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...
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-preference (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-prefering 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 > NP | [46,49] |
| Sardinian Alcohol Preferring versus Non-Preferring | sP > sNP | [45,48] |
| High alcohol drinking versus Low alcohol drinking | HAD > LAD | [51,54] |
| Alko Alcohol versus Alko Non-Alcohol | AA > ANA | [50,55] |

**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) | Floripa L < Floripa H | [57] |
| 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 had 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 γ-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 Fos, 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 anxiety. 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-prefering 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, V5, 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-prefering 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/BL6J mice and low alcohol consuming DBA/2J mice [118]. Amygdalar expression of NPY is not different between the AA and ANA lines, but alcohol-prefering AA rats do have lower NPY expression in the hippocampus, another brain region known to mediate both emotional and alcohol-related behaviors, than non-prefering 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 naive 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 amygdalar 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 higher 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-response 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 Ca2+-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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References

1. American Psychiatric Association (2013). Diagnostic and statistical manual of mental disorders (DSM V) Washington DC, American Psychiatric Association [5th edn].

2. Hasin DS, Stinson FS, Ogburn E, Grant BF (2007) Prevalence, correlates, disability, and comorbidity of DSM-IV alcohol abuse and dependence in the United States: results from the National Epidemiologic Survey on Alcohol and Related Conditions. Arch Gen Psychiatry 64: 830-842.

3. Volpicelli JR (2001) Alcohol abuse and alcoholism: an overview. J Clin Psychiartry 62 Suppl 20: 4-10.

4. Doremus TL, Brunell SC, Rajendran P, Spear LP (2005) Factors influencing elevated ethanol consumption in adolescent relative to adult rats. Alcohol Clin Exp Res 29: 1796-1808.

5. Eckardt MJ, File SE, Gessa GL, Grant KA, Guerri C, et al. (1998) Effects of moderate alcohol consumption on the central nervous system. Alcohol Clin Exp Res 22: 988-1040.

6. Hefner K, Holmes A (2007) An investigation of the behavioral actions of ethanol across adolescence in mice. Psychopharmacology (Beri) 191: 311-322.

7. Kuntsche E, Müller S (2012) Why do young people start drinking? Motives for first-time alcohol consumption and links to risky drinking in early adolescence. Eur Addict Res 18: 34-39.

8. Norberg MM, Norton AR, Olivier J, Zvolensky MJ (2010) Social anxiety, reasons for drinking, and college students. Behav Ther 41: 555-566.

9. Kushner M.G., Abrams K., Borchardt C. (2000) The relationship between anxiety disorders and alcohol use disorders: a review of major perspectives and findings. Clin.Psychol.Rev 20: 149-171.

10. Kushner MG, Wall MM, Krueger RF, Sherr KJ, Maurer E, et al. (2012) Alcohol dependence is related to overall internalizing psychopathology load rather than to particular internalizing disorders: evidence from a national sample. Alcohol Clin Exp Res 36: 325-331.

11. Svendsen J, Conway KP, Degenhardt L, Giantz M, Jin R, et al. (2010) Mental disorders as risk factors for substance use, abuse and dependence: results from the 10-year follow-up of the National Comorbidity Survey. Addiction 105: 1117-1128.

12. Kliehemnes CL (2005) Anxiety-like behaviors following chronic ethanol exposure. Neurosci Biobehav Rev 28: 837-850.

13. Valdez G.R., Roberts A.J., Chan K, Davis H., Brennan M et al. (2002) Increased ethanol self-administration and anxiety-like behavior during acute ethanol withdrawal and protracted abstinence: regulation by corticotropin-releasing factor. Alcohol Clin Exp Res 26: 1494-1501.

14. Zhang Z, Morse AC, Koob GF, Schulteis G (2007) Dose- and time-dependent expression of anxiety-like behavior in the elevated plus-maze during withdrawal from acute and repeated intermittent ethanol intoxication in rats. Alcohol Clin Exp Res 31: 1811-1819.

15. Heilig M, Egl M, Crabbe JC, Becker HC (2010) Acute withdrawal, protracted abstinence and negative affect in alcoholism: are they linked? Addict Biol 15: 169-184.

16. Goodwin RD, Stein DJ (2013) Anxiety disorders and drug dependence: evidence on sequence and specificity among adults. Psychiatry Clin Neurosci 67: 167-173.

