences in anxiety responses influence the initiation and maintenance of alcohol consumption.

### Alcohol, Anxiety and the Amygdala

The prevalence with which AUDs co-occur with anxiety disorders suggests that the development and expression of these disorders may share overlapping neurobiological pathways. The amygdala has been of particular interest, as this region is well documented to be involved in fear and anxiety and is believed to play a critical role in anxiety disorders [71]. The amygdala is comprised of several nuclei, including the central nucleus (CeA) and the basolateral complex (BLA), which work in concert to process responses to fearful stimuli. The BLA receives and integrates sensory input from the thalamus and cortex and modulates neuronal excitation in the CeA through glutamatergic projections [72]. The CeA then coordinates the behavioral and physiological responses to the stimuli through mainly  $\gamma$ -aminobutyric acid-ergic (GABAergic) projections to the bed nucleus of the stria terminalis (BNST) and brainstem nuclei [73]. Alcohol's anxiolytic effects are thought to be mediated by these signaling pathways, as well. In slice preparations, acute alcohol augments inhibitory GABAergic neurotransmission in the CeA via increased presynaptic GABA release [74]. Acute alcohol also inhibits excitatory glutamatergic transmission in the BLA [75,76] and BNST [77], predominantly through inhibition of glutamate receptor function. It is postulated that this combination of enhanced inhibitory output from the CeA and reduced excitatory activity in the BLA and BNST following alcohol administration results in an overall inhibition of downstream effector regions and a decrease in anxiety-like behavior [78].

Studies examining neuronal activation in the CeA with alcohol administration support that this region appears to be of particular significance for the expression alcohol-induced reductions in anxiety behaviors. Quantifying the expression of cFos, an immediately early gene, has frequently been used to identify brain regions that are activated by a specific stimulus or challenge, as changes in Fos expression are temporally regulated [79]. Using this technique, a number of studies have demonstrated that acute alcohol exposure increases Fos expression in the CeA. Moderate to high doses (0.75 to 3 g/kg) of alcohol increased Fos immunoreactivity in the CeA without altering Fos levels in the BLA [80-83]. Fos immunoreactivity was also elevated in the CeA following alcohol consumption in a limited access paradigm [84], suggesting that animals will consume enough alcohol to activate the CeA. Work from our laboratory further explored the relationship between acute alcohol consumption, amygdalar activation, and alcohol-induced anxiolysis. Using a four day limited access drinking paradigm, we demonstrated Fos immunoreactivity in the CeA was positively correlated with alcohol consumption and open arm time in the plus maze, suggesting that increases in Fos expression may be related to increased expression of alcohol-induced anxiolysis [34]. In contrast, chronic high dose

alcohol treatment (3 g/kg for 17-24 days) results in a desensitization of alcohol-induced Fos activation in the CeA [85]. Determining if this desensitization correlates with changes in sensitivity to the anxiolytic effects of alcohol would aid in determining how amygdalar activation relates to the expression of alcohol-induced anxiolysis. It must be noted that the CeA is further divided into medial, lateral and paracapsular divisions and has several distinct neuronal populations (with distinct projections) that may be differentially responsive to acute alcohol. Preliminary evidence indicates that acute alcohol administration (1g/kg) selectively increases Fos expression in the lateral division of the CeA [32], but further analysis is required to determine if this subregion is an important mediator of alcohol's anxiolytic effects. Additionally, determining the phenotypic identity of these Fos-positive neurons is an essential step in fully characterizing the role of the CeA in anxiety and the anxiolytic effects of alcohol. Current evidence suggests that these activated neurons are enkephalin-containing GABAergic neurons, but the CeA also includes somatostatin, corticotropin-releasing factor (CRF), and NPY containing GABAergic neurons [73,80,83]. Despite the evidence suggesting a role for amygdalar NPY in anxiety and ethanol effects, current evidence from our laboratory suggests that acute, low dose alcohol does not activate NPY expressing neurons of the CeA. Dual labeling studies have shown no co localization of Fos and NPY immunoreactivity in this region two hours after alcohol treatment (unpublished observations); however, further studies are planned to explore the temporal regulation of amygdalar NPY responses to alcohol challenge.

