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Neuropeptide-Y in the paraventricular nucleus increases ethanol self-administration

Stephen P. Kelley, Michelle A. Nannini, Alison M. Bratt, and Clyde W. Hodge*

Department of Neurology, Gallo Research Center, University of California at San Francisco, 5858 Horton Street, Suite 200, Emeryville, CA 94608 USA

Abstract

The paraventricular nucleus (PVN) of the hypothalamus is known to modulate feeding, obesity, and ethanol intake. Neuropeptide-Y (NPY), which is released endogenously by neurons projecting from the arcuate nucleus to the PVN, is one of the most potent stimulants of feeding behavior known. The role of NPY in the PVN on ethanol self-administration is unknown. To address this issue, rats were trained to self-administer ethanol via a sucrose fading procedure and injector guide cannulae aimed at the PVN were surgically implanted. Microinjections of NPY and NPY antagonists in the PVN were conducted prior to ethanol self-administration sessions. All doses of NPY significantly increased ethanol self-administration and preference, and decreased water intake. The NPY antagonist D-NPY partially reduced ethanol self-administration and completely blocked the effects of an intermediate dose of NPY (10 fmol) on ethanol intake, preference, and water intake. The competitive non-peptide Y1 receptor antagonist BIBP 3226 did not significantly alter ethanol self-administration or water intake when administered alone in the PVN but it completely blocked the effect of NPY (10 fmol) on ethanol intake. NPY infused in the PVN had no effect on ethanol self-administration when tested in rats that did not have a long history of ethanol self-administration. The doses of NPY tested produced no effect on food intake or body weight measured during the 24-h period after infusion in either ethanol-experienced or ethanol-inexperienced rats. These results indicate that elevation of NPY levels in the PVN potentially increases ethanol self-administration and that this effect is mediated through NPY Y1 receptors.

Keywords

Neuropeptide Y; NPY; Paraventricular nucleus; PVN; Hypothalamus; Alcohol self-administration; Alcohol drinking; Feeding; Rats

1. Introduction

The mechanisms by which alcohol interacts with biochemical and behavioral processes to produce addiction are not fully characterized. Research on other excessive consummatory behaviors, such as obesity, has identified the hypothalamus as a primary CNS regulatory system [2,19]. Recent evidence indicates that alcohol and food intake is similarly regulated

by hypothalamic transmitter systems. Injections of serotonin (5-HT) in the paraventricular nucleus (PVN) inhibit norepinephrine-induced carbohydrate intake [24]. Similarly, we have shown that site-specific norepinephrine infusion in the PVN also produces increases in alcohol self-administration, which are completely blocked by co-infusion of 5-HT [16]. This suggests that feeding and alcohol-seeking behavior may be regulated by common hypothalamic mechanisms [33].

Neuropeptide Y (NPY) is the most potent stimulant of feeding behavior known. Infusions of NPY into the cerebral ventricles or into nuclei of the hypothalamus [38,40] increase food intake [39], and repeated injections lead to hyperphagia and obesity [37]. Injection of NPY into a number of brain regions leads to increased food intake, but the PVN is the primary site of action [36,40]. Consistent with these effects of NPY, when animals are food-deprived, hypothalamic NPY immunoreactivity [1], mRNA levels [35], and NPY release [8,20,23] are increased. Moreover, reduction of endogenous NPY in the PVN reduces food intake [22]. Food intake and body weight, therefore, appear to be influenced by NPY activity in the PVN.

