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ROLE OF NEUROPEPTIDE TYROSINE (NPY) IN ETHANOL ADDICTION

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*Here, an overview of neurophysiological, pharmacological and genetic research on the role of neuropeptide tyrosine (NPY) in ethanol consumption and withdrawal is presented. NPY is abundantly expressed in the extended amygdala and is critically involved in the regulation of negative affective states in rats, also is involved with neurobiological responses to ethanol and other drug of abuse. Genetic, molecular and pharmacological evidences suggest that NPY is an important neurobiological substrate for the predisposition to alcoholism. Administration, as well as the withdrawal of ethanol, alters central NPY expression. Alcohol-preferring rats exhibit basal NPY deficits in central amygdala. In the latter, NPY may rescue dependence-induced increases in anxiety and alcohol drinking. Low NPY levels in some brain regions following ethanol withdrawal contribute to the increased sensitivity to seizure and the heightened levels of anxiety characteristic of withdrawal responses. Mice with deletion of NPY gene exhibit a high-anxiety, high-alcohol-drinking phenotype. Pharmacological and genetic manipulations suggest that central NPY signaling modulates ethanol consumption via Y1, Y2, and Y5 receptors. Analysis of chromosomal regions (QTLs) associated with alcohol consumption identified NPY as one of the genes that influence alcohol dependence and as a promising target for pharmacotherapeutics to combat alcohol associated disorders. Consequently, NPY is a potentially new pharmacological target for the treatment of alcohol diseases. **Biomed Rev 2016; 27: 27-39***

Key words: NPY, alcohol consumption, alcohol dependence, alcohol pharmacogenetics, alcohol neuropharmacology, amygdala

Abbreviations used

AA: Alko, Alcohol
BLA: basolateral amygdala
BNST: bed nucleus of stria terminalis
cAMP: cyclic AMP
CeA: central nucleus of the amygdala
CFR: corticotropin releasing factor
eIPSCs: evoked inhibitory postsynaptic currents

GABAARs: GABAA receptors
GABAergic: γ -aminobutyricacidergic
HAD: high alcohol-drinking
HEP: high-ethanol preferring
HPA: hypothalamic-pituitary-adrenal
LAD: low alcohol-drinking
MAPK: mitogen-activated protein kinase

NAc: nucleus accumbens
NP: Alcohol-Nonpreferring
NPY: neuropeptide tyrosine
P: Alcohol-Preferring
QTL: Quantitative Trait Locus
Y1Rs: post-synaptic NPY receptors
Y2Rs: pre-synaptic NPY receptors

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INTRODUCTION

Neuropeptide tyrosine (NPY) is a 36-amino acid neuropeptide, belonging to the PP-fold family of peptides (1) that results from cleavage of a 97-amino acid precursor, preproNPY. NPY exhibits a high degree of phylogenetic conservation across species (2,3) and is widely expressed in the central nervous system, particularly in the striatum, amygdala, cortex and hypothalamus (4,5). The neuropeptide functions through G protein-coupled receptors to suppress adenylyl cyclase, switch on mitogen-activated protein kinase (MAPK), trigger potassium channels and balance intracellular levels of calcium. At least five NPY receptor subtypes have been identified in mammals (6), of which the best characterized are Y1, Y2, and Y5 receptors (7).

In the brain NPY is produced in a lot of regions and controls several functions as neuronal development (8,9), food intake (10,11), seizure activity (12,13), cardiovascular homeostasis (14), anxiety and stress behaviors (15,16), thermogenesis (17), circadian rhythms (18,19), pain modulation (20), and reproduction (21,22). In recent years, genetic, pharmacological and molecular evidences have emerged suggesting that NPY is also associated with neurobiological responses to ethanol and drugs of abuse (23–25) and is important for the predisposition to alcohol-seeking behaviors (26).

NPY is abundant in the extended amygdala, a conceptual macrostructure in the basal forebrain holding the central nucleus of the amygdala (CeA), lateral division of the bed nucleus of *stria terminalis* (BNST), and shell of *nucleus accumbens* (NAc) (27). These regions of the extended amygdala display similar cytoarchitecture, overlapping afferents from limbic cortices, hippocampus, and basolateral amygdala (BLA). The outputs of the extended amygdala largely project to effector regions, including lateral hypothalamus and various brain stem regions important for physiological and behavioral responses to relevant emotive stimuli (e.g., stressors, alcohol, and drugs) (28,29).

The extended amygdala plays an important role in regulation of negative affective (e.g., anxiety) states (30). The constituent regions of the extended amygdala are densely populated by neuropeptides with pro-stress (e.g., corticotropin releasing factor, CRF) and anti-stress (e.g., NPY and nociceptin) profiles (7).

The CeA is composed mostly of γ -aminobutyric acidergic (GABAergic) projection neurons and interneurons (31), and the BNST is a major target of CeA projection neurons (32). CeA and BNST connections often contain neuropeptide co-transmitters. For example, CeA is a major source of CRF in the BNST (33,34).

EFFECTS OF ETHANOL INTAKE ON NPY EXPRESSION

Long-Evans rats feeding a liquid diet that include 6% ethanol for 12 weeks show significant increases in NPY levels in the arcuate and ventromedial nuclei of the hypothalamus, the median eminence, and the suprachiasmatic nucleus when compared to rats that drank a control diet (35). Similarly, peripheral injection of 1.5 and 3.5 g/kg ethanol causes activation of NPY-containing neurons in the ventrolateral medulla of Long-Evans rats (36). It has been hypothesized that the increment of NPY activity in response to ethanol may serve as a mechanism of protection to limit further ethanol intake (37). However, ethanol administration and withdrawal from ethanol reduce NPY signaling mRNA^{NPY} levels in *arcuate nucleus* of the hypothalamus are reduced in Sprague-Dawley rats after a single peripheral injection of a 1.0 g ethanol/kg dose (38). Twenty-four hours after withdrawal from a diet containing 9% ethanol (after 15 days of exposure), Sprague-Dawley rats show decreased NPY immunoreactivity in the cingulate gyrus, various regions of the cortex, the central and medial nuclei of the amygdala, and the paraventricular and arcuate nuclei of the hypothalamus (39). Wistar rats exposed to ethanol vapor for 14 h/day show no differences in brain NPY expression after 7 weeks of exposure, but do have increased NPY expression in the hypothalamus 7 weeks after withdrawal from ethanol (40). More recently, in Sprague-Dawley rats an increase of NPY immunoreactivity in the hippocampus 72-h after withdrawal from an ethanol diet was observed. The authors argue that increased NPY expression may be protective against seizure activity that develops after ethanol withdrawal (41). Thus, immediately after withdrawal from ethanol, NPY levels are low in some brain regions that control increased seizure activity and heightened levels of anxiety characteristic of ethanol withdrawal (23,42,43). This is followed by an upregulation of NPY in the hippocampus, a possible protective response. The evidences supporting this hypothesis comes from the finding that intracerebroventricular (i.c.v.) infusion of NPY significantly weakens ethanol withdrawal responses in Wistar rats (44). These data indicate that central NPY signaling regulates neurobiological responses to ethanol and ethanol withdrawal (26).

THE IMPACT OF ALCOHOL ON GABAERGIC TRANSMISSION AND NPY

γ -Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain. It acts via two receptor subtypes called GABAA and GABAB. GABA activity in the brain is

increased by alcohol through two general mechanisms: the action on the GABA-releasing presynaptic neurons, that increase GABA release; or the action on the signal-receiving postsynaptic neurons, that facilitate the activity of the GABA_A receptors (GABA_ARs). Alcohol drinking is suppressed by compounds that interfere with the actions of the GABA_ARs antagonists as well as compounds that stimulate GABA_B receptor agonists in the NAs, ventral pallidum, BNST, and amygdala (45). Of these, CeA, a brain region relevant in the control of emotional states, is highly responsive to suppression of alcohol drinking by compounds acting on the GABA systems (46). Indeed, GABA transmission in this brain region increases after acute and chronic alcohol exposure (47,48). GABA_A receptors consist of several proteins subunits. There are several types of GABA_A subunits, and the subunit composition of the receptors varies among different brain regions and remodels in response to environmental changes. Chronic alcohol exposure also leads to alterations in the GABA systems. For instance, in some brain regions, alcohol affects the expression of genes that encode components of the GABA_A receptors, resulting in changes in the composition of the subunit of the receptors, the most consistent of which are decreases in $\alpha 1$ - and increases in $\alpha 4$ -subunits (49). The function of GABA_A receptors also is regulated by neuroactive steroids (50) that are produced both in the brain and in other organs. Alcohol increases the brain levels of many neuroactive steroids (51).

