NPY Family of Peptides in Neurobiology, Cardiovascular and Metabolic Disorders:
from Genes to Therapeutics
Edited by Zofia Zukowska and Giora Z. Feuerstein

NPY in alcoholism and psychiatric disorders

Annika Thorsell, Rose-Marie Karlsson, and Markus Heilig

NIH/NIAAA, LCS, Building 10-CRC/Room 1-5330, 10 Center Drive, Bethesda, Maryland 20892, USA

NPY in affective disorders

Symptoms of anxiety and depression commonly co-exist and both disorders are thought to reflect maladaptive changes in stress-responsive systems [1]. Known genetic factors increase vulnerability for both anxiety and depression [2]. It has furthermore been suggested that the present classification of depressive and anxiety disorders may be artificial, and that for a large proportion of subjects with affective symptoms, a more appropriate categorization would be "major depression – generalized anxiety disorder" [3]. Thus, the role of NPY in these two conditions is dealt with jointly.

In rodent models, injections of nanomolar doses of NPY have been shown to decrease anxiety-like responses in a variety of tasks, including the elevated plus-maze [4, 5], social interaction task [6], fear potentiated startle and fear conditioned responses [4, 5, 7, 8]. In addition, intracerebroventricular (icv) administration of NPY to a large extent prevented gastric ulceration induced by water restraint, a strong stressor [9]. Mutant mice lacking NPY show increased anxiety-like behavior [10]. Although a transgenic mouse overexpressing NPY has been developed, only a limited phenotypic characterization for this line is available [11]. However, transgenic rats overexpressing NPY in hippocampus were shown to be resistant to stress-induced increases in anxiety-like behavior [12, 13].

These studies together indicate that pharmacological or transgenic activation of NPY signaling is stress reducing. The physiological involvement of endogenous NPY in mediation of stress responses and anxiety related behavior was demonstrated in two studies showing that NPY gene expression in amygdala and cortex is regulated by stress. Acute stress downregulates NPY-IR and NPY mRNA expression within 1 h, with mRNA levels returning to normal levels within 10 h and peptide levels within 2 h [14]. This stress has been shown to be anxiogenic on the elevated plus-maze. Interestingly, with repeated stress exposure, leading to a behavioral habituation, this effect is reversed. Under these conditions, NPY expression is instead upregulated [15]. On the basis of these pharmacological and expression studies, it was proposed that an upregulation of NPY expression may contribute to successful behavioral adap-

tation to stress. This extends a previously introduced hypothesis that NPY may act to "buffer" behavioral effects of stress-promoting signals such as CRF [16].

For depression, a differential NPY expression has been detected in a genetic animal model, the Flinders Sensitive rats (FSL) [17–20]. This is in agreement with the finding that chronic cocaine reduces NPY expression in the prefrontal cortex [21], since clinical hallmarks of cocaine withdrawal and dependence are symptoms of depression. Treatment with clinically effective antidepressants was early reported to increase NPY expression in several brain regions in rats, with frontal cortex being the most consistent region [22]. Initial attempts to replicate the effects of chronic antidepressant treatment and extend them to mRNA level were unsuccessful [23, 24] for reasons which remain unclear but may be related to assay specificity or, more likely, the half life of the drugs used being insufficient to maintain adequate plasma concentrations. Subsequently, a region-specific regulation of NPY and Y1 receptor expression was reported following chronic treatment with the serotonin-selective reuptake inhibitor (SSRI) fluoxetine, both in the "depressed" (FSL) line and the corresponding control line (FRL) [17, 25]. In these studies, fluoxetine elevated NPY-IR in the hypothalamic arcuate nucleus and anterior cingulate cortex, and increased Y1 binding sites in the medial amygdala and occipital cortex in both lines. In agreement with these findings, an increase in the NPY mRNA was found in the arcuate nucleus in both lines. In other brain regions, fluoxetine treatment caused a differential effect on the induction of NPY-related genes in these two rat strains: in hippocampus, NPY mRNA expression was increased in the "depressed" (FSL) subjects, but decreased in the "non-depressed" (FRL) line. In contrast, Y1 mRNA levels tended to decrease by fluoxetine in the nucleus accumbens of the FSL rats, but increased in the FRL. On the basis of these findings, an involvement of NPY was suggested in the antidepressant effect of fluoxetine.