Several studies have implicated NPY in the biochemical, physiological, and behavioral effects of ethanol. Selectively bred alcohol preferring (P) rats have lower levels of NPY-like immunoreactivity in hippocampus, amygdala, and frontal cortex as compared to alcohol non-preferring (NP) rats [9]. Genetic linkage analysis of P and NP rats identified a significant quantitative trait locus on a chromosomal region that includes the NPY gene [4]. Additional evidence shows that ethanol and NPY also have similar effects on event related potentials recorded from cortex and amygdala [10]. Comparisons of mutant mice that lack NPY with transgenic mice that overexpress NPY show that ethanol intake and acute sensitivity are inversely related to total levels of NPY in the brain [42]. However, no studies to date have examined the effects of direct modulation of NPY levels in the hypothalamus on ethanol self-administration. To address this, we tested the effects of PVN infusions of NPY on ethanol self-administration, and food and water intake, in rats.

2. Method

2.1. Animals

Thirty-six male Long-Evans rats (Harlan, Indianapolis, In) were housed individually in Plexiglas cages. Access to water was restricted during the first day of behavioral training, but was continuously available for the remainder of the experiment. Food was always available in the home cage. In experimental chambers, ethanol (10% v/v) and water were available concurrently during daily (M–F) sessions. The animal colony room was maintained on a 12L:12D cycle with the lights on at 06:30. Experimental sessions were run during the light portion of the cycle. All experimental procedures were conducted under institutional and NIH guidelines.

2.2. Apparatus

Experimental sessions were conducted in Plexiglas chambers (27 × 37 × 21 cm) located in sound attenuating cubicles (MED Associates, model ENV 016M, Lafayette, IN). Chambers

were equipped with exhaust fans that masked external noise. The right wall of each chamber contained two 50-ml drinking bottles. Drug solutions were administered bilaterally through stainless steel injectors (Plastics One, Roanoke, VA), which were connected via plastic tubing to two 1.0 μ l syringes (Hamilton, Reno, NV). Syringes were mounted on a micro-infusion pump (Harvard Apparatus, Model 22, Natick, MA) set to deliver 0.5 μ l/min/syringe.

2.3. Self-Administration Procedures

After 2 weeks of adaptation to laboratory housing conditions, fluid access was restricted to 1-h per day (for 2 days only) and two groups of rats ($n \times 12$ per group) were trained to drink from the bottles in the experimental chambers by overnight access to sucrose (10% w/v) vs. water during one overnight session. Daily 1-hour sessions were then conducted with sucrose (10% w/v) vs. water available concurrently. The location (i.e., left or right side of the chamber) of the sucrose and water solutions was alternated daily. When sucrose and water intake patterns stabilized, the rats were trained to self-administer concurrent ethanol (10% v/v) vs. water using a sucrose fading procedure [34] as previously described (e.g., [15]). Briefly, ethanol was gradually added to the sucrose solution and then sucrose was faded out of the solution until rats were self-administering 10% ethanol vs. water. During the 2-month sucrose fading procedure, the location of the ethanol/sucrose solution and water were alternated daily. All animals preferred the ethanol/sucrose solution to water (data not shown). After sucrose fading, the rats were allowed to self-administer ethanol (10% v/v) versus water 5 days per week (M–F) for 4 months to establish a long-term history of ethanol self-administration. At the end of the 4-month baseline procedure, all animals underwent stereotaxic surgery. They were then allowed to self-administer ethanol for an additional 3 months prior to microinjection procedures.

Since NPY has potent effects on consummatory behavior, it is possible that NPY infusions in the PVN could alter ethanol intake via mechanisms that are unrelated to experience with the pharmacological effects of ethanol. Thus, as a control for nonspecific effects of NPY on consumption, the effects of NPY were tested in a separate group of rats ($n = 12$) that did not have a long-term history of ethanol self-administration. Experimentally naïve rats were allowed 1-week to adapt to the laboratory. They were then implanted with bilateral guide cannulae aimed at the PVN. Experimental sessions began one week after surgery to allow for recovery. During daily 60-min sessions, rats were placed in the self-administration chambers with concurrent ethanol (10% v/v) vs. water available. Baseline levels of ethanol and water intake were measured for one week. Microinjection procedures were then conducted.