Antagonists of GABA_ARs in CeA lower alcohol self-administration in rats (46). Acute alcohol increases GABAergic synaptic transmission in CeA (47), and this effect is fast, reversible, and with a significant pre-synaptic component. Chronic alcohol exposure facilitates GABA release in the CeA, largely *via* actions at presynaptic GABAergic terminals (48,52). Acute alcohol enhances GABAergic transmission similarly in the CeA of alcohol-naïve and alcohol-dependent rats, suggesting that this brain region is particularly sensitive to the acute effects of alcohol (48). Because antagonists of GABA_A receptors in BNST also reduce alcohol self-administration in rats (46), it is reasonable to hypothesize that the effects of alcohol on inhibitory transmission in BNST may be similar to effects in CeA.

NPY prevents and reverses acute alcohol-induced increases in evoked GABAergic transmission in CeA *via* pre-synaptic effects on GABA release (53). NPY blocks alcohol effects on GABA release *via* activation of pre-synaptic receptors (Y2Rs) in slice electrophysiology recordings from the CeA of rats. NPY alone does not decrease GABAergic transmission in CeA

unless post-synaptic receptors (Y1Rs) are blocked, suggesting that functional Y1Rs in CeA “buffer” the effects of NPY at pre-synaptic Y2Rs. Notably, NPY normalizes GABA release in CeA, increased by alcohol dependence, suggesting that chronic alcohol produces neuroadaptations in NPY system that affect inhibitory transmission in CeA (53). In agreement with these findings, NPY lowers evoked inhibitory postsynaptic currents (eIPSCs) in the CeA of mice, and this effect intensifies in slices from mice with a history of binge alcohol drinking (54). Similar to its effects in CeA, NPY modulates GABA release in BNST (55) *via* activation of pre-synaptic Y2Rs, supporting the notion that Y2Rs function not only as autoreceptors regulating NPY release (56) but also as heteroreceptors modulating the release of other neurotransmitters (57).

CORTICOTROPIN-RELEASING FACTOR AND NPY SYSTEMS

Recent research has led to the hypothesis that the transitions to alcohol dependence dysregulates not only neural circuits of reward but also circuits that are involved in behavioral responses to stressors. Perturbation of the brain stress and antistress systems induced by alcohol contributes to the negative emotional state characteristic of alcohol withdrawal. One stress system involves the signaling molecule CRF. The latter, being produced and released from hypothalamus, activates the body's major stress system that is the hypothalamic-pituitary-adrenal (HPA) axis. However, activation of extrahypothalamic CRF systems also produces high anxiety states in animals. Several observations indicate that the development of alcohol dependence is triggered by extrahypothalamic CRF. For example, alcohol-dependent rats exhibit increased extracellular CRF content in CeA (58). Moreover, the injection of CRF antagonists directly into this brain structure suppress both the anxiety-like behavior (59) and the increase in alcohol drinking (60) associated with alcohol dependence. NPY is also involved in regulating the body's stress response. It has a neural and behavioral profile that in almost every aspect is opposite to that of CRF. NPY has potent anxiety-reducing effects in animals and alcohol-dependent rats exhibit decreased NPY content in CeA during withdrawal (39). In contrast, as stated above, CRF levels in this brain region are increased in alcohol-dependent animals. Furthermore, stimulation of NPY activity in this brain structure suppresses anxiety-like behavior (61) and increased in alcohol drinking induced by dependence (62). The anatomical distributions of CRF and NPY are highly overlapping, suggesting that one might serve as a “buffer” for the effects of the other.

NPY EFFECTS ON ALCOHOL DEPENDENCE-RELATED BEHAVIORS

Alcohol dependence is a disease characterized by a negative emotional state in absence of the drug (63, 64). The transition to alcohol dependence causes a dysregulation in neurotransmission in CeA mediated by neuropeptide systems (65). NPY is highly expressed in CeA and controls negative affective states in rats. Alcohol dependence produces neuroadaptation in amygdala NPY systems. This neuropeptide displays anxiolytic properties when infused into amygdala (16,66) and can rescue the high-anxiety associated with excessive drinking state in alcohol dependence. Studies on rats showed that NPY microinjected into the CeA blocks alcohol consumption in alcohol-dependent rats (67). This effect involves both postsynaptic Y1Rs and presynaptic Y2Rs. In the mouse, studies observed that acute stress and withdrawal increase Y1R expression in the amygdala.

Increases in alcohol self-administration during subsequent withdrawals (53), is a hallmark of the transition to alcohol dependence. This effect is blocked by repeated intraventricular NPY administration during prior alcohol withdrawals. It may be due to NPY reversal of anxiety-like behavior induced by withdrawal, as it has been observed with other anti-anxiety compounds chronically administered in a similar protocol (68). Activation of NPY systems in the CeA suppresses alcohol self-administration in alcohol-dependent rats at doses that do not affect alcohol self-administration in non-dependent rats (61,67). Chronic high-dose alcohol exposure and withdrawals in rats cause deficits in mRNA^{NPY} and protein in CeA and a parallel increase in anxiety-like behavior (39,69). Finally, NPY reverts the alcohol-seeking behavior induced by stress (70, 71), probably through its effects on inhibitory neurotransmission in CeA (53). NPY effects on post dependent alcohol-related behaviors are likely mediated by presynaptic Y2Rs. Indeed intraventricular administration of a Y2R antagonist (BIIE0246) reduces alcohol consumption by rats (71) and mice (54), and during protracted abstinence, in alcohol-dependent rats, it was observed that the sensitivity to the suppressive effects of BIIE0246 on alcohol drinking increases (72). Systemic administration of an Y2R antagonist (JNJ-31020028), able to cross the blood-brain barrier, dose-dependently reverses the increased anxiety-like behavior in rats during withdrawal from a single bolus injection of alcohol (73). NPY systems in the extended amygdala may constitute a promising target in the search for potential pharmacotherapeutics to combat alcohol use disorders in humans (7).

HOW NPY SIGNALING MODULATES ETHANOL INTAKE: SOME HYPOTHESES

To understand the mechanism by which NPY signaling modulates ethanol consumption, some assumptions have been made. One possibility is that NPY influences ethanol consumption by regulating anxiety. While there are data that supports this hypothesis, there is evidence that NPY can modulate ethanol consumption independent of its effects on anxiety. Anxiety-like behavior (regardless of alcohol history) is affected by NPY release regulated by Y2R, whereas NPY modulation of alcohol-drinking behavior in alcohol-dependent animals occurs *via* Y2R regulation of GABA release. Alcohol dependence produces changes in expression of NPY and its receptors, but the effects of NPY on excessive alcohol drinking by alcohol-dependent (and perhaps alcohol-preferring) animals is mostly mediated by the recruitment of Y2R heteroreceptor function and NPY-GABA interactions in the amygdala. Electrophysiological data have shown that Y2R antagonist (BIIE0246) applied to rat CeA slices increases evoked GABAergic transmission and blocks the ability of NPY to abolish alcohol effects on GABA release (53). Therefore, Y2R antagonists mimic the effects of NPY in the CeA on anxiety-like behavior, but have the opposite result of the effects of NPY on alcohol consumption in alcohol-dependent animals and also in NPY effects on GABA release in CeA. Therefore, there would appear to be a delicate balance between the autoreceptor and heteroreceptor functions of pre-synaptic Y2Rs in CeA, a balance that may differ in non-dependent and alcohol-dependent states, and also across individuals. Another set of predictions that come out from the electrophysiological data is that infusion of a GABAAR antagonist into the CeA should reduce alcohol drinking and that pre-treatment with a GABAAR antagonist bypasses the effect of manipulation of pre-synaptic NPY and CRF receptors on alcohol drinking. The latter of these hypotheses has not been tested, but the infusion of a competitive GABAAR antagonist (SR 95531) into the amygdala, in particular, the CeA, does reduce alcohol self-administration in rats (46). Blocking GABAergic transmission with a drug peripherally administered is not viable as a long-term strategy for treating alcohol dependence in humans. As such, a more promising approach may be to target neuromodulators (e.g., NPY, CRF) of inhibitory transmission that is recruited (e.g., in the extended amygdala) during the transition to alcohol dependence.