Another established and effective antidepressive treatment, electroconvulsive shock (ECS), has been much more consistent in upregulating brain NPY-levels, with hippocampus as a seemingly central target. An elevated NPY level was demonstrated after repeated, but not single ECS, paralleling the requirements for clinical effect in depressed subjects [26–28]. These data has been both replicated and extended [29–31] and this effect seems robust in both "normal" laboratory rats and in the genetically selected FSL and FRLs. The mechanism is an upregulation of preproNPY expression which leads to an increased extracellular availability of the NPY peptide. Against the background of our behavioral finding in the transgenic rat model [12], upregulated hippocampal NPY-expression might be of importance both for therapeutic and amnesic effects of ECS.

The anti-anxiety and anti-depressive actions of NPY appear to be predominantly mediated via the Y1 receptor system. This was initially based on the observation that full length NPY peptide produced an anti-anxiety effect in elevated plus-maze, Vogel test [5] and Geller-Seifter test [7], while the C-terminal, presumably Y2-selective fragment, NPY_{13-36}, did not generate this

action. The *in vivo* use of intracerebral antisense oligonucleotides targeting Y1 receptor transcript made it possible to demonstrate a selectively lowered density of Y1 binding sites, with an outcome of decreased behavioral effects on the elevated plus-maze [32]. With the development of more selective pharmacological tools, Y1 mediation of anti-stress effects of NPY appears to have been confirmed.

Y2 receptors may also play a role in the regulation of emotionality. NPY-Y2 receptors are located presynaptically on NPY-ergic neurons, and control the release of endogenous NPY [33, 34]. Antagonizing this receptor is expected to potentiate the release of NPY and through this mechanism offer an "NPY-mimetic" effect without developing an Y1 agonist. This mechanism would therefore be an attractive target in the drug development efforts. Studies of NPY Y2 receptor knockout mice have supported this idea [35, 36] and results are consistent with the anxiogenic-like effects of intra-amygdala treatment of Y2-preferring agonists in the rat social interaction test [37, 38]. Another more direct involvement of Y2 receptors has been suggested within the locus coeruleus, where an anxiolytic-like effect was detected after a 10 pmol NPY microinjection into this structure, mimicked by NPY_{13-36} but not by [Leu31, Pro34]NPY, a "non-Y2" ligand [39].

The amygdala has so far been the most prominent region of interest with regard to emotionality. Central amygdala was initially suggested to be the mediating site of anxiolytic NPY actions [40]. However, subsequent microinjection studies using smaller injection volumes have prompted a re-evaluation of the data, suggesting that the lateral/basolateral complex in fact mediates anti-stress effects of NPY within the amygdala [6]. Periaqueductal grey matter (PAG) is involved in the behavioral output of fear responses, with subcompartments that are differentially involved in defensive behaviors [41, 42]. Its dorsolateral compartment (DPAG) has been suggested to tonically inhibit the amygdala. Microinjections of Y1 antagonists within DPAG produced an anxiogenic effect in elevated plus-maze [43] and social interaction task [44].

Septum has been implicated to be part of another important "behavioral inhibition system" but septal lesion that studies demonstrated effects on anxiety-like behaviors most likely reflected effects on fibers passing through this structure, probably belonging to hippocampal output through fornix fimbriae [45]. Hippocampus is an important component of neuronal circuitry controlling anxiety-related behaviors and stress responses, and in particularly dorsal hippocampus [46, 47], and septo-hippocampal circuits are likely to be important for fear related behaviors. NPY microinjections into lateral septum reproduced anxiolytic-like actions of intracerebroventricular administration of NPY, and reversed the anxiogenic action of corticotrophin releasing factor (CRF). This was clearly mediated by the Y1 receptor, since a highly selective Y1 receptor antagonist, BIBO 3304, blocked this anxiolytic-like action [48].

Human studies support an involvement of NPY in depression and anxiety disorders. An early study stated decreased levels of NPY in the cerebrospinal fluid (CSF) of patients with major depression [49], which could reflect a

decrease in central availability of NPY. Low levels of NPY in brain tissue were also reported in suicide victims [50]. These studies were followed by reports which failed to replicate their results [51, 52], although issues of assay specificity are particularly likely to complicate matters in this case. In a recent re-examination of this issue in a large number of therapy-refractory depressed patients, a highly significant, 30% reduction of CSF NPY was found [53]. Interestingly, postmortem studies have meanwhile shown a decreased NPY mRNA expression which is most prominent in bipolar disorder [54]. It is a well-known fact that a proportion of patients diagnosed with unipolar disorder in fact has a genetic vulnerability for bipolar disorder, but has not yet presented with their first manic episode, and may never do so. It is therefore possible that the involvement of NPY is primarily related to bipolar traits and that the discrepant CSF results are partly due to varying proportion of this patient category in the different clinical populations.