2.4. Stereotaxic Surgery

When ethanol and water intake stabilized, bilateral stainless steel guide cannulae (26 gauge) aimed at the PVN were surgically implanted. Rats were anesthetized with pentobarbital (60 mg/kg, i.p.) combined with atropine (0.4 mg/kg, i.p.) and placed in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA). Injector cannulae (Plastics One, Roanoke, VA), aimed to terminate 1 mm dorsal to the PVN, were implanted and secured to the skull with cranial screws and dental cement. Removable wire obturators were inserted in the full length

of the guide cannulae to limit obstruction by tissue and contamination by external debris. The stereotaxic coordinates used for the PVN were -1.8 mm from bregma, $+1.0$ mm lateral to the midline, and -6.5 mm ventral to the cortical surface at 5° lateral to the vertical plane [29]. All measurements were taken from flat skull. Following surgery, all rats were given buprenorphine (0.2 mg/kg, sc.) for post-operative pain management. Daily sessions were resumed one week after surgery.

2.5. Microinjection Procedure

When ethanol and water intake stabilized again, micro-injections were conducted once per week. Unanesthetized rats were placed in plastic containers ($15 \times 30 \times 15$ cm deep) to reduce movement. Obturators were removed and sterile 33-gauge injectors were inserted bilaterally to a depth 1 mm beyond the end of the guide cannulae. Drug solutions were infused bilaterally in distilled water vehicle in a total volume of $1 \mu\text{l}$ ($0.5 \mu\text{l}/\text{side}$) over a 1 -min period. The injectors were left in place for an additional 30 -sec period to allow drug diffusion. Precise flow of the solutions was verified before and after each injection to ensure compound delivery. Self-administration sessions began immediately after microinjections. Sterile obturators were reinserted at the end of the behavioral sessions. When co-administered, D-NPY or BIBP 3226 was infused 15 -min prior to NPY. Vehicle injections were also performed to control for local pressure or osmotic changes caused by infusions. During one month prior to drug testing, the animals were handled and placed in the plastic tubs to minimize the effects of procedural changes on subsequent drug effects. The data from these sessions were not used in the analysis. After completion of the microinjection protocol, the rats were sacrificed and their brains were removed for histological verification of injection sites.

2.6. Drug and Dosing

The drugs used in this study for central administration were NPY, the non-selective NPY antagonist [D-Tyr^{27,36}, D-Thr³²] Neuropeptide Y [27–36] (D-NPY), and the Y1 selective antagonist N2-(Diphenylacetyl)-N-[(4-hydroxyphenyl) methyl]-D-arginine amide (BIBP 3226). All drugs were obtained from Research Biochemicals International, Natick, MA. All drugs were dissolved in sterile distilled water for central administration. Drug solutions were prepared immediately prior to administration and were infused bilaterally in a total volume of $1 \mu\text{l}$ ($0.5 \mu\text{l}/\text{side}/\text{min}$). Drug doses were administered in a randomized order by an experimenter not blinded to dose. Ethanol (95%) was diluted in tap water for self-administration.

2.7. Histology

After completion of the experiment, the rats were administered a lethal dose of sodium pentobarbital (200 mg/kg, ip.) and perfused transcardially with 0.9% NaCl followed by 10% formalin. The brains were removed and stored in a solution of 10% formalin/ 30% sucrose for a minimum of 10 days. Fixed brains were frozen, sectioned ($40 \mu\text{m}$), and stained with cresyl violet and examined under a light microscope to determine injection locations. Data were used only from rats that were verified to have clearly definable injector tracks that terminated bilaterally in the PVN.

2.8. Data Analysis and Statistics

Volume of ethanol and water consumed was measured to the nearest 0.5 milliliter at the end of each 1-h session. Ethanol intake was converted from mls consumed to g/kg body weight. Relative ethanol intake (preference) was calculated as mls of ethanol consumed divided by total fluid intake (ethanol + water mls). Drug dose effects were analyzed by one-way repeated measures analysis of variance (ANOVA). Post-hoc comparisons between drug doses and vehicle control were conducted using paired t-test or Dunnett's t-test where appropriate (SigmaStat, Jandel, San Rafael, CA).