While it is tempting to hypothesize that activation of the CeA is the mechanism by which alcohol exerts its anxiolytic effects, there are several caveats to this postulate. It must be noted that there are no differences in Fos activation in the CeA between the alcohol-preferring P and AA rats and their associated non-preferring lines following acute alcohol challenge [86]. Although acute alcohol treatment decreases anxiety-like behaviors in P rats, NP rats appear to be insensitive to the anxiolytic effects of alcohol [46], suggesting that this increase in amygdalar activation following acute alcohol treatment is not directly related to alcohol's anxiolytic effects, but to another pharmacological effect of alcohol. In fact, we found a positive correlation between locomotor activity on the EPM and Fos activation in the CeA [34], supporting other literature suggesting that amygdalar activation may be related to the locomotor effects of alcohol [87,88]. Further, several anxiogenic stimuli can also increase immediate early gene expression in this region. Acute treatment with anxiogenic drugs has been shown to increase amygdalar expression of Fos and *egr-1*, both measures of neuronal activation, suggesting that activation of the CeA may be related to any perturbation of the fear and anxiety circuits [89,90]. This hypothesis is supported by some evidence that acute exposure to anxiogenic stressors can increase markers of neuronal activation in the CeA [91-94]. Thus, the neuronal activation of the CeA with ethanol could be related to the more anxiogenic or psychostimulant-like properties of alcohol.

### Alcohol, Anxiety, and NPY

In addition to common neuroanatomical pathways, many of the neurochemical systems and their signaling cascades that mediate anxiety-related responses are also modulated by alcohol use. In the CeA, NPY has been identified as one of the key mediators of both anxiety- and alcohol-related behaviors [78], suggesting that this neuropeptide may play a significant role in the comorbidity of anxiety disorders and AUDs. NPY, a 36 amino acid peptide, is one of the most abundant peptides in the central nervous system. Widely distributed throughout

the brain, NPY is found in cortical and limbic structures, the striatum and the brain stem, and is often colocalized with GABA, as well as other neuropeptides [95,96]. The actions of NPY are mediated by a family of five G-protein coupled receptors (Y1, Y2, Y4, Y5, Y6), with the Y1 and Y2 receptors being the most abundant in the central nervous system [96]. NPY has been implicated in a diverse set of behavioral functions, including regulation of food intake [97,98], circadian rhythms [99], and seizure activity [100,101]. NPY is also significantly involved in the expression and regulation of emotional behavior [102,103]. Specifically, NPY is thought to act as an endogenous anxiolytic in the amygdala, counteracting the behavioral stress responses mediated by CRF and protecting the brain from the negative effects of chronic stress via the opposing actions of NPY and CRF on the BLA output cells [104-106]. NPY is also heavily implicated in the neuronal mechanisms that mediate the behavioral effects of alcohol and the anxiety associated with discontinuation of alcohol consumption [107-109], making NPY a likely point of convergence in the neurobiological mechanisms that underlie both anxiety responses and AUDs.