In summary, compelling evidence exists for a role of NPY as an endogenous anti-stress compound, which is physiologically recruited to cope with prolonged stress. Dysfunction of this system seems to be present in affective illness. Targeting the NPY system, possibly through antagonism at presynaptic Y2 autoreceptors, offers an attractive strategy to develop novel antidepressant and anti-anxiety treatments.

NPY in alcoholism

In addition to involvement in mood disorders such as depression and anxiety syndromes, NPY has been demonstrated to have a role in alcohol intake, dependence, and withdrawal. The effect profile of NPY shows numerous similarities with not only that of established anti-anxiety compounds, but also that of alcohol. Furthermore, in clinical studies of alcohol dependence a correlation between initial anxiety and subsequent alcohol abuse, possibly due to the anxiolytic action of alcohol, has been demonstrated [55, 56]. While this may only be true for a subgroup of alcoholics, it may partially explain some of the changes and effects seen for NPY in alcoholism.

A direct link between NPY signaling and regulation of alcohol consumption was first shown in a study where mice with a transgenic overexpression of NPY consumed less alcohol, while mice with a null-mutation, i.e. inactivation, in the NPY gene had an increased alcohol consumption [11]. Genetic studies in both experimental animals and humans provide tentative support for a role of NPY in regulation of ethanol intake. Within the genome of a genetic rat model of high alcohol drinking state, the P-rat (see below), a quantitative trait locus was identified which spans the locus for the NPY gene [57, 58]. Furthermore, associations between alcoholism and polymorphisms within the NPY gene have been suggested. A substitution (Pro7 for Leu7) in the signal peptide region of the NPY precursor, prepro-NPY, leads to increased plasma NPY in response to stress compared to control subjects without the substitu-

tion [59, 60]. A 34% higher average alcohol consumption was reported in Finnish men with this substitution compared to matched control subjects [59]. Another report showed alcoholic European/American men had a 5–5.5% Pro7 allele frequency while the frequency in the non-alcoholic control group was 2% [61]. However, this polymorphism has also been reported to be of lower frequency in alcoholics or to not be significantly different between alcoholics and controls [61, 62]. We have recently reanalyzed this issue by reconstructing the haplotype structure of the preproNPY – gene using five polymorphic markers. This has yielded two preliminary insights: The coding Leu7Pro 1128 SNP is in strong linkage disequilibrium with a novel promoter polymorphism, and is present almost exclusively on a common haplotype. The frequency of this haplotype differs significantly between alcohol dependent subjects and normals. All of this difference can be attributed to type I alcoholics, i.e., patients with late onset of alcohol problems. Interestingly, this clinical subtype is characterized by high trait anxiety, which makes the association of particular interest considering the established role of NPY in anxiety. Finally, another polymorphism, a C-to-T substitution at the 5671 locus of the NPY gene, was reported to be more frequent in a Japanese alcoholic patient population [63].

In animals, selective breeding for ethanol consumption or preference has created several lines of mice and rats which have been well characterized with regards to numerous behavioral, pharmacological, and biological traits. Mouse lines include the high-alcohol-preference (HAP) and low-alcohol-preference (LAP) line, and rat lines include the Sardinian preferring (SP) and non-preferring (SNP) lines, the Indiana alcohol-preferring (P) and non-preferring (NP) lines, the Alko alcohol (AA) and Alko non-alcohol (ANA) lines, as well as the high-alcohol drinking (HAD) and low alcohol drinking (LAD) lines [64]. Each high drinking/preferring line consumes sufficient amounts of alcohol to achieve pharmacologically significant blood levels (50–250 mg%), is motivated by ethanol's pharmacological properties rather than smell, taste, or caloric content, and develops physiological tolerance after long term access to alcohol. NPY and NPY receptor expression patterns have been examined in these 'genetic models of alcohol dependence'. For example, P rats have been shown to have low levels of NPY in amygdala, frontal cortex, and hippocampus compared to the non-preferring NP-line, but higher levels in the paraventricular nucleus, arcuate nucleus, and cingulate cortex [65, 66]. In the HAD line NPY-IR was decreased in central nucleus of the amygdala, paraventricular nucleus of the hypothalamus, and the arcuate nucleus as compared to LAD rats [67]. In the AA/ANA, a different pattern was seen, with lower hippocampal NPY mRNA expression compared to the non-preferring line [68]. The NPY Y2 receptor subtype was also found to be reduced in the medial amygdala of the AA line as compared to the ANA line.