3. Results

The behavioral procedures resulted in stable ethanol self-administration in the majority of animals. Ethanol intake averaged over the 25 days preceding microinjection procedures was (0.44 ± 0.05 g/kg). Data from 3 rats in the group that received NPY and BIBP-3226 were excluded from analysis due to deterioration of baseline performance.

3.1. Histology

Results of histological examination of coronal brain sections are shown in Fig. 1. Analysis showed that 11 of 12 animals that received sucrose fading followed by NPY and D-NPY infusions received bilateral injections in the PVN (Fig. 1A, closed circles). Examination of brain sections from rats that received sucrose fading followed by NPY and BIBP 3226 showed that 9 of 9 animals received injections in the PVN (Fig. 1A, open squares). Ten of 12 control animals received bilateral injections in the PVN (Fig. 1B). The injection procedure produced minimal gliosis or tissue damage in the PVN (Fig. 1C). Data are presented only for animals that received bilateral injections in the PVN.

3.2. Effects of NPY on Ethanol Intake and Preference

The results of NPY infusions in the PVN are shown in Fig. 2. Repeated-measures ANOVA indicated that NPY significantly increased ethanol intake [$F(3,30) = 7.6$, $p < 0.001$]. Post-hoc Dunnett's comparisons showed that all doses of NPY significantly increased ethanol intake, as compared to vehicle control (Fig. 2A). Inspection of data from individual rats indicated that all eleven rats tested showed increased ethanol intake (g/kg) at all doses of NPY. Although baseline ethanol preference was high ($>70\%$), NPY administration in the PVN also significantly increased ethanol preference [$F(3,30) = 3.3$, $p < 0.05$] in a dose-dependent manner (Fig. 2B). At the two highest doses of NPY tested, ethanol represented greater than 90% of fluid consumption during daily sessions ($p < 0.05$; Fig. 2B). The increases in ethanol intake and preference were associated with a significant overall reduction in water intake [$F(3,30) = 5.4$, $p < 0.01$] during experimental sessions (Fig. 2C). Post-hoc comparisons demonstrated that NPY decreased water intake in a dose-dependent manner ($p < 0.05$).

The nonselective NPY receptor antagonist D-NPY produced a partial, but significant, decrease in ethanol intake (Fig. 2A, right). When co-administered with NPY (10 fmol), D-NPY completely blocked the effects of NPY on ethanol and water intake (Fig. 2A and 2C,

right). Although there was a trend in the preference data, D-NPY did not significantly alter NPY-induced increase in ethanol preference, $P < 0.09$ (Fig. 2B)

Table 1 shows the effects of NPY infusion in the PVN of rats that had no long-term history of ethanol self-administration. Under these conditions, no dose of NPY tested produced a change in ethanol intake or preference, or water intake.

3.3. Effects of NPY on Home-cage Food and Water Consumption

NPY infusion in the PVN had no effect on body weight (Fig. 3A) or food consumption (Fig. 3B) measured in the home cage (Fig. 3B). However, this dose of NPY significantly $F(1,10) = 9.625$, $p < 0.05$] reduced water intake in the home cage (Fig. 3C). Administration of D-NPY either alone or in combination with NPY produced no effect on body weight (Fig. 3A, right). NPY also produced no effect on body weight, food intake, or water intake in the home cage of sucrose-inexperienced animals.

3.4. Effects of Y1 antagonist on NPY-induced Changes in Ethanol Intake and Preference

Fig. 4 shows the results of intra-PVN infusion of NPY and the NPY Y1 selective antagonist BIBP 3226. Infusion of BIBP 3226 (10.6 μM) alone in the PVN produced no significant effect on ethanol intake or preference, or water intake (Fig. 4A, B, and C). NPY (10 fmol) significantly increased ethanol intake above control values (Fig. 4A). Co-administration of BIBP 3226 with NPY in the PVN completely blocked the ability of this dose of NPY to increase ethanol intake (Fig. 4A).