17. Marquenie LA, Schade á, van Balkom AJ, Comijs HC, de Graaf R, et al. (2007) Origin of the comorbidity of anxiety disorders and alcohol dependence: findings of a general population study. Eur Addict Res 13: 39-49.

18. Merkangas K.R., Mehta R.L., Molnar B.E., Walters E.E., Swendsen J.D. et al. (1998) Comorbidity of substance use disorders with mood and anxiety disorders: results of the International Consortium in Psychiatric Epidemiology. Addict.Behav. 23: 893-907.

19. Cappell TL, Herman CP (1972) Alcohol and tension reduction. A review. J Q Stud Alcohol 33: 33-64.

20. CONGER JJ (1956) Alcoholism: theory, problem and challenge. II. Reinforcement theory and the dynamics of alcoholism. Q J Stud Alcohol 17: 296-305.

21. Koob GF (2003) Alcoholism: allostasis and beyond. Alcohol Clin Exp Res 27: 232-243.

22. Schuckit MA, Hesselbrock V (1994) Alcohol dependence and anxiety disorders: what is the relationship? Am J Psychiatry 151: 1723-1734.

23. Cooney NL, Litt MD, Morse PA, Bauer LO, Gauppi L (1997) Alcohol cue reactivity, negative-mood reactivity, and relapse in treated alcoholic men. J Abnorm Psychol 106: 243-250.

24. de Wit H, Søderpalm AH, Nikolayev L, Young E (2003) Effects of acute social stress on alcohol consumption in healthy subjects. Alcohol Clin Exp Res 27: 1270-1277.

25. Mason BJ, Light JM, Escher T, Droses DJ (2008) Effect of positive and negative affective stimuli and beverage cues on measures of craving in non-treatment-seeking alcoholics. Psychopharmacology (Berl) 200: 141-150.

26. Pohorecky LA (1991) Stress and alcohol interaction: an update of human research. Alcohol Clin Exp Res 15: 438-459.

27. Søderpalm AH, de Wit H (2002) Effects of stress and alcohol on subjective state in humans. Alcohol Clin Exp Res 26: 819-826.

28. Bertoglio L.J, Carabrese AP (2002) Anxiolytic effects of ethanol and phenobarbital are abolished in test-experienced rats submitted to the elevated plus maze. Pharmacol Biochem Behav 73: 963-969.

29. Criswell HE, Knapp DJ, Overstreet DH, Breese GR (1994) Effects of ethanol, chlordiazepoxide, and MK-801 on performance in the elevated-plus maze and on locomotor activity. Alcohol Clin Exp Res 18: 596-601.

30. Wilson MA, Burghardt PR, Ford KA, Wilkinson MB, Primeaux SD (2004) Anxiolytic effects of diazepam and ethanol in two behavioral models: comparison of males and females. Pharmacol Biochem Behav 78: 445-456.

31. Correa M, Manrique HM, Font L, Esrig MA, Aragon CM (2008) Reduction in the anxiolytic-effects of ethanol by centrally formed acetaldehyde: the role of catalase inhibitors and acetaldehyde-sequestering agents. Psychopharmacology (Berl) 200: 455-464.

32. Sharko A. C., Kaigler K. F., Fadel J., Wilson M. (2013) A Individual differences in basal anxiety-like behavior and ethanol-induced anxiety.

33. Gallate JE, Morley KC, Ambermoon P, McGregor IS (2003) The consequences of beer consumption in rats: acute anxiolytic and ataxic effects and withdrawal-induced anxiety. Psychopharmacology (Berl) 166: 51-60.

34. Sharko AC, Kaigler KF, Fadel JR, Wilson MA (2013) Individual differences in voluntary ethanol consumption lead to differential activation of the central amygdala in rats: relationship to the anxiolytic and stimulant effects of low dose ethanol. Alcohol Clin Exp Res 37 Suppl 1: E172-180.

35. Carrigan MH, Randall CL (2003) Self-medication in social phobia: a review of the alcohol literature. Addict Behav 28: 269-284.

36. Connord PJ, Phil RJ, Vassileva J (1998) Differential sensitivity to alcohol reinforcement in groups of men at risk for distinct alcoholism subtypes. Alcohol Clin Exp Res 22: 585-597.