The effect of NPY on alcohol consumption appears to be in part dependent on the individual's history and state of alcohol consumption. In animal studies, central administration of NPY into the lateral ventricles, central nucleus of the amygdala, or the third ventricle leaves level of ethanol intake unaffected in

normal, out-bred rat strains [69–72]. However, a significant suppression of alcohol intake was found in the P-line as compared to NP and normal Wistar rats, and in the HAD rat line [73, 71]. The lack of effect in states of low intake but efficacy in the preferring lines which consume ethanol for its pharmacological properties is key to understanding a basic distinction, which is further highlighted by experiments in animals with or without a history of dependence. Thus, a basal component of ethanol consumption seems to be unrelated to the pharmacological/rewarding actions of ethanol, but might instead be related to its properties as caloric nutrient, regulated by factors modulating appetite. This component is not suppressed by NPY; in contrary, it is stimulated by hypothalamic NPY injections, as would be expected from NPYs well established effect to stimulate appetite [74]. In contrast to the suppressive effects of NPY on ethanol intake in high-preferring animals, the modulation of the low level intake component appears to be the same in rats genetically selected for low and high preference, making it further unlikely that it is related to the addictive properties of ethanol [75].

Further evidence for the dichotomy between effects of NPY on ethanol consumption related to addictive properties of this drug, *versus* effects on low level intake, has been provided using animals in which dependence and high alcohol preference was induced using 8 weeks exposure to intermittent ethanol vapor (14 h on/10 h off per day; target BAL 200 mg%). This models chronic alcohol consumption and leads to similar clinical manifestations as well as long-term changes in neurochemistry and increases in alcohol intake [76]. In this model, NPY was shown to significantly suppress alcohol intake in exposed animals as compared to saline treatment. Notably, consumption was reduced back to but not below pre-vapor exposure levels [77].

Thus, the NPY system may offer an attractive target for developing novel therapies for alcohol dependence. The likelihood of this has been strengthened by recent findings that mice in which the Y1 receptor gene as been inactivated consume increased amounts of ethanol [78]. Furthermore, icv administration of the selective Y2 antagonist BIIE0246 lead to decreased ethanol intake in non-dependent rats, and a sensitization to this effect was shown in post-dependent (vapor exposed) rats [79, 80].

Conclusion

The NPY system may well be one of the most interesting target systems for development of treatments for alcohol dependence as well as mood disorders such as depression and anxiety syndromes. NPY is an endogenous anxiolytic compound, functions as an antidepressant, and is effective in modifying alcohol intake in high drinking states. Through receptor subtype specific compounds, the NPY system offers an interesting and innovative future approach for treatment designs. Selective Y2 receptor antagonists and/or Y1 agonists that are peripherally available and effectively penetrate the CNS are possible

candidates. In conclusion, the NPY system offers attractive targets for development of future treatments for depression, anxiety, and alcohol dependence.