Another possibility is that central NPY signaling controls the rewarding properties associated with ethanol. The NAc

is a brain structure in which the highest levels of NPY immunoreactivity has been observed (74). Also, the NAc is a crucial brain region for the modulation of ethanol reward (75). NPY infused directly into the NAc supports conditioned place preference in rats, indicating that NPY signaling in the NAc is rewarding (76,77). Furthermore, pretreatment with a dopamine receptor antagonist prevents NPY from producing conditioned place preference, suggesting that NPY-mediated reward involves an interaction with the dopamine system (77). A recent report found that the high ethanol drinking C57BL/6 strain of mice has a significantly lower expression of NPY in the shell of the NAc relative to the low ethanol drinking DBA/2 strain (78). Thus, low NPY function in the NAc may, in part, drive the high ethanol drinking in C57BL/6 mice (26).

NPY KNOCKOUT AND TRANSGENIC RODENTS

Genetic manipulations demonstrated that central NPY signaling modulates neurobiological responses to ethanol and ethanol withdrawal. The phenotype of mice with deletion of the NPY gene is characterized by excessive alcohol consumption and reduced sensitivity to the sedative effects of high alcohol doses (37). Conversely, transgenic mice that overexpress NPY consume less alcohol than controls and display increased sensitivity to the sedative effects of high alcohol doses (37). Mice with deletion of the Y1R gene are very similar to NPY KO mice because they consume high quantities of alcohol and exhibit reduced sensitivity to the sedative effects of alcohol (79). Mice lacking the Y2R consume normal or lower quantities of alcohol relative to controls (80).

Pharmacological and/or genetic manipulations have implicated the Y1R, Y2R and Y5Rs in the modulation of ethanol intake. In the mouse, NPY acts through at least five receptor subtypes, namely Y1R, Y2R, Y4R, Y5R and Y6Rs, all of which couple to G proteins that inhibit the production of cyclic AMP (cAMP) (81). The Y1R is located post-synaptically and is found in brain regions that are involved with neurobiological responses to ethanol, including the hippocampus, the hypothalamus and the amygdala (82). The Y2R is primarily located presynaptically where it acts as an autoreceptor, inhibiting further release of NPY (83,84). The Y5R is located post-synaptically, primarily in the hypothalamus, and has been hypothesized to be critically involved in the regulation of food intake (85). Voluntary ethanol intake by Y1R knockout mice (Y1R^{-/-}) and by normal wild-type (Y1R^{+/+}) mice was recently examined (79).

Y1R^{-/-} mice display increased consumption of solutions

containing 3%, 6%, and 10% (v/v) ethanol but show normal consumption of sucrose and quinine solutions suggesting that increased consumption of ethanol is not associated with altered taste preference or caloric need. If presynaptic Y2 receptors control voluntary ethanol consumption and sensitivity, the Y2R^{-/-} mice should exhibit ethanol-related phenotypes opposite to those found with the Y1R^{-/-} mice. That is, in the absence of presynaptic inhibition of NPY release in Y2R^{-/-} mice there should be an augment of NPY signaling. The Y2R^{-/-} mice drink significantly less of solutions containing 3% and 6% ethanol and have significantly lower ethanol preference ratios relative to wild-type (Y2R^{+/+}) mice, an effect that is genetic background-dependent. However, the Y2R^{-/-} mice show normal consumption of solutions containing either sucrose or quinine and normal metabolism of ethanol. The observation that mRNA^{Y2R} is reduced in the high ethanol drinking AA (Alko, Alcohol) line of rats is additional evidence for a role of the Y2R in modulating alcohol drinking (86). Recent work indicates that mutant mice lacking Y5R (Y5R^{-/-}) drink normal amounts of 3%, 6%, 10% and 20% (v/v) ethanol (87).

Together, data from NPY receptor knockout mice suggest that voluntary consumption of ethanol is modulated by Y1R and Y2R, but not the Y5R. Pharmacological data strengthen genetic studies and suggest a role for Y1R, Y2R and Y5R. First, direct infusion of femtomolar doses of NPY into the PVN of the hypothalamus increases consumption of ethanol by Long–Evans rats, and this effect is blocked by pretreatment with the Y1R selective antagonist, BIBP 3226 (88). Recently, it was shown that site-directed infusion of BIBP 3226 into the amygdala increases ethanol self-administration by Long–Evans rats (89). Second, i.c.v. infusion of the selective Y2 receptor antagonist, BIIE0246, reduces operant self-administration of ethanol in Wistar rats at doses that do not interfere with normal locomotor activity (71). Finally, a recent report showed that i.p. injection of a selective Y5R antagonist does not alter absolute ethanol self-administration over a 16-h test but increases the latency to initiate operant responding for ethanol by C57BL/6J mice. Thus, also the NPY Y5R may regulate the onset of ethanol self-administration (90).

NPY IN ANIMAL MODELS OF ALCOHOL DEPENDENCE

Several animal models have been proposed to investigate alcohol related diseases (91–96) mechanisms and effects. Indeed, many studies aimed to unravel brain neurotransmitters' changes, including NPY. Some of the best evidence that NPY plays a role in neurobiological responses to ethanol comes

from studies comparing the Indiana Alcohol-Preferring (P) and Alcohol-Nonpreferring (NP) rats.

The P and NP lines were selectively bred from Wistar rats for high and low alcohol consumption, respectively (97). In this model, P rats exhibit several features that are consistent with alcoholism in humans (98). For instance, P rats (a) orally self-administer ethanol in pharmacologically relevant amounts; (b) consume ethanol for its pharmacological effects (not caloric value or taste); (c) show positive reinforcement; (d) develop tolerance; and (e) exhibit withdrawal symptoms (99, 100). To date, a plethora of correlative studies has been conducted to study the behavioral and neurobiological differences between the P and NP lines (100).

P and NP rats show opposite electrophysiological activity in the amygdala following i.c.v. infusion of NPY (101), suggesting that altered NPY signaling in the amygdala of P rats contributes to their high alcohol drinking. It was later discovered that P rats have low levels of NPY in the amygdala, frontal cortex, and hippocampus relative to NP rats, but higher levels of NPY in the hypothalamus and cingulate cortex (40,102). Similarly, the high alcohol-drinking (HAD) rats, bred by the technique used to generate the P rats, also have low levels of NPY in the amygdala when compared with low alcohol drinking (LAD) rats. Unlike the P rats, however, the HAD rats have lower levels of NPY in hypothalamic nuclei (102). Taken together the high alcohol drinking by the P and HAD rats are best explained by the low levels of endogenous NPY in the amygdala observed in each of these lines, i.c.v. infusion of 5.0 or 10.0 lg doses of NPY significantly reduce 2-h voluntary consumption of an 8% ethanol solution in P rats, but do not alter ethanol drinking in NP or outbred Wistar rats (103). Similarly, 5.0 or 10.0 lg doses of NPY reduce ethanol drinking in HAD, but not LAD, rats (104). P rats given i.c.v. infusion of a 10.0-lg dose of NPY show suppressed ethanol drinking for up to 2-days following a 2-week period of abstinence from ethanol. Without ethanol abstinence, a 10.0 lg dose of NPY reduces ethanol drinking for only 24-h. These data demonstrate that centrally infused NPY alters ethanol drinking by P rats for long periods of time, an effect that is potentiated by abstinence (105). The overall pattern of results from pharmacological studies suggests the possibility that central infusion of NPY can reduce ethanol drinking in animal models showing high levels of voluntary ethanol consumption (i.e., the P and HAD rats) but not in animals with low or moderate levels of ethanol consumption (i.e., NP, LAD, and Wistar rats). Genetic linkage analyses (e.g., quantitative trait locus) in P and NP rats have

also suggested a role for NPY.