37. DeMartini KS, Carey KB (2011) The role of anxiety sensitivity and drinking motives in predicting alcohol use: a critical review. Clin Psychol Rev 31: 169-177.

38. Becker HC, Lopez MF, Doremus-Fitzwater TL (2011) Effects of stress on alcohol drinking: a review of animal studies. Psychopharmacology (Berl) 218: 131-156.

39. Enoch MA (2011) The role of early life stress as a predictor for alcohol and drug dependence. Psychopharmacology (Berl) 214: 17-31.

40. Heim C, Nemeroff CB (2001) The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. Biol Psychiatry 49: 1023-1039.

41. Huot RL, Thrivikraman KV, Meaney MJ, Pflotsky PM (2001) Development of adult ethanol preference and anxiety as a consequence of neonatal maternal separation in Long Evans rats and reversal with antidepresant treatment. Psychopharmacology (Berl) 158: 366-373.

42. Ploj K, Roman E, Nylander I (2003) Long-term effects of maternal separation on ethanol intake and brain opioid and dopamine receptors in male Wistar rats. Neuroscience 121: 787-799.

43. Daoura L, Haaker J, Nylander I (2011) Early environmental factors differentially affect voluntary ethanol consumption in adolescent and adult male rats. Alcohol Clin Exp Res 35: 506-515.

44. Lindell SG, Schwant MD, Sun H, Sparenborg JD, Björk K, et al. (2010) Functional NPY variation as a factor in stress resilience and alcohol consumption in thses macaques. Arch Gen Psychiatry 67: 423-431.
45. Colombo G, Agabio R, Lobina C, Reali R, Zocchi A, et al. (1995) Sardinian alcohol-prefering rats: a genetic animal model of anxiety. Physiol Behav 57: 1181-1185.

46. Pandey SC, Zhang H, Roy A, Xu T (2005) Deficits in amygdaloid cAMP-responsive element-binding protein signaling play a role in genetic predisposition to anxiety and alcoholism. J Clin Invest 115: 2762-2773.

47. Richter RM, Zorrilla EP, Basso AM, Koob GF, Weiss F (2000) Altered amygdalar CRF release and increased anxiety-like behavior in Sardinian alcohol-prefering rats: a microdialysis and behavioral study. Alcohol Clin Exp Res 24: 1765-1772.

48. Stewart RB, Gatto GJ, Lumeng L, Li TK, Murphy JM (1993) Comparison of alcohol-prefering (P) and nonprefering (NP) rats on tests of anxiety and for the anxiolytic effects of ethanol. Alcohol 10: 1-10.

49. Vignilayskaia IV, Overstreet DH, Kashevskaya OP, Badishlov BA, Kampov-Polevoy AB, et al. (1995) To drink or not to drink: tests of anxiety and immunity in alcohol-prefering and alcohol-nonpreferring rat strains. Physiol Behav 57: 937-941.

50. Badia-Elder NE, Stewart RB, Powrozek TA, Murphy JM, Li TK (2003) Effects of neuropeptide Y on sucrose and ethanol intake and on anxiety-like behavior in high alcohol drinking (HAD) and low alcohol drinking (LAD) rats. Alcohol Clin Exp Res 27: 894-899.

51. Möller C, Winklnd L, Thorsell A, Hyltén P, Heilig M (1997) Decreased measures of experimental anxiety in rats bred for high alcohol preference. Alcohol Clin Exp Res 21: 566-660.

52. Overstreet DH, Halikas JA, Seredenin SB, Kampov-Polevoy AB, Vignilayskaia IV, et al. (1997) Behavioral similarities and differences among alcohol-prefering and nonpreferring rats: confirmation by factor analysis and extension to additional groups. Alcohol Clin Exp Res 21: 840-848.

53. Roman E, Stewart WB, Belthorpe ML, Jensen ML, Colombo G, et al. (2012) Behavioral profiling of multiple pairs of rats selectively bred for high and low alcohol intake using the MCSF test. Addict Biol 17: 33-46.

54. Tuominen K, Hilakivi LA, Päivärinta P, Korpi ER (1990) Behavior of alcohol-preferring rats: gradual versus abrupt ethanol presentation. Physiol Behav 48: 1181-1185.