4. Discussion

The neurobiological mechanisms by which alcohol interacts with brain and behavioral processes to produce addiction are not fully characterized. Research on excessive food consumption, such as that seen in obesity, has identified the PVN of the hypothalamus as the primary CNS regulatory system and NPY as one of the most important orexigenic agents [19]. The present study was designed to test the effects of direct manipulation of NPY levels in the PVN on ethanol self-administration. Our primary finding was that infusion of exogenous NPY in the PVN potentially increased ethanol intake and preference in rats. The non-selective NPY antagonist D-NPY partially reduced baseline ethanol intake and completely blocked the increase produced by NPY. Moreover, the selective non-peptide NPY Y1 antagonist BIBP 3226 completely blocked NPY-induced increases in ethanol intake. These data indicate that NPY, acting at Y1 receptors in the PVN, is a potent stimulant of alcohol self-administration.

Stimulation of alcohol self-administration by NPY in the PVN is consistent with several other reports. Selectively bred alcohol preferring P rats have more NPY immunoreactivity in the PVN and arcuate nucleus than alcohol non-preferring NP rats [18]. Rats exposed to chronic ethanol vapor demonstrate significantly higher NPY immunoreactivity in the hypothalamus than non-exposed controls when measured 1 month after ethanol withdrawal [9]. Similarly, long-term exposure to a 6% ethanol-containing diet produced elevated NPY content in the PVN and altered feeding patterns of Long-Evans rats [5]. The present study

adds to these findings by showing that elevated NPY levels in the PVN produce the immediate behavioral consequence of increasing ethanol self-administration and preference.

Other evidence obtained with genetically manipulated animals, however, is inconsistent with the present data. Selectively bred high alcohol drinking (HAD) rats have less NPY immunoreactivity in the PVN than low alcohol drinking (LAD) rats [18]. In addition, null mutant mice lacking NPY drink more ethanol than wild-type control mice and transgenic mice that over express NPY drink less ethanol than wild-type control mice [42]. Taken together, these data suggest that reduced NPY levels are associated with increases in ethanol self-administration. However, HAD rats also have lower levels of NPY in the central amygdala as compared to LAD rats [18] indicating that alterations in NPY levels in other brain regions might influence ethanol self-administration in these rats. In addition, the behavior of NPY null mutant and transgenic mice was likely influenced by global changes in NPY as well as potential developmental compensation in other functionally related peptidergic systems, which makes it difficult to draw any specific conclusions regarding the role of hypothalamic NPY in ethanol self-administration from that study. When taken together with the present data, these studies suggest that NPY may produce brain region-specific effects on ethanol self-administration.

The present data demonstrate that NPY-induced increases in ethanol self-administration are mediated by Y1 receptors. Similarly, considerable attention has been paid to the receptor subtype mediating the orexigenic effect of NPY [14,17,26]. The Y1 receptor was originally proposed as the receptor involved in this effect, since the Y1 agonist [Pro³⁴]-NPY stimulates feeding. However, potent orexigenic effects are also produced by an N-terminal truncated NPY fragment, NPY 2–36, which has low potency at the Y1 receptor, which led to the concept that a novel ‘Y1-like’ receptor may mediate the effect [41]. In addition, [Pro³⁴]-NPY stimulates approximately 50% of the food intake seen following the injection of NPY, suggesting that the Y1 receptor may be responsible for some portion of the orexigenic effect of NPY [26]. Thus, like feeding, ethanol intake may be controlled at least in part by NPY Y1 receptors in the PVN.