A quantitative trait locus (QTL) is a section of DNA (the locus) that is associated with a particular phenotypic trait and correlates with variation in a phenotype (the quantitative trait). Usually, the QTL is linked to, or contains, the genes that control that phenotype. QTLs are mapped by identifying which molecular markers (such as SNPs or AFLPs) correlate with an observed trait. This is often an early step in identifying and sequencing the actual genes that cause the trait variation.

Another use of QTLs is to identify candidate genes underlying a trait. Once a region of DNA is identified as contributing to a phenotype, it can be sequenced. The DNA sequence of any genes in this region can then be compared to a database of DNA for genes whose function is already known. Quantitative trait loci analyses identified a region of chromosome 4 that significantly correlates with differences in alcohol drinking between P and NP rats. The NPY precursor gene is located in this chromosomal region (106,107). It is interesting to note that a similar QTL located on chromosome 4 has been correlated with the high ethanol drinking of the high-ethanol preferring (HEP) line of rats (108). This QTL region defines a finite chromosomal interval with a limited number of genes. With current bioinformatics tools, all possible genes underlying a QTL region can now be identified. Combined with other bioinformatics resources, each of these genes can be screened for their potential relevance to the quantitative trait of interest, thus, providing targets for molecular-based research (109). Therefore, the literature and bioinformatics afford a complementary tool that can help demarcate a specific gene of interest from a broad QTL region with a multitude of differentially expressed genes (110). Ultimately, the genes for neuropeptide Y (*Npy*), *Snca* and corticotrophin-releasing factor receptor 2 (*Crhr2*) were prioritized for further characterization using molecular-based research because they: (a) exhibit a close proximity to the peak of the linkage signal; (b) show biological correlates that are relevant to alcohol dependence or an alcohol-related phenotype; and (c) display differences in gene expression (RNA, protein) between the P and NP strains. Molecular-based strategies can be effectively used to screen and target genes that contribute to a QTL. Three candidate genes (*Npy*, *Snca*, *Crhr2*) were initially screened and selected based on their proximity to the peak of the QTL and their gene expression profile. The identification of promising genetic targets (i.e. *Npy*, *Snca*, *Crhr2*) that map to a QTL and show strain-specific molecular and phenotypic correlations can provide a starting point for hypothesis-driven research that

better define the relevance of each candidate gene (*Npy*, *Sncα*, *Crhr2*) to alcohol dependence. In humans, a polymorphism in *Npy* (Leu7Pro) was significantly associated with alcohol dependence in human alcoholics (111,112). It has been well-documented that the P and NP strains exhibit marked differences in anxiety-like behavior with studies suggesting that P rats are more “anxious” than NP rats. Compared with NP rats, P rats: (a) showed greater foot-shock-induced suppression of operant responding in an approach-avoidance conflict test; (b) spent less time in the open arms of an elevated plus maze; and (c) took longer in a passive avoidance test to step down from a platform to a grid floor where foot shock was received 24 hours earlier (113). In addition, both acoustic startle and potentiated startle response were consistently greater in P than NP rats, and only P rats showed significant fear-conditioned startle (114). Therefore, due to NPY’s role in the modulation of behavioral effects of stress, particularly anxiety-like behavior (115), *Npy* may represent an important biologic target for alcohol-seeking behavior, especially in the P and NP model. The identification of the genetic factors that influence addictive behavior can provide important targets for both basic and clinical research. In the P and NP model, QTL mapping (QTL analysis, fine-mapping) and molecular-based research were implemented as complementary approaches to identify and prioritize promising genetic targets for alcohol-seeking behavior. With the recent advancements in molecular biology and bioinformatics, QTL mapping and molecular-based strategies applied to selectively bred and inbred strains provide a multifaceted approach to target high-priority genes that contribute to alcohol dependence. NPY may represent an important biologic target for alcohol-seeking behavior, especially in the P and NP model. The identification of the genetic factors that influence addictive behavior can provide important targets for both basic and clinical research in the P and NP model. The overall objective of this research is to implement a multifaceted approach to target high-priority genes that contribute to alcohol dependence and are eligible for pharmaceutical development of new therapies.

REFERENCES

1. Dumont Y, Martel J-C, Fournier A, St-Pierre S, Quirion R. Neuropeptide Y and neuropeptide Y receptor subtypes in brain and peripheral tissues. *Prog Neurobiol* 1992;38:125-167. [http://dx.doi.org/10.1016/0301-0082\(92\)90038-G](http://dx.doi.org/10.1016/0301-0082(92)90038-G)
2. Allen J, Novotný J, Martin J, Heinrich G. Molecular structure of mammalian neuropeptide Y: analysis by molecular cloning and computer-aided comparison with crystal structure of avian homologue. *Proc Natl Acad Sci USA* 1987;84:2532-2536.
3. Larhammar D, Ericsson A, Persson H. Structure and expression of the rat neuropeptide Y gene. *Proc Natl Acad Sci USA* 1987; 84:2068-2072.
4. Emson P, Quidt MD. NPY - a new member of the pancreatic polypeptide family. *Trends Neurosci* 1984; 7: 31–35. DOI: 10.1016/S0166-2236(84)80271-4
5. Gray TST, Morley JEJ. Neuropeptide Y: anatomical distribution and possible function in mammalian nervous system. *Life Sci* 1986;38:389-401. DOI: 10.1016/0024-3205(86)90061-5
6. Larhammar D, Salaneck E. Molecular evolution of NPY receptor subtypes. *Neuropeptides* 2004; Aug;38(4):141-51. DOI: 10.1016/j.npep.2004.06.002
7. Gilpin NW. Neuropeptide Y (NPY) in the extended amygdala is recruited during the transition to alcohol dependence. *Neuropeptides*. 2012 Dec;46(6):253–9. DOI: 10.1016/j.npep.2012.08.001
8. Hansel D, Eipper B. Regulation of olfactory neurogenesis by amidated neuropeptides. *J Neurosci*. 2001; 66:1-7. DOI: 10.1002/jnr.1191
9. Hansel D, Eipper B, Ronnett G. Neuropeptide Y functions as a neuroproliferative factor. *Nature*, 2001 Apr 19;410(6831):940-4.2001 DOI: 10.1038/35073601
10. Clark J, Kalra P, Crowley W, Kalra S. Neuropeptide Y and human pancreatic polypeptide stimulate feeding behavior in rats. *Endocrinology* 1984 Jul;115(1):427-9. DOI: 10.1210/endo-115-1-427
11. Levine A, Morley J. Neuropeptide Y: a potent inducer of consummatory behavior in rats. *Peptides* 1984; Nov-Dec;5(6):1025-9. [http://dx.doi.org/10.1016/0196-9781\(84\)90165-7](http://dx.doi.org/10.1016/0196-9781(84)90165-7)
12. Woldbye D, Madsen T, Larsen P, Mikkelsen J. Neuropeptide Y inhibits hippocampal seizures and wet dog shakes. *Brain Res*. 1996; Oct 21;737(1-2):162-8. [http://dx.doi.org/10.1016/0006-8993\(96\)00730-5](http://dx.doi.org/10.1016/0006-8993(96)00730-5)
13. Woldbye D, Larsen P, Mikkelsen J, Klemp K. Powerful inhibition of kainic acid seizures by neuropeptide Y via Y5-like receptors. *Nat Med*. 1997; Jul;3(7):761-4. doi:10.1038/nm0797-761
14. Pedrazzini T, Seydoux J, Künstner P, Aubert JF, Grouzmann E, Beermann F, et al. Cardiovascular response, feeding behavior and locomotor activity in mice lacking the NPY Y1 receptor. *Nat Med*. 1998;4(6):722–6. doi:10.1038/nm0698-722