55. Henniger MS, Spanagel R, Wigger A, Landgraf R, Höller SM (2002) Alcohol self-administration in two rat lines selectively bred for extremes in anxiety-related behavior. Neuropsychopharmacology 26: 729-736.

56. Da Silva GE, Ramos A, Takahashi RN (2004) Comparison of voluntary ethanol intake by two pairs of rat lines used as genetic models of anxiety. Braz J Med Biol Res 37: 1511-1517.

57. Izidio GS, Ramos A (2007) Positive association between ethanol consumption and anxiety-related behaviors in two selected rat lines. Alcohol 41: 517-524.

58. Manzo L, Gómez MJ, Callejas-Aguilera JE, Fernández-Teruel A, Papini MR, et al. (2012) Oral ethanol self-administration in inbred Roman high- and low-alcohol avoidance rats: gradual versus abrupt ethanol presentation. Physiol Behav 108: 1-5.

59. Singewald N, Hord C, Rapoport SI, Waters D, Doenges RL (2005) Ethanol intake using the MCSF test. Addict Biol 17: 33-46.

60. Da Silva GE, Vendruscolo LF, Takahashi RN (2005) Effects of ethanol on locomotor and anxiety-like behaviors and the acquisition of ethanol intake in Lewis and spontaneously hypertensive rats. Life Sci 77: 693-706.

61. Hayton SJ, Mahoney MK, Olmstead MC (2012) Behavioral traits predicting alcohol drinking in outbred rats: an investigation of anxiety, novelty seeking, and cognitive flexibility. Alcohol Clin Exp Res 36: 594-603.

62. McCool BA, Chappel AM (2007) Strychnine and taurine modulation of amygdala-associated anxiety-like behavior is ‘state’ dependent. Behav Brain Res 178: 70-81.

63. Primeaux SD, Wilson SP, Bray GA, York DA, Wilson MA (2006) Overexpression of neuropeptide Y in the central nucleus of the amygdala decreases ethanol self-administration in “anxious” rats. Alcohol Clin Exp Res 30: 791-801.

64. White L C, Ford K A, Fadel J R, Wilson M (2009) A individual variation in anxiety and ethanol self-administration: a phenotypic analysis of limbic system neuronal activation. International Behavioral Neuroscience Society Meeting, Nassau, Bahamas.

65. Bahl A (2013) Individual differences in elevated plus-maze exploration predicted higher ethanol consumption and preference in outbred mice. Pharmacol Biochem Behav 105: 83-88.

66. Spanagel R, Montkowski A, Allingham K, Stöhr T, Shoaib M, et al. (1995) Anxiety: a potential predictor of vulnerability to the initiation of ethanol self-administration in rats. Psychopharmacology (Berl) 122: 369-373.

67. Rhodes JS, Best K, Kelnap JK, Finn DA, Crabbe JC (2005) Evaluation of a simple model of ethanol drinking to intoxication in C57BL/6J mice. Physiol Behav 84: 53-63.

68. Phelps EA, LeDoux JE (2005) Contributions of the amygdala to emotion processing: from animal models to human behavior. Neuron 48: 175-187.

69. LeDoux J (2007) The amygdala. Curr Biol 17: R686-R74.

70. McDonald AJ (2003) Is there an amygdala and how far does it extend? An anatomical perspective. Ann N Y Acad Sci 985: 1-21.

71. Roberto M, Madamba SG, Moore SD, Tallent MK, Siggins GR (2003) Ethanol increases GABAergic transmission at both pre- and postsynaptic sites in rat central amygdala neurons. Proc Natl Acad Sci U S A 100: 2053-2058.

72. Lock AK, Arwoodola OJ, Chappell AM, Weins LI, McCool BA (2008) Ethanol inhibition of kainate receptor-mediated excitatory neurotransmission in the rat basolateral nucleus of the amygdala. Neuropharmacology 55: 661-668.

73. Roberto M, Schweitzer P, Madamba SG, Stouffer DG, Parsons LH, et al. (2004) Acute and chronic ethanol alter glutamatergic transmission in rat central amygdala: an in vitro and in vivo analysis. J.Neurosci. 24:1594-1603.

74. Kash TL, Matthews RT, Winder DG (2008) Alcohol inhibits NR2B-containing NMDA receptors in the ventral bed nucleus of the stria terminals. Neuropharmacology 33: 1379-1390.