References

1 Holsboer F (2000) The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology* 23: 477–501
2 Kendler KS, Neale MC, Kessler RC, Heath AC, Eaves LJ (1992) Major depression and generalized anxiety disorder. Same genes, (partly) different environments? *Arch Gen Psychiatry* 49: 716–722
3 Sullivan PF, Kendler KS (1998) Typology of common psychiatric syndromes. An empirical study. *Br J Psychiatry* 173: 312–319
4 Broqua P, Wettstein JG, Rocher MN, Gauthier-Martin B, Junien JL (1995) Behavioral effects of neuropeptide Y receptor agonists in the elevated plus-maze and fear-potentiated startle procedures. *Behav Pharmacol* 6: 215–222
5 Heilig M, Soderpalm B, Engel JA, Widerlov E (1989) Centrally administered neuropeptide Y (NPY) produces anxiolytic-like effects in animal anxiety models. *Psychopharmacology (Berl)* 98: 524–529
6 Sajdyk TJ, Vandergriff MG, Gehlert DR (1999) Amygdalar neuropeptide Y Y1 receptors mediate the anxiolytic-like actions of neuropeptide Y in the social interaction test. *Eur J Pharmacol* 368: 143–147
7 Heilig M, McLeod S, Koob GK, Britton KT (1992) Anxiolytic-like effect of neuropeptide Y (NPY), but not other peptides in an operant conflict test. *Regul Pept* 41: 61–69
8 Tovote P, Meyer M, Beck-Sickinger AG, von HS, Ove OS, Spiess J, Stiedl O (2004) Central NPY receptor-mediated alteration of heart rate dynamics in mice during expression of fear conditioned to an auditory cue. *Regul Pept* 120: 205–214
9 Heilig M, Murison R (1987) Intracerebroventricular neuropeptide Y protects against stress-induced gastric erosion in the rat. *Eur J Pharmacol* 137: 127–129
10 Bannon AW, Seda J, Carmouche M, Francis JM, Norman MH, Karbon B, McCaleb ML (2000) Behavioral characterization of neuropeptide Y knockout mice. *Brain Res* 868: 79–87
11 Thiele TE, Marsh DJ, Ste ML, Bernstein IL, Palmiter RD (1998) Ethanol consumption and resistance are inversely related to neuropeptide Y levels. *Nature* 396: 366–369
12 Thorsell A, Michalkiewicz M, Dumont Y, Quirion R, Caberlotto L, Rimondini R, Mathe AA, Heilig M (2000) Behavioral insensitivity to restraint stress, absent fear suppression of behavior and impaired spatial learning in transgenic rats with hippocampal neuropeptide Y overexpression. *Proc Natl Acad Sci USA* 97: 12852–12857
13 Carvajal CC, Vercauteren F, Dumont Y, Michalkiewicz M, Quirion R (2004) Aged neuropeptide Y transgenic rats are resistant to acute stress but maintain spatial and non-spatial learning. *Behav Brain Res* 153: 471–480
14 Thorsell A, Svensson P, Wiklund L, Sommer W, Ekman R, Heilig M (1998) Suppressed neuropeptide Y (NPY) mRNA in rat amygdala following restraint stress. *Regul Pept* 75–76: 247–254
15 Thorsell A, Carlsson K, Ekman R, Heilig M (1999) Behavioral and endocrine adaptation, and up-regulation of NPY expression in rat amygdala following repeated restraint stress. *Neuroreport* 10: 3003–3007
16 Heilig M, Thorsell A (2002) Brain neuropeptide Y (NPY) in stress and alcohol dependence. *Rev Neurosci* 13: 85–94
17 Caberlotto L, Fuxe K, Overstreet DH, Gerrard P, Hurd YL (1998) Alterations in neuropeptide Y and Y1 receptor mRNA expression in brains from an animal model of depression: region specific adaptation after fluoxetine treatment. *Brain Res Mol Brain Res* 59: 58–65
18 Caberlotto L, Jimenez P, Overstreet DH, Hurd YL, Mathe AA, Fuxe K (1999) Alterations in neuropeptide Y levels and Y1 binding sites in the Flinders Sensitive Line rats, a genetic animal model of depression. *Neurosci Lett* 265: 191–194
19 Jimenez-Vasquez PA, Overstreet DH, Mathe AA (2000) Neuropeptide Y in male and female brains of Flinders Sensitive Line, a rat model of depression. Effects of electroconvulsive stimuli. *J Psychiatr Res* 34: 405–412