Several studies have demonstrated the anxiolytic-like effects of NPY in rodent models. On the EPM, central infusion of NPY, either into the ventricles or directly into the amygdala, increased open arm time and open arm entries [110,111]. Similarly, virally-mediated over expression of NPY in the amygdala decreased anxiety-like behaviors in the EPM [106]. Selective modulation of the putative postsynaptic NPY receptors further confirms the anxiolytic actions of this neuropeptide. Central infusion of selective agonists for the Y1 and Y5 receptors resulted in increased open arm time and open arm entries in the EPM [111], and amygdalar infusion of a Y1 selective antagonist decreased these same open arm measures [106]. With such strong evidence for the anxiolytic properties of NPY, it is surprising that congenital differences in NPY expression are not consistently predictive of innate anxiety in rodent models. In mice, NPY ablation increases anxiety-like behaviors in a number of behavioral tests of anxiety as compared to wild type mice, but transgenic over expression of NPY does not affect these behaviors, at least on the EPM [112-114]. Although central NPY levels have not yet been reported in rat lines selectively bred for high and low anxiety-like behavior, NPY expression has been assessed in some of the selected alcohol-preferring and non-preferring lines. In the P/NP rats, NPY expression in the CeA is inversely related to anxiety phenotype, with P rats having lower NPY levels and greater anxiety-like behavior as compared to NP rats [46,115]. In contrast, the HAD/LAD rats show the opposite relationship, as HAD rats have lower NPY levels in the CeA, but lower anxiety-like behavior, as compared to LAD rats [116]. As NPY expression levels do not correlate with anxiety phenotype among these lines, it has been posited that these behavioral variations might be the result of differential effects of amygdalar Y1 versus Y2 receptors in anxiety-related responses. Infusion of a selective antagonist for the presynaptic Y2 receptor or Y2 receptor knockdown has anxiolytic effects, similar to the Y1 and Y5 receptor agonists, suggesting that NPY may have both anxiolytic and anxiogenic effects via different receptor subtypes in the amygdala and indicating that NPY-induced signaling cascades exert complex control over anxiety-related responses. [111,117].

The relationship between alcohol consumption and NPY expression appears to be more consistent, with alcohol intake and preference inversely related to NPY levels. Both P rats and HAD rats have lower NPY levels in the CeA as compared to their respective non-preferring lines (NP and LAD rats) [46,115,116]. These results are consistent with evidence that NPY expression is also inversely related to alcohol consumption in transgenic mouse models. Mice lacking NPY drink

more alcohol than wild-type littermates, while transgenic mice that over express NPY consume less alcohol [114]. This difference was also evident in comparisons of amygdalar NPY expression in high alcohol consuming C57/BL6 mice and low alcohol consuming DBA/2J mice [118]. Amygdalar expression of NPY is not different between the AA and ANA lines, but alcohol-preferring AA rats do have lower NPY expression in the hippocampus, another brain region known to mediate both emotional and alcohol-related behaviors, than non-preferring ANA rats [119].

While these data indicate that higher NPY levels are generally associated with reduced alcohol consumption, studies examining NPY-induced changes in drinking behaviors prove that the relationship between NPY and alcohol consumption is more complex. Central NPY infusion decreases alcohol consumption, but only under certain conditions. Thorsell et al. [120,121] showed that NPY reduced alcohol intake in animals that had been chronically exposed to alcohol vapor, but not alcohol naïve animals. In another study, amygdalar NPY administration suppresses alcohol self-administration in dependent animals but not in non-dependent animals [122]. More specifically, NPY reduces alcohol intake in animals that have gone through multiple withdrawals and it has been proposed that NPY effects this change by opposing the anxiogenic effects of abstinence in these animals [122]. That anxiety may be an important facet of NPY's effects on drinking behavior is borne out by evidence that over expression of NPY in the amygdala reduced alcohol consumption in animals with high innate anxiety [66].