15. Heilig M. Antisense inhibition of neuropeptide Y (NPY)-Y1 receptor expression blocks the anxiolytic-like action of NPY in amygdala and paradoxically increases feeding. *Regul Pept.* 1995;59(2):201–5. [http://dx.doi.org/10.1016/0167-0115\(95\)00103-I](http://dx.doi.org/10.1016/0167-0115(95)00103-I)
16. Heilig M, McLeod S, Brot M, Heinrichs S. Anxiolytic-like action of neuropeptide Y: mediation by Y1 receptors in amygdala, and dissociation from food intake effects. *Neuropsychopharmacology.* 1993 Jun;8(4):357–63. DOI: 10.1038/npp.1993.35
17. Lopez-Valpuesta F, Nyce J, Myers R. NPY-Y1 receptor antisense injected centrally in rats causes hyperthermia and feeding. *Neuroreport.* 1996 Nov 4;7(15-17):2781–4.
18. Biello S, Golombek D, Schak K. Circadian phase shifts to neuropeptide Y in vitro: cellular communication and signal transduction. *J Neurosci.* 1997 Nov 1;17(21):8468–75.
19. Golombek D, Biello S, Rendon R. Neuropeptide Y phase shifts the circadian clock in vitro via a Y2 receptor. *Neuroreport.* 1996 May 17;7(7):1315–9.
20. Shi T-J, Tandrup T, Bergman E, Xu Z-Q, Ulfhake B, Hökfelt T. Effect of peripheral nerve injury on dorsal root ganglion neurons in the C57 BL/6J mouse: marked changes both in cell numbers and neuropeptide expression. *Neuroscience.* 2001;105(1):249–63. [http://dx.doi.org/10.1016/S0306-4522\(01\)00148-8](http://dx.doi.org/10.1016/S0306-4522(01)00148-8)
21. Kalra SP, Xu B, Dube MG, Moldawer LL, Martin D, Kalra PS. Leptin and ciliary neurotrophic factor (CNTF) inhibit fasting-induced suppression of luteinizing hormone release in rats: role of neuropeptide Y. *Neurosci Lett.* 1998;240(1):45–9. [http://dx.doi.org/10.1016/S0304-3940\(97\)00896-3](http://dx.doi.org/10.1016/S0304-3940(97)00896-3)
22. Kasuya E, Mizuno M, Watanabe G, Terasawa E. Effects of an antisense oligodeoxynucleotide for neuropeptide Y mRNA on in vivo luteinizing hormone-releasing hormone release in ovariectomized female rhesus monkeys. *Regul Pept.* 1998;75:319–25. [http://dx.doi.org/10.1016/S0167-0115\(98\)00084-6](http://dx.doi.org/10.1016/S0167-0115(98)00084-6)
23. Pandey SC. Anxiety and alcohol abuse disorders: A common role for CREB and its target, the neuropeptide Y gene. *Trends Pharmacol Sci.* 2003 Sep;24(9):456–60. DOI: 10.1016/S0165-6147(03)00226-8
24. Thiele TE, Badia-Elder NE. A role for neuropeptide Y in alcohol intake control: evidence from human and animal research. *Physiol Behav.* 2003 Jun;79(1):95–101. [http://dx.doi.org/10.1016/S0031-9384\(03\)00109-4](http://dx.doi.org/10.1016/S0031-9384(03)00109-4)
25. Thiele TE, Navarro M, Sparta DR, Fee JR, Knapp DJ, Cubero I. Alcoholism and obesity: overlapping neuropeptide pathways? *Neuropeptides.* 2003 Dec;37(6):321–37. <http://dx.doi.org/10.1016/j.npep.2003.10.002>
26. Thiele TE, Sparta DR, Hayes DM, Fee JR. A role for neuropeptide Y in neurobiological responses to ethanol and drugs of abuse. *Neuropeptides.* 2004 Aug;38(4):235–43. DOI: 10.1016/j.npep.2004.04.005
27. Alheid GF, Heimer L. New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: the striatopallidal, amygdaloid, and corticopetal components of substantia innominata. *Neuroscience.* 1988;27(1):1–39. [http://dx.doi.org/10.1016/0306-4522\(88\)90217-5](http://dx.doi.org/10.1016/0306-4522(88)90217-5)
28. Davis M, Walker DL, Miles L, Grillon C. Phasic vs sustained fear in rats and humans: role of the extended amygdala in fear vs anxiety. *Neuropsychopharmacology.* 2010;35(1):105–35. DOI: 10.1038/npp.2009.109
29. Gilpin NW, Herman MA, Roberto M. The Central Amygdala as an Integrative Hub for Anxiety and Alcohol Use Disorders. *Biol Psychiatry.* 2015 May;77(10):859–69. DOI: 10.1016/j.biopsych.2014.09.008
30. Koob GF. A role for brain stress systems in addiction. *Neuron.* 2008;59(1):11–34. DOI: 10.1016/j.neuron.2008.06.012
31. Sun N, Cassell MD. Intrinsic GABAergic neurons in the rat central extended amygdala. *J Comp Neurol.* 1993;330:381–404. DOI: 10.1002/cne.903300308
32. Veinante P, Freund-Mercier M-J. Intrinsic and extrinsic connections of the rat central extended amygdala: an in vivo electrophysiological study of the central amygdaloid nucleus. *Brain Res.* 1998;794(2):188–98. [http://dx.doi.org/10.1016/S0006-8993\(98\)00228-5](http://dx.doi.org/10.1016/S0006-8993(98)00228-5)
33. Roberto M, Gilpin NW, Siggins GR. The central amygdala and alcohol: Role of γ -aminobutyric acid, glutamate, and neuropeptides. *Cold Spring Harb Perspect Med* 2012;2:a012195–a012195. DOI: 10.1101/cshperspect.a012195
34. Sakanaka M, Shibasaki T, Lederis K. Distribution and efferent projections of corticotropin-releasing factor-like immunoreactivity in the rat amygdaloid complex. *Brain Res.* 1986;382:213–238. DOI: 10.1016/0006-8993(86)91332-6
35. Clark JT, Keaton AK, Sahu A, Kalra SP, Mahajan SC, Gudger JN. Neuropeptide Y (NPY) levels in alcoholic and food restricted male rats: implications for site selective function. *Regul Pept.* 1998;75-76:335–345. DOI: 10.1016/S0167-0115(98)00088-3