20 Jimenez Vasquez PA, Salmi P, Ahlenius S, Mathe AA (2000) Neuropeptide Y in brains of the Flinders Sensitive Line rat, a model of depression. Effects of electroconvulsive stimuli and d-amphetamine on peptide concentrations and locomotion. *Behav Brain Res* 111: 115–123
21 Wahlestedt C, Karoum F, Jaskiw G, Wyatt RJ, Larhammar D, Ekman R, Reis DJ (1991) Cocaine-induced reduction of brain neuropeptide Y synthesis dependent on medial prefrontal cortex. *Proc Natl Acad Sci USA* 88: 2078–2082
22 Heilig M, Wahlestedt C, Ekman R, Widerlöv E (1988) Antidepressant drugs increase the concentration of neuropeptide Y (NPY)-like immunoreactivity in the rat brain. *European Journal of Pharmacology* 147: 465–467
23 Heilig M, Ekman R (1995) Chronic parenteral antidepressant treatment in rats: unaltered levels and processing of neuropeptide Y (NPY) and corticotropin-releasing hormone (CRH). *Neurochem Int* 26: 351–355
24 Bellman R, Sperk G (1993) Effects of antidepressant drug treatment on levels of NPY or prepro-NPY-mRNA in the rat brain. *Neurochemistry International* 22: 183–187
25 Overstreet DH, Pucilowski O, Rezvani AH, Janowsky DS (1995) Administration of antidepressants, diazepam and psychomotor stimulants further confirms the utility of Flinders Sensitive Line rats as an animal model of depression. *Psychopharmacology (Berl)* 121: 27–37
26 Mathe AA, Gruber S, Jimenez PA, Theodorsson E, Stenfors C (1997) Effects of electroconvulsive stimuli and MK-801 on neuropeptide Y, neurokinin A, and calcitonin gene-related peptide in rat brain. *Neurochem Res* 22: 629–636
27 Mathe AA, Jimenez PA, Theodorsson E, Stenfors C (1998) Neuropeptide Y, neurokinin A and neurotensin in brain regions of Fawn Hooded "depressed", Wistar, and Sprague Dawley rats. Effects of electroconvulsive stimuli. *Prog Neuropsychopharmacol Biol Psychiatry* 22: 529–546
28 Wahlestedt C, Blendy JA, Kellar KJ, Heilig M, Widerlov E, Ekman R (1990) Electroconvulsive shocks increase the concentration of neocortical and hippocampal neuropeptide Y (NPY)-like immunoreactivity in the rat. *Brain Res* 507: 65–68
29 Husum H, Gruber SH, Bolwig TG, Mathe AA (2002) Extracellular levels of NPY in the dorsal hippocampus of freely moving rats are markedly elevated following a single electroconvulsive stimulation, irrespective of anticonvulsive Y1 receptor blockade. *Neuropeptides* 36: 363–369
30 Husum H, Mikkelsen JD, Hogg S, Mathe AA, Mork A (2000) Involvement of hippocampal neuropeptide Y in mediating the chronic actions of lithium, electroconvulsive stimulation and citalopram. *Neuropharmacology* 39: 1463–1473
31 Zachrisson O, Mathe AA, Stenfors C, Lindefors N (1995) Limbic effects of repeated electroconvulsive stimulation on neuropeptide Y and somatostatin mRNA expression in the rat brain. *Brain Res Mol Brain Res* 31: 71–85
32 Wahlestedt C, Pich EM, Koob GF, Yee F, Heilig M (1993) Modulation of anxiety and neuropeptide Y-Y1 receptors by antisense oligodeoxynucleotides. *Science* 259: 528–531
33 King PJ, Williams G, Doods H, Widdowson PS (2000) Effect of a selective neuropeptide Y Y(2) receptor antagonist, BIIE0246 on neuropeptide Y release. *Eur J Pharmacol* 396: R1–R3
34 King PJ, Widdowson PS, Doods HN, Williams G (1999) Regulation of neuropeptide Y release by neuropeptide Y receptor ligands and calcium channel antagonists in hypothalamic slices. *J Neurochem* 73: 641–646
35 Redrobe JP, Dumont Y, Herzog H, Quirion R (2003) Neuropeptide Y (NPY) Y2 receptors mediate behaviour in two animal models of anxiety: evidence from Y2 receptor knockout mice. *Behav Brain Res* 141: 251–255
36 Tschenett A, Singewald N, Carli M, Balducci C, Salchner P, Vezzani A, Herzog H, Sperk G (2003) Reduced anxiety and improved stress coping ability in mice lacking NPY-Y2 receptors. *Eur J Neurosci* 18: 143–148
37 Sajdyk TJ, Schober DA, Gehlert DR (2002) Neuropeptide Y receptor subtypes in the basolateral nucleus of the amygdala modulate anxiogenic responses in rats. *Neuropharmacology* 43: 1165–1172
38 Sajdyk TJ, Schober DA, Smiley DL, Gehlert DR (2002) Neuropeptide Y-Y2 receptors mediate anxiety in the amygdala. *Pharmacol Biochem Behav* 71: 419–423
39 Kask A, Eller M, Oreland L, Harro J (2000) Neuropeptide Y attenuates the effect of locus coeruleus denervation by DSP-4 treatment on social behaviour in the rat. *Neuropeptides* 34: 58–61
40 Heilig M, McLeod S, Brot M, Heinrichs SC, Menzaghi F, Koob GF, Britton KT (1993) Anxiolytic-like action of neuropeptide Y: mediation by Y1 receptors in amygdala, and dissociation from food intake effects. *Neuropsychopharmacology* 8: 357–363