Although little is known about the effects of NPY administration on behavior in humans, some clinical evidence suggests that genetic variation in NPY expression may underlie differences in both innate anxiety and susceptibility to AUDs. As in the macaques subjected to maternal separation stress [44], genetic variation in human NPY expression has been linked to differences in stress and emotional responses. Haplotype-driven NPY expression was found to predict responses to emotional and stress stimuli in humans, with lower NPY expression predicting higher trait anxiety and contributing to greater amygdala responses to emotional challenges using functional magnetic resonance imaging (fMRI) [123]. A single nucleotide polymorphism (SNP) in the promoter region of the NPY gene accounted for more than half of the variation in NPY expression seen in this study, indicating that small variations in genetically driven NPY expression may contribute significantly to differences in susceptibility to high innate anxiety. The report by Zhou et al. [123] also showed differences between NPY expression in a group of alcoholic patients compared to controls, supporting the notion that genetically determined differences in NPY expression may represent one of the contributing factors in alcohol use and abuse. In fact, several different SNPs in the NPY gene have also been linked to increased risk for alcoholism. The Leu7Pro SNP has been reported to predict an increased risk for alcohol dependence among Americans of European descent [124], although no significant difference in genotype frequencies were found between alcoholics and non-alcoholics in Finland and Sweden [125] or in Germany [126]. In Mediterranean populations, a 1258G >A NPY SNP is associated with increased alcohol consumption [127]. Variation in the NPY Y2 receptor gene has also been shown to associate with alcohol dependence in Americans of European descent [128]. Clearly, further work is needed to elucidate the contribution of these genetic variations on anxiety, alcohol consumption and the development of AUDs.

## Potential Mechanisms

Much of the evidence connecting anxiety responses and AUDs is correlational in nature and little is currently known about the specific molecular mechanisms by which a preexisting anxiety disorder might confer increased risk for alcohol abuse or dependence. As indicated above, variations in NPY expression due to SNPs in the NPY gene itself may predispose some individuals to developing anxiety disorders and AUDs. Genetic variations in the expression of cAMP-responsive binding element protein (CREB), the transcription factor that regulates NPY expression, have also been implicated in increased risk for elevated anxiety and increased alcohol consumption [129,130]. P rats innately express lower levels of CREB and have less of the active, phosphorylated form of CREB in the central amygdala than NP rats and these data suggest that the increased alcohol consumption and elevated anxiety phenotype seen in P rats may arise from reduced CREB activity [46,131]. This hypothesis is supported by the fact that doses of alcohol that reduce alcohol consumption and anxiety-like behavior in P rats also increase CREB expression to the levels seen in NP rats [46]. Further support for CREB as a mediator of both anxiety- and alcohol-related behaviors comes from studies in transgenic mice. Mutations that reduce CREB expression increase both anxiety-like behavior and alcohol consumption [132,133]. Modulation of CREB activity further upstream in the signaling cascade produces analogous results. CREB is activated by a kinase cascade including Protein Kinase A (PKA) and Ca<sup>2+</sup> calmodulin-dependent protein kinase (CaMK). In P rats, infusion of a PKA activator into the CeA increased CREB phosphorylation and NPY mRNA expression, while decreasing anxiety-like behavior alcohol consumption [46]. These results indicate that a deficiency in this signaling cascade in the CeA may be involved in anxiety- and alcohol related-behaviors. Interestingly, both NPY and alcohol have been shown to modulate this cascade. Acting through Y1 receptors, which couple to cAMP, NPY can activate this signaling cascade. Infusion of anxiolytic doses of NPY into the CeA increased levels of CaMK and phosphorylated CREB (pCREB), as well as mRNA and protein levels of NPY [134], suggesting that this cascade might serve as an important feed forward loop that might be disrupted in individuals with anxiety disorders. Alcohol consumption also increased pCREB and NPY levels [134], and this effect may be important for the anxiolytic actions of alcohol.

## Conclusions

A variety of experimental approaches indicate that there is a relationship between trait anxiety and alcohol consumption, although the causative contribution of pre-existing anxiety to alcohol consumption remains unclear. Current evidence demonstrates a critical role for the amygdala, particularly amygdalar NPY systems, in mediating both anxiety-related responses and the acute effects of alcohol and suggests that variations in NPY signaling in this region may be of particular importance in determining how trait anxiety influences alcohol consumption patterns. Although the exact nature of how genetic and alcohol-induced changes in the amygdalar NPY system control anxiety responses and alcohol consumption remains to be elucidated, continued efforts to understand the role of this system in both anxiety and alcohol abuse may provide a novel therapeutic target for treating co-morbid anxiety disorders and AUDs.

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