75. Gilpin NW (2012) Corticotropin-releasing factor (CRF) and neuropeptide Y (NPY): effects on inhibitory transmission in central amygdala, and anxiety- and alcohol-related behaviors. Alcohol 46: 329-337.

76. Curran T, Morgan JI (1995) Fos: an immediate-early transcription factor in neurons. J Neurobiol 26: 403-412.

77. Criado JR, Morales M (2000) Acute ethanol induction of c-Fos immunoreactivity in pre-pro-enkephalin expressing neurons of the central nucleus of the amygdala. Brain Res 861: 173-177.

78. Hansson AC, Rimondini R, Neznanova O, Sommer WH, Heilig M (2008) Neuroplasticity in brain reward circuitry following a history of ethanol dependence: from animal models to human behavior. Neuron 48: 175-187.

79. Demarest K, Patel N.A., Romero A.A. (1995) Activation and desensitization of Fos immunoreactivity in the extended amygdala and hypothalamus of the rat brain: focus on cholinergic interneurons of the nucleus accumbens. Alcohol Clin Exp Res 28: 588-597.

80. Morales M, Criado J.R., Sanna P.P., Henrikson S.J., Bloom F.E. (1998) Acute ethanol induces c-fos immunoreactivity in GABAergic neurons of the central nucleus of the amygdala. Brain Res. 798: 333-336.

81. Bachtell R.K., Wang Y.M., Freeman P., Risinger F.O., Ryabinin A.E. (1999) Alcohol drinking produces brain region-selective changes in expression of inducible transcription factors. Brain Res. 847: 157-165.

82. Chang S.L., Patel N.A., Romero A.A. (1995) Activation and desensitization of Fos immunoreactivity in the rat brain following ethanol administration. Brain Res.679: 89-98.

83. Thiele TE, van Dijk G, Bernstein IL (1997) Ethanol-induced c-Fos expression in rat lines selected for low and high alcohol consumption. Brain Res 756: 278-282.

84. Demarest K, Hitzemann B, Mahjubi E, McCaughran J Jr, Hitzemman R (1998) Further evidence that the central nucleus of the amygdala is associated with the ethanol-induced locomotor response. Alcohol Clin Exp Res 22: 1531-1537.

85. McBride WJ (2002) Central nucleus of the amygdala and the effects of alcohol and alcohol-drinking behavior in rodents. Pharmacol Biochem Behav 71: 509-515.
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89. Singewald N, Saltchner P, Sharp T (2003) Induction of c-Fos expression in specific areas of the fear circuitry in rat forebrain by anxiogenic drugs. Biol Psychiatry 53: 275-283.

90. Thompson BL, Rosen JB (2006) Immediate-early gene expression in the central nucleus of the amygdala is not specific for anxiolytic or anxiogenic drugs. Neuropharmacology 50: 57-68.

91. Butler RK, White LC, Frederick-Duus D, Kaigler KR, Fadel JR, et al. (2012) Comparison of the activation of somatostatin- and neuropeptide Y-containing neuronal populations of the rat amygdala following two different anxiogenic stressors, Exp Neurol 236: 52-63.

92. Möller C, Bing O, Heilig M (1994) c-Fos expression in the amygdala: in vivo antisense modulation and role in anxiety. Cell Mol Neurobiol 14: 415-423.

93. Porter K, Hayward LF (2011) Stress-induced changes in c-Fos and corticotropin releasing hormone immunoreactivity in the amygdala of the spontaneously hypertensive rat. Behav Brain Res 216: 543-551.

94. Sterrenburg L, Gassner B, Boerrigter J, Santbergen L, Bramini M (2012) Sex-dependent and differential responses to acute restraint stress of corticotropin-releasing factor-producing neurons in the rat paraventricular nucleus, central amygdala, and ventral area of the stria terminalis. J Neurosci Res. 90: 179-192.

95. Heilig M, Widerlov E (1990) Neuropeptide Y: an overview of central distribution, functional aspects, and possible involvement in neuropsychiatric illnesses. Acta Psychiatr Scand 82: 95-114.

96. Kask A, Harro J, von Hörsten S, Redrobe JP, Dumont Y, et al. (2002) The neurocircuitry and receptor subtypes mediating anxiolytic-like effects of neuropeptide Y. Neurosci Biobehav Rev 26: 259-283.