36. Thiele TE, Cubero I, Van Dijk G, Mediavilla C, Bernstein IL. Ethanol- Induced c- Fos Expression in Catecholamine- and Neuropeptide Y- Producing Neurons in Rat Brainstem. *Alcohol Clin Exp Res.* 2000;24(6):802–9. DOI: 10.1111/j.1530-0277.2000.tb02059.x
37. Thiele TE, Marsh DJ, Ste Marie L, Bernstein IL, Palmiter RD. Ethanol consumption and resistance are inversely related to neuropeptide Y levels. *Nature.* 1998 Nov;396(6709):366–9. DOI: 10.1038/24614
38. Kinoshita H, Jessop DS, Finn DP, Coventry TL, Roberts DJ, Ameno K, et al. Acute ethanol decreases NPY mRNA but not POMC mRNA in the arcuate nucleus. *Neuroreport.* 2000;11(16):3517–9.
39. Roy A, Pandey SC. The decreased cellular expression of neuropeptide Y protein in rat brain structures during ethanol withdrawal after chronic ethanol exposure. *Alcohol Clin Exp Res.* 2002;26(6):796–803. DOI: 10.1111/j.1530-0277.2002.tb02607.x
40. Ehlers CL, Li TK, Lumeng L, Hwang BH, Somes C, Jimenez P, et al. Neuropeptide Y levels in ethanol-naive alcohol-preferring and nonpreferring rats and in Wistar rats after ethanol exposure. *Alcohol Clin Exp Res.* 1998;22(8):1778–82. DOI: 10.1111/j.1530-0277.1998.tb03979.x
41. Bison S, Crews F. Alcohol withdrawal increases neuropeptide Y immunoreactivity in rat brain. *Alcohol Clin Exp Res.* 2003;27(7):1173–83. DOI: 10.1097/01.ALC.0000075827.74538.FE
42. Becker HC, Diaz-Granados JL, Weathersby RT. Repeated ethanol withdrawal experience increases the severity and duration of subsequent withdrawal seizures in mice. *Alcohol.* 1997;14(4):319–26. [http://dx.doi.org/10.1016/S0741-8329\(97\)87949-9](http://dx.doi.org/10.1016/S0741-8329(97)87949-9)
43. Becker HC, Jaime LD, Hale RL. Exacerbation of ethanol withdrawal seizures in mice with a history of multiple withdrawal experience. *Pharmacol Biochem Behav.* 1997;57(1):179–83. [http://dx.doi.org/10.1016/S0091-3057\(96\)00303-6](http://dx.doi.org/10.1016/S0091-3057(96)00303-6)
44. Woldbye DP, Ulrichsen J, Haugbol S, Bolwig TG. Ethanol withdrawal in rats is attenuated by intracerebroventricular administration of neuropeptide Y. *Alcohol Alcohol.* 2002;37(4):318–21. DOI: <https://doi.org/10.1093/alcalc/37.4.318>
45. Koob GF, Ahmed SH, Boutrel B, Chen SA, Kenny PJ, Markou A, et al. Neurobiological mechanisms in the transition from drug use to drug dependence. *Neurosci Biobehav Rev.* 2004;27(8):739–49. DOI: 10.1016/j.neubiorev.2003.11.007
46. Hyytiä P, Koob GF. GABAA receptor antagonism in the extended amygdala decreases ethanol self-administration in rats. *Eur J Pharmacol.* 1995;283(1):151–9. [http://dx.doi.org/10.1016/0014-2999\(95\)00314-B](http://dx.doi.org/10.1016/0014-2999(95)00314-B)
47. Roberto M, Madamba SG, Moore SD, Tallent MK, Siggins GR. Ethanol increases GABAergic transmission at both pre-and postsynaptic sites in rat central amygdala neurons. *Proc Natl Acad Sci USA.* 2003;100(4):2053–8. DOI: 10.1073/pnas.0437926100
48. Roberto M, Madamba SG, Stouffer DG, Parsons LH, Siggins GR. Increased GABA release in the central amygdala of ethanol-dependent rats. *J Neurosci.* 2004;24(45):10159–66. DOI: 10.1523/JNEUROSCI.3004-04.2004
49. Biggio G, Concas A, Follesa P, Sanna E, Serra M. Stress, ethanol, and neuroactive steroids. *Pharmacol Ther.* 2007;116(1):140–71. DOI: 10.1016/j.pharmthera.2007.04.005
50. Lambert PD, Anderson KD, Sleeman MW, Wong V, Tan J, Hjarunguru A, et al. Ciliary neurotrophic factor activates leptin-like pathways and reduces body fat, without cachexia or rebound weight gain, even in leptin-resistant obesity. *Proc Natl Acad Sci USA.* 2001;98(8):4652–7. doi: 10.1073/pnas.061034298
51. VanDoren MJ, Matthews DB, Janis GC, Grobin AC, Devaud LL, Morrow AL. Neuroactive steroid 3 α -hydroxy-5 α -pregnan-20-one modulates electrophysiological and behavioral actions of ethanol. *J Neurosci.* 2000;20(5):1982–9.
52. Roberto M, Cruz MT, Gilpin NW, Sabino V, Schweitzer P, Bajo M, et al. Corticotropin Releasing Factor–Induced Amygdala Gamma-Aminobutyric Acid Release Plays a Key Role in Alcohol Dependence. *Biol Psychiatry.* 2010;67(9):831–9. doi: 10.1016/j.biopsych.2009.11.007.
53. Gilpin NW, Misra K, Herman MA, Cruz MT, Koob GF, Roberto M. Neuropeptide y opposes alcohol effects on gamma-aminobutyric acid release in amygdala and blocks the transition to alcohol dependence. *Biol Psychiatry.* 2011;69(11):1091–9. doi: 10.1016/j.biopsych.2011.02.004.
54. Sparrow AM, Lowery-Gionta EG, Pleil KE, Li C, Sprow GM, Cox BR, et al. Central neuropeptide Y modulates binge-like ethanol drinking in C57BL/6J mice via Y1 and Y2 receptors. *Neuropsychopharmacology.* 2012;37(6):1409–21. doi: 10.1038/npp.2011.327.

55. Kash TL, Winder DG. Neuropeptide Y and corticotropin-releasing factor bi-directionally modulate inhibitory synaptic transmission in the bed nucleus of the stria terminalis. *Neuropharmacology*. 2006;51(5):1013–22. DOI: 10.1016/j.neuropharm.2006.06.011
56. Chen X, Dimaggio DA, Han SP, Westfall TC. Autoreceptor-induced inhibition of neuropeptide Y release from PC-12 cells is mediated by Y2 receptors. *Am J Physiol-Heart Circ Physiol*. 1997;273(4):H1737–44.
57. Greber S, Schwarzer C, Sperk G. Neuropeptide Y inhibits potassium-stimulated glutamate release through Y2 receptors in rat hippocampal slices in vitro. *Br J Pharmacol*. 1994;113(3):737–40.
58. Pich EM, Lorang M, Yeganeh M, De Fonseca FR, Raber J, Koob GF, et al. Increase of extracellular corticotropin-releasing factor-like immunoreactivity levels in the amygdala of awake rats during restraint stress and ethanol withdrawal as measured by microdialysis. *J Neurosci*. 1995;15(8):5439–47.
59. Rassnick S, Heinrichs SC, Britton KT, Koob GF. Microinjection of a corticotropin-releasing factor antagonist into the central nucleus of the amygdala reverses anxiogenic-like effects of ethanol withdrawal. *Brain Res*. 1993;605(1):25–32.
60. Funk D, Li Z, Le AD. Effects of environmental and pharmacological stressors on c-fos and corticotropin-releasing factor mRNA in rat brain: relationship to the reinstatement of alcohol seeking. *Neuroscience*. 2006;138(1):235–43. DOI: 10.1016/j.neuroscience.2005.10.062
61. Thorsell A, Repunte-Canonigo V, O'Dell LE, Chen SA, King AR, Lekic D, et al. Viral vector-induced amygdala NPY overexpression reverses increased alcohol intake caused by repeated deprivations in Wistar rats. *Brain*. 2007;130(5):1330–7. DOI: 10.1093/brain/awm033
62. Gilpin NW, Misra K, Koob GF. Neuropeptide Y in the central nucleus of the amygdala suppresses dependence-induced increases in alcohol drinking. *Pharmacol Biochem Behav*. 2008;90(3):475–80. DOI: 10.1016/j.pbb.2008.04.006
63. Ceccanti M, Hamilton D, Coriale G, Carito V, Aloe L, Chaldakov G, et al. Spatial learning in men undergoing alcohol detoxification. *Physiol Behav*. 2015;149:324–30. DOI: 10.1016/j.physbeh.2015.06.034
64. Ceccanti M, et al. Serum BDNF and NGF Modulation by Olive Polyphenols in Alcoholics during Withdrawal. *J Alcohol & Drug Dep* 2015, 3:214. doi:10.4172/23296488.1000214
65. Koob GF. Theoretical frameworks and mechanistic aspects of alcohol addiction: alcohol addiction as a reward deficit disorder. In: *Behavioral Neurobiology of Alcohol Addiction*. Springer; 2011. p. 3–30. DOI: 10.1007/7854_2011_129
66. Heilig M, Wahlestedt C, Widerlöv E. Neuropeptide Y (NPY)-induced suppression of activity in the rat: evidence for NPY receptor heterogeneity and for interaction with alpha-adrenoceptors. *Eur J Pharmacol*. 1988;157(2–3):205–13.
67. Gilpin NW, Stewart RB, Badia-Elder NE. Neuropeptide Y administration into the amygdala suppresses ethanol drinking in alcohol-preferring (P) rats following multiple deprivations. *Pharmacol Biochem Behav*. 2008;90(3):470–4. DOI: 10.1016/j.pbb.2008.04.005
68. Breese GR, Overstreet DH, Knapp DJ. Conceptual framework for the etiology of alcoholism: a “kindling”/stress hypothesis. *Psychopharmacology (Berl)*. 2005;178(4):367–80. DOI: 10.1007/s00213-004-2016-2
69. Zhang H, Pandey SC. Effects of PKA modulation on the expression of neuropeptide Y in rat amygdaloid structures during ethanol withdrawal. *Peptides*. 2003;24(9):1397–402. <http://dx.doi.org/10.1016/j.peptides.2003.08.008>
70. Cippitelli A, Damadzic R, Hansson AC, Singley E, Sommer WH, Eskay R, et al. Neuropeptide y (NPY) suppresses yohimbine-induced reinstatement of alcohol seeking. *Psychopharmacology (Berl)*. 2010;208(3):417–26. DOI: 10.1007/s00213-009-1741-y
71. Thorsell A, Rimondini R, Heilig M. Blockade of central neuropeptide Y (NPY) Y2 receptors reduces ethanol self-administration in rats. *Neurosci Lett*. 2002;332(1):1–4. [http://dx.doi.org/10.1016/S0304-3940\(02\)00904-7](http://dx.doi.org/10.1016/S0304-3940(02)00904-7)
72. Rimondini R, Thorsell A, Heilig M. Suppression of ethanol self-administration by the neuropeptide Y (NPY) Y2 receptor antagonist BIIE0246: Evidence for sensitization in rats with a history of dependence. *Neurosci Lett*. 2005;375(2):129–33. DOI: 10.1016/j.neulet.2004.10.084
73. Cippitelli A, Rezvani AH, Robinson JE, Eisenberg L, Levin ED, Bonaventure P, et al. The novel, selective, brain-penetrant neuropeptide Y Y2 receptor antagonist, JNJ-31020028, tested in animal models of alcohol consumption, relapse, and anxiety. *Alcohol*. 2011;45(6):567–76. DOI: 10.1016/j.alcohol.2010.09.003