41 Brandao ML (1993) Involvement of opioid mechanisms in the dorsal periaqueductal gray in drug abuse. *Rev Neurosci* 4: 397–405
42 Fendt M, Fanselow MS (1999) The neuroanatomical and neurochemical basis of conditioned fear. *Neurosci Biobehav Rev* 23: 743–760
43 Kask A, Rago L, Harro J (1998) Anxiogenic-like effect of the NPY Y1 receptor antagonist BIBP3226 administered into the dorsal periaqueductal gray matter in rats. *Regul Pept* 75–76: 255–262
44 Kask A, Rago L, Harro J (1998) NPY Y1 receptors in the dorsal periaqueductal gray matter regulate anxiety in the social interaction test. *Neuroreport* 9: 2713–2716
45 Lee Y, Davis M (1997) Role of the septum in the excitatory effect of corticotropin-releasing hormone on the acoustic startle reflex. *J Neurosci* 17: 6424–6433
46 Andrews N, File SE, Fernandes C, Gonzalez LE, Barnes NM (1997) Evidence that the median raphe nucleus–dorsal hippocampal pathway mediates diazepam withdrawal-induced anxiety. *Psychopharmacology (Berl)* 130: 228–234
47 Gonzalez LE, Ouagazzal AM, File SE (1998) Stimulation of benzodiazepine receptors in the dorsal hippocampus and median raphe reveals differential GABAergic control in two animal tests of anxiety. *Eur J Neurosci* 10: 3673–3680
48 Kask A, Nguyen HP, Pabst R, von HS (2001) Neuropeptide Y Y1 receptor-mediated anxiolysis in the dorsocaudal lateral septum: functional antagonism of corticotropin-releasing hormone-induced anxiety. *Neuroscience* 104: 799–806
49 Widerlov E, Lindstrom LH, Wahlestedt C, Ekman R (1988) Neuropeptide Y and peptide YY as possible cerebrospinal fluid markers for major depression and schizophrenia, respectively. *J Psychiatr Res* 22: 69–79
50 Widdowson PS, Ordway GA, Halaris AE (1992) Reduced neuropeptide Y concentrations in suicide brain. *J Neurochem* 59: 73–80
51 Berrettini WH, Doran AR, Kelsoe J, Roy A, Pickar D (1987) Cerebrospinal fluid neuropeptide Y in depression and schizophrenia. *Neuropsychopharmacology* 1: 81–83
52 Ordway GA, Stockmeier CA, Meltzer HY, Overholser JC, Jaconetta S, Widdowson PS (1995) Neuropeptide Y in frontal cortex is not altered in major depression. *J Neurochem* 65: 1646–1650
53 Heilig M, Zachrisson O, Thorsell A, Ehnvall A, Mottagui-Tabar S, Sjogren M, Asberg M, Ekman R, Wahlestedt C, Agren H (2004) Decreased cerebrospinal fluid neuropeptide Y (NPY) in patients with treatment refractory unipolar major depression: preliminary evidence for association with preproNPY gene polymorphism. *J Psychiatr Res* 38: 113–121
54 Caberlotto L, Hurd YL (1999) Reduced neuropeptide Y mRNA expression in the prefrontal cortex of subjects with bipolar disorder. *Neuroreport* 10: 1747–1750
55 Pandey 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
56 Pandey SC, Carr LG, Heilig M, Ilveskoski E, Thiele TE (2003) Neuropeptide y and alcoholism: Genetic, molecular, and pharmacological evidence. *Alcohol Clin Exp Res* 27: 149–154
57 Bice P, Foroud T, Bo R, Castelluccio P, Lumeng L, Li TK, Carr LG (1998) Genomic screen for QTLs underlying alcohol consumption in the P and NP rat lines. *Mamm Genome* 9: 949–955
58 Carr LG, Foroud T, Bice P, Gobbett T, Ivashina J, Edenberg H, Lumeng L, Li TK (1998) A quantitative trait locus for alcohol consumption in selectively bred rat lines. *Alcohol Clin Exp Res* 22: 884–887
59 Kauhanen J, Karvonen MK, Pesonen U, Koulu M, Tuomainen TP, Uusitupa MI, Salonen JT (2000) Neuropeptide Y polymorphism and alcohol consumption in middle-aged men. *Am J Med Genet* 93: 117–121
60 Karvonen MK, Pesonen U, Koulu M, Niskanen L, Laakso M, Rissanen A, Dekker JM, Hart LM, Valve R, Uusitupa MIJ (1998) Association of a leucine(7)-to-proline(7) polymorphism in the signal peptide of neuropeptide Y with high serum cholesterol and LDL cholesterol levels. *Nature Medicine* 4: 1434–1437
61 Ilveskoski E, Kajander OA, Lehtimaki T, Kunnas T, Karhunen PJ, Heinala P, Virkkunen M, Alho H (2001) Association of neuropeptide y polymorphism with the occurrence of type 1 and type 2 alcoholism. *Alcohol Clin Exp Res* 25: 1420–1422
62 Zhu G, Pollak L, Mottagui-Tabar S, Wahlestedt C, Taubman J, Virkkunen M, Goldman D, Heilig M (2003) NPY Leu7Pro and alcohol dependence in Finnish and Swedish populations. *Alcohol Clin Exp Res* 27: 19–24
63 Okubo T, Harada S (2001) Polymorphism of the neuropeptide Y gene: An association study with