97. Clark JT, Kalra PS, Kalra SP (1985) Neuropeptide Y stimulates feeding but inhibits sexual behavior in rats. Endocrinology 117: 2435-2442.

98. Politi C, Cicciocoppo R, Regoli D, Massi M (2000) Neuropeptide Y receptor(s) mediating feeding in the rat: characterization with antagonists. Peptides 21: 29-35.

99. Dyzma M, Boudjeltia KZ, Faraut B, Kerkhofs M (2010) Neuropeptide Y and sleep. Sleep Med Rev 14: 161-165.

100. Babab SC, Hollopetter G, Erickson JC, Schwartzkoeln PA, Palmiter RD (1997) Knock-out mice reveal a critical antiepileptic role for neuropeptide Y. J Neurosci 17: 80-85.

101. Mathé AA, Husum H, El Khoury A, Jiménez-Vasquez P, Gruber SH, et al. (2007) Search for biological correlates of depression and mechanisms of action of antidepressant treatment modalities. Do neuropeptides play a role? Physiol Behav 92: 226-231.

102. Giesbrecht C.J., Mackay J.P., Silveira H.B., Urban J.H., Colmers W.F. (2010) A functional neuropeptide Y Leu7Pro polymorphism associated with alcohol dependence in a large population sample from the United States. Arch Gen Psychiatry 67: 133-140.

103. Zhou Z, Zhu G, Hani AR, Enno MA, Scott D, et al. (2008) Genetic variation in human NPY expression affects stress response and emotion. Nature 452: 997-1001.

104. Lappalainen J, Kranzler HR, Malison R, Price LH, Van Dyck C, et al. (2005) A functional neuropeptide Y Leu7Pro polymorphism associated with alcohol dependence in a large population sample from the United States. Arch Gen Psychiatry 62: 113-120.

105. Zhu G, Pollak L, Mottagui-Tabriz S, Walsehledt C, Taubman J, et al. (2003) NPY Leu7Pro and alcohol dependence in Finnish and Swedish populations. Alcohol Clin Exp Res 27: 19-24.

106. Zill P, Preuss UW, Koller G, Bondy B, Soyka M (2008) Analysis of single nucleotide polymorphisms and haplotypes in the neuropeptide Y gene: no evidence for association with alcoholism in a German population sample. Alcohol Clin Exp Res 32: 430-434.

107. François F, Guille M, Verdu F, Portolés O, Casteló A, et al. (2011) The 1258 G>A polymorphism in the neuropeptide Y gene is associated with greater alcohol consumption in a Mediterranean population. Alcohol 45: 131-136.

108. Wetherill L, Schuckit MA, Hessellbrock V, Xuei X, Liang T, et al. (2008) Neuropeptide Y receptor genes are associated with alcohol dependence, alcohol withdrawal phenotypes, and cocaine dependence. Alcohol Clin Exp Res 32: 2031-2040.

109. Pande SC (2003) Anxiety and alcohol abuse disorders: a common role for CREB and its target, the neuropeptide Y gene. Trends Pharmacol Sci 24: 456-460.

110. Wond G (2005) The anxious amygdala: CREB signaling and predisposition to anxiety and alcoholism. J Clin Invest 115: 2697-2699.
131. Pandey SC, Mittal N, Lumeng L, Li TK (1999) Involvement of the cyclic AMP-responsive element binding protein gene transcription factor in genetic preference for alcohol drinking behavior. Alcohol Clin Exp Res 23: 1425-1434.

132. Graves L, Dalvi A, Lucki I, Blendy JA, Abel T (2002) Behavioral analysis of CREB alphadelta mutation on a B6/129 F1 hybrid background. Hippocampus 12: 18-26.

133. Pandey SC, Roy A, Zhang H, Xu T (2004) Partial deletion of the cAMP response element-binding protein gene promotes alcohol-drinking behaviors. J Neurosci 24: 5022-5030.

134. Zhang H, Sakharkar AJ, Shi G, Ugale R, Prakash A, et al. (2010) Neuropeptide Y signaling in the central nucleus of amygdala regulates alcohol-drinking and anxiety-like behaviors of alcohol-preferring rats. Alcohol Clin Exp Res 34: 451-461.