74. Hendry SHC. Organization of neuropeptide Y neurons in the mammalian central nervous system. In: *The Biology of Neuropeptide Y and Related Peptides*. Springer; 1993. p. 65–156. DOI 10.1007/978-1-59259-465-8_3
75. Koob GF. Alcoholism: allostasis and beyond. *Alcohol Clin Exp Res*. 2003;27(2):232–43. DOI: 10.1097/01.ALC.0000057122.36127.C2
76. Brown CM, Coscina DV, Fletcher PJ. The rewarding properties of neuropeptide Y in perifornical hypothalamus vs. nucleus accumbens. *Peptides*. 2000;21(8):1279–87. [http://dx.doi.org/10.1016/S0196-9781\(00\)00270-9](http://dx.doi.org/10.1016/S0196-9781(00)00270-9)
77. Josselyn SA, Beninger RJ. Neuropeptide Y: intraaccumbens injections produce a place preference that is blocked by cis-flupenthixol. *Pharmacol Biochem Behav*. 1993;46(3):543–52. [http://dx.doi.org/10.1016/0091-3057\(93\)90542-2](http://dx.doi.org/10.1016/0091-3057(93)90542-2)
78. Misra K, Pandey SC. Differences in Basal Levels of CREB and NPY in Nucleus Accumbens Regions between C57BL/6 and DBA/2 Mice Differing in Inborn Alcohol Drinking Behavior. *J Neurosci Res*. 2003;74(6):967–75. DOI: 10.1002/jnr.10831
79. Thiele TE, Koh MT, Pedrazzini T. Voluntary alcohol consumption is controlled via the neuropeptide Y Y1 receptor. *J Neurosci Off J Soc Neurosci*. 2002;22:RC208-RC208.
80. Thiele TE, Naveilhan P, Ernfors P. Assessment of ethanol consumption and water drinking by NPY Y2 receptor knockout mice. *Peptides*. 2004;25(6):975–83. DOI: 10.1016/j.peptides.2004.03.009
81. Palmiter RD, Erickson JC, Hollopeter G, Baraban SC, Schwartz MW. Life without neuropeptide Y. *Recent Prog Horm Res*. 1998;53:163–99.
82. Naveilhan P, Neveu I, Arenas E, Ernfors P. Complementary and overlapping expression of Y1, Y2 and Y5 receptors in the developing and adult mouse nervous system. *Neuroscience*. 1998;87(1):289–302. [http://dx.doi.org/10.1016/S0306-4522\(98\)00141-9](http://dx.doi.org/10.1016/S0306-4522(98)00141-9)
83. King PJ, Williams G, Doods H, Widdowson PS. Effect of a selective neuropeptide YY 2 receptor antagonist, BIIE0246 on neuropeptide Y release. *Eur J Pharmacol*. 2000;396(1):R1–3. [http://dx.doi.org/10.1016/S0014-2999\(00\)00230-2](http://dx.doi.org/10.1016/S0014-2999(00)00230-2)
84. Smith-White MA, Hardy TA, Brock JA, Potter EK. Effects of a selective neuropeptide Y Y2 receptor antagonist, BIIE0246, on Y2 receptors at peripheral neuroeffector junctions. *Br J Pharmacol*. 2001;132(4):861–8. DOI: 10.1038/sj.bjp.0703879
85. Berglund MM, Schober DA, Statnick MA, McDonald PH, Gehlert DR. The use of bioluminescence resonance energy transfer 2 to study neuropeptide Y receptor agonist-induced β -arrestin 2 interaction. *J Pharmacol Exp Ther*. 2003;306(1):147–56. DOI: 10.1124/jpet.103.051227
86. Caberlotto L, Thorsell A, Rimondini R, Sommer W, Hyttiä P, Heilig M. Differential expression of NPY and its receptors in alcohol-preferring AA and alcohol-avoiding ANA rats. *Alcohol Clin Exp Res*. 2001;25(11):1564–9. DOI: 10.1111/j.1530-0277.2001.tb02162.x
87. Thiele TE, Miura GI, Marsh DJ, Bernstein IL, Palmiter RD. Neurobiological responses to ethanol in mutant mice lacking neuropeptide Y or the Y5 receptor. *Pharmacol Biochem Behav*. 2000;67(4):683–91. [http://dx.doi.org/10.1016/S0091-3057\(00\)00413-5](http://dx.doi.org/10.1016/S0091-3057(00)00413-5)
88. Kelley SP, Nannini MA, Bratt AM, Hodge CW. Neuropeptide-Y in the paraventricular nucleus increases ethanol self-administration. *Peptides*. 2001;22(3):515–22. [http://dx.doi.org/10.1016/S0196-9781\(01\)00361-8](http://dx.doi.org/10.1016/S0196-9781(01)00361-8)
89. Schroeder JP, Olive F, Koenig H, Hodge CW. Intra-amygdala infusion of the NPY Y1 receptor antagonist BIBP 3226 attenuates operant ethanol self-administration. *Alcohol Clin Exp Res*. 2003;27(12):1884–91. DOI: 10.1097/01.ALC.0000098875.95923.69
90. Schroeder JP, Iller KA, Hodge CW. Neuropeptide- Y Y5 Receptors Modulate the Onset and Maintenance of Operant Ethanol Self- Administration. *Alcohol Clin Exp Res*. 2003;27(12):1912–20. DOI: 10.1097/01.ALC.0000098873.80433.BA
91. Ceccanti M, Mancinelli R, Tirassa P, Laviola G, Rossi S, Romeo M, et al. Early exposure to ethanol or red wine and long-lasting effects in aged mice. A study on nerve growth factor, brain-derived neurotrophic factor, hepatocyte growth factor, and vascular endothelial growth factor. *Neurobiol Aging*. 2010;33(2):359–67. DOI: 10.1016/j.neurobiolaging.2010.03.005
92. Fiore M, Laviola G, Aloe L, di Fausto V, Mancinelli R, Ceccanti M. Early exposure to ethanol but not red wine at the same alcohol concentration induces behavioral and brain neurotrophin alterations in young and adult mice. *Neurotoxicology*. 2009;30(1):59–71. DOI: 10.1016/j.neuro.2008.11.009