alcohol withdrawal. *Alcohol Clin Exp Res* 25: 59S–62S
64 Li TK, Lumeng L, McBride WJ, Murphy JM (1987) Rodent lines selected for factors affecting alcohol consumption. *Alcohol and Alcoholism* Suppl 1: 91–96
65 Murphy JM, Stewart RB, Bell RL, Badia-Elder NE, Carr LG, McBride WJ, Lumeng L, Li TK (2002) Phenotypic and genotypic characterization of the Indiana University rat lines selectively bred for high and low alcohol preference. *Behav Genet* 32: 363–388
66 Ehlers CL, Li TK, Lumeng L, Hwang BH, Somes C, Jimenez P, Mathe AA (1998) Neuropeptide Y levels in ethanol-naive alcohol-preferring and nonpreferring rats and in Wistar rats after ethanol exposure. *Alcohol Clin Exp Res* 22: 1778–1782
67 Hwang BH, Zhang JK, Ehlers CL, Lumeng L, Li TK (1999) 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* 23: 1023–1030
68 Caberlotto L, Thorsell A, Rimondini R, Sommer W, Hyytia P, Heilig M (2001) Differential expression of NPY and its receptors in alcohol-preferring AA and alcohol-avoiding ANA rats. *Alcohol Clin Exp Res* 25: 1564–1569
69 Katner SN, Slawecki CJ, Ehlers CL (2002) Neuropeptide Y administration into the amygdala does not affect ethanol consumption. *Alcohol* 28: 29–38
70 Katner SN, Slawecki CJ, Ehlers CL (2002) Neuropeptide Y administration into the third ventricle does not increase sucrose or ethanol self-administration but does affect the cortical EEG and increases food intake. *Psychopharmacology (Berl)* 160: 146–154
71 Badia-Elder NE, Stewart RB, Powrozek TA, Roy KF, Murphy JM, Li TK (2001) Effect of neuropeptide Y (NPY) on oral ethanol intake in Wistar, alcohol-preferring (P), and -nonpreferring (NP) rats. *Alcohol Clin Exp Res* 25: 386–390
72 Slawecki CJ, Betancourt M, Walpole T, Ehlers CL (2000) Increases in sucrose consumption, but not ethanol consumption, following ICV NPY administration. *Pharmacol Biochem Behav* 66: 591–594
73 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
74 Kelley SP, Nannini MA, Bratt AM, Hodge CW (2001) Neuropeptide-Y in the paraventricular nucleus increases ethanol self-administration. *Peptides* 22: 515–522
75 Gilpin NW, Stewart RB, Murphy JM, Badia-Elder NE (2004) Neuropeptide Y in the paraventricular nucleus of the hypothalamus increases ethanol intake in high- and low-alcohol-drinking rats. *Alcohol Clin Exp Res* 28: 1492–1498
76 Rimondini R, Arlinde C, Sommer W, Heilig M (2002) Long-lasting increase in voluntary ethanol consumption and transcriptional regulation in the rat brain after intermittent exposure to alcohol. *FASEB J* 16: 27–35
77 Thorsell A, Slawecki CJ, Ehlers CL (2005) Effects of neuropeptide Y and corticotropin-releasing factor on ethanol intake in Wistar rats: Interaction with chronic ethanol exposure. *Behav Brain Res* 161: 133–140
78 Thiele TE, Koh MT, Pedrazzini T (2002) Voluntary alcohol consumption is controlled via the neuropeptide Y Y1 receptor. *J Neurosci* 22: 1–6
79 Thorsell A, Rimondini R, Heilig M (2002) Blockade of central neuropeptide Y (NPY) Y2 receptors reduces ethanol self-administration in rats. *Neurosci Lett* 332: 1–4
80 Rimondini R, Thorsell A, Heilig M (2005) 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* 375: 129–133