93. Ceccanti M, De Nicolo S, Mancinelli R, Chaldakov G, Carito V, Laviola G, et al. NGF and BDNF long-term variations in the thyroid, testis and adrenal glands of a mouse model of fetal alcohol spectrum disorders. *Ann Ist Super Sanita*. 2013;49(4):383–90. DOI: 10.4415/ANN_13_04_11
94. Fiore M., Mancinelli R., Laviola G., Aloe L., Sornelli F., Ceccanti M. Hepatocyte growth factor, vascular endothelial growth factor, glial cell-derived neurotrophic factor and nerve growth factor are differentially affected by early chronic ethanol or red wine intake. *Toxicol Let* 2009, 188:208–213. DOI: 10.1016/j.toxlet.2009.04.013
95. Carito V, Ceccanti M, Cestari V, Natella F, Bello C, Coccurello R, et al. Olive polyphenol effects in a mouse model of chronic ethanol addiction. *Nutrition*. 2017;33:65–9. DOI: 10.1016/j.nut.2016.08.014
96. Camarini R, Marcos Pautassi R, Méndez M, Marian Quadros I, Lucia Souza-Formigoni M, Boerngen-Lacerda R. Behavioral and neurochemical studies in distinct animal models of ethanol's motivational effects. *Curr Drug Abuse Rev*. 2010;3(4):205–21. DOI: 10.2174/1874473711003040205
97. Li TK, Lumeng L, Doolittle DP, Carr LG. Molecular associations of alcohol-seeking behavior in rat lines selectively bred for high and low voluntary ethanol drinking. *Alcohol Alcohol Oxf Oxf Suppl*. 1991;1:121–121.
98. Cicero TJ. A critique of animal analogues of alcoholism. *Biochem Pharmacol Ethanol*. 1979;2:533–60.
99. McBride WJ, Li TK. Animal models of alcoholism: neurobiology of high alcohol-drinking behavior in rodents. *Crit Rev Neurobiol*. 1998;12:339–369.
100. Murphy JM, Stewart RB, Bell RL, Badia-Elder NE, Carr LG, McBride WJ, et al. Phenotypic and genotypic characterization of the Indiana University rat lines selectively bred for high and low alcohol preference. *Behav Genet*. 2002;32:363–388. DOI: 10.1023/A:1020266306135
101. Ehlers CL, Somes C, Lumeng L, Li TK. Electrophysiological response to neuropeptide Y (NPY): In alcohol-naïve preferring and non-preferring rats. *Pharmacol Biochem Behav*. 1999;63(2):291–9. DOI: 10.1016/S0091-3057(99)00012-X
102. Hwang BH, Zhang JK, Ehlers CL, Lumeng L, Li TK. Innate differences of neuropeptide Y (NPY) in hypothalamic nuclei and central nucleus of the amygdala between selectively bred rats with high and low alcohol preference. *Alcohol Clin Exp Res*. 1999;23:1023–30. DOI: 10.1111/j.1530-0277.1999.tb04220.x
103. Badia-Elder NE, Stewart RB, Powrozek TA, Roy KF, Murphy JM, Li TK. Effect of neuropeptide Y (NPY) on oral ethanol intake in Wistar, alcohol-preferring (P), and -nonpreferring (NP) rats. *Alcohol Clin Exp Res*. 2001;25(3):386–90. DOI: 10.1111/j.1530-0277.2001.tb02225.x
104. Badia-Elder NE, Stewart RB, Powrozek TA, Murphy JM, Li TK, Badia-Elder NE, et al. 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*. 2003;27(6):894–9. DOI: 10.1097/01.ALC.0000071929.17974.DA
105. Gilpin NW, Stewart RB, Murphy JM, Li T-K, Badia-Elder NE. Neuropeptide Y reduces oral ethanol intake in alcohol-preferring (P) rats following a period of imposed ethanol abstinence. *Alcohol Clin Exp Res*. 2003;27(5):787–94. DOI: 10.1097/01.ALC.0000065723.93234.1D
106. Bice P, Foroud T, Bo R, Castelluccio P, Lumeng L, Li T-K, et al. Genomic screen for QTLs underlying alcohol consumption in the P and NP rat lines. *Mamm Genome*. 1998;9(12):949–55. DOI: 10.1007/s003359900905
107. Carr LG, Foroud T, Bice P, Gobbett T, Ivashina J, Edenberg H, et al. A quantitative trait locus for alcohol consumption in selectively bred rat lines. *Alcohol Clin Exp Res*. 1998;22(4):884–7. DOI: 10.1111/j.1530-0277.1998.tb03883.x
108. Terenina-Rigaldie E, Moisan M-P, Colas A, Beaugé F, Shah KV, Jones BC, et al. Genetics of behaviour: phenotypic and molecular study of rats derived from high-and low-alcohol consuming lines. *Pharmacogenet Genomics*. 2003;13(9):543–54. DOI: 10.1097/01.fpc.0000054120.14659.8c
109. Carr LG, Kimpel MW, Liang T, McClintick JN, McCall K, Morse M, et al. Identification of candidate genes for alcohol preference by expression profiling of congenic rat strains. *Alcohol Clin Exp Res*. 2007;31(7):1089–98. DOI: 10.1111/j.1530-0277.2007.00397.x
110. Spence J, Liang T, Foroud T, Lo D, Carr L. Expression profiling and QTL analysis: a powerful complementary strategy in drug abuse research. *Addict Biol*. 2005;10(1):47–51. DOI: 10.1080/13556210412331308958

111. Lappalainen J, Kranzler HR, Malison R, Price LH, Van Dyck C, Rosenheck RA, et al. A functional neuropeptide Y Leu7Pro polymorphism associated with alcohol dependence in a large population sample from the United States. *Arch Gen Psychiatry*. 2002;59(9):825–31. doi:10.1001/archpsyc.59.9.825
112. Zhu G, Pollak L, Mottagui-Tabar S, Wahlestedt C, Taubman J, Virkkunen M, et al. NPY Leu7Pro and alcohol dependence in Finnish and Swedish populations. *Alcohol Clin Exp Res*. 2003;27:19-24. DOI: 10.1097/01.ALC.0000050642.62233.44
113. Stewart R, Gatto G, Lumeng L, Li T-K, Murphy J. Comparison of alcohol-preferring (P) and nonpreferring (NP) rats on tests of anxiety and for the anxiolytic effects of ethanol. *Alcohol*. 1993;10:1-10. DOI: 10.1016/0741-8329(93)90046-Q
114. McKinzie D, Sajdyk T, McBride W, Murphy J, Lumeng L, Li T-K, et al. Acoustic startle and fear-potentiated startle in alcohol-preferring (P) and nonpreferring (NP) lines of rats. *Pharmacol Biochem Behav* 2000;65:691-696. DOI: 10.1016/S0091-3057(99)00252-X
115. Sajdyk TJ, Johnson PL, Leitermann RJ, Fitz SD, Dietrich A, Morin M, et al. Neuropeptide Y in the amygdala induces long-term resilience to stress-induced reductions in social responses but not hypothalamic–adrenal–pituitary axis activity or hyperthermia. *J Neurosci*. 2008;28:893-903. DOI:10.1523/JNEUROSCI.0659-07.2008