Another finding of the present study is that NPY infusions in the PVN only increased ethanol self-administration in rats that underwent the sucrose fading procedure followed by a long-term history of ethanol self-administration [34]. Comparable effects of carbohydrate and alcohol intake have been known for some time [21,31]. Alcohol intake decreases carbohydrate intake in a nutrient-selective manner without altering protein or fat consumption of rats [12]. Accordingly, human studies indicate that intake of sweets is inversely related to alcohol consumption [6]. Other studies have demonstrated that low carbohydrate diets increase alcohol intake, but high carbohydrate diets decrease alcohol drinking [3,13,30]. Simple carbohydrates have been reported to help delay relapse in alcoholics [11]. Furthermore, recovering alcoholic women have been noted to eat more sweet foods during periods of strong alcohol craving [32]. The present data raise the hypothesis that pairing ethanol with a carbohydrate, such as sucrose, during the development of self-administration behavior might engage homeostatic neural systems that mediate food intake (i.e., the hypothalamus). Alternatively, it is possible that the higher level of ethanol intake by sucrose-trained animals influenced the effect of NPY. Additional studies are

needed to clarify the potential importance of sucrose/ethanol pairings and history of ethanol self-administration.

In contrast to the well-known effects of this peptide, NPY infusions in the PVN had no effect on body weight or consummatory behavior in the home cage environment. The absence of NPY-related effects on food intake and body weight might be accounted for by several factors. First, the concentrations of NPY used in the present study were in the fmol range, which is significantly less than concentrations typically used to induce feeding, which are in the pmol—nmol range (e.g., [38]). Thus, ethanol self-administration behavior may be more sensitive to alterations in NPY levels than feeding. Second, elevated NPY levels in the PVN may produce immediate increases in consumption of the most relevant substance in the environment. This did not appear to be the case in the present experiment because NPY produced no effect on ethanol intake in rats trained with a sucrose-independent method. Third, since food intake was not recorded until 24-h after NPY infusion, measurement may have occurred after any NPY-induced effects on feeding. This premise does not seem likely, however, because NPY infusions produce long lasting changes in feeding behavior that may peak at 12-h post-infusion and continue for up to 24-h [7]. Fourth, it is also not likely that the rats were overly sedated by the self-administered ethanol, which could have inhibited feeding, because the doses achieved were only mildly sedative. A final, plausible, explanation for the lack of effect of NPY on feeding is that pairing the pharmacological effects of alcohol with sucrose may engage hypothalamic regulatory systems that are primarily involved in eating and drinking behavior [33]. This raises the interesting hypothesis that hypothalamic “feeding” systems could be usurped by alcohol during the development of addiction, which could lead to a re-focusing of NPY systems in the hypothalamus to control alcohol-seeking behavior rather than food-seeking behavior. Clearly, more research is required to address this possibility.

The role of NPY in the control of food intake is well characterized, but its involvement in water consumption is not as well understood. Central administration of NPY stimulates water intake in rodents and rabbits, an effect believed to be independent of its orexigenic effects [25,28]. Consistent with the effects of food restriction, water deprivation increased prepro-NPY mRNA levels in the arcuate nucleus [27]. This suggests that changes in central NPY can independently stimulate water as well as food intake. In the present study, NPY significantly decreased water intake during ethanol self-administration sessions, which contributed to the increase in ethanol preference. We also observed relatively small but significant reductions in 24-h water intake in the home cage after NPY infusion in the PVN. The non-selective NPY antagonist D-NPY significantly reversed NPY-induced decreases in water intake during ethanol self-administration sessions and the Y1 selective antagonist reversed a trend toward decreased water intake. Taken together, these data suggest that NPY has differential effects on water intake in ethanol-experienced versus ethanol-inexperienced rats.

In conclusion, results of this study showed that NPY infusions in the PVN increase ethanol self-administration and preference. Both the nonselective NPY antagonist D-NPY and the Y1 selective antagonist BIBP 3226 blocked the effects of NPY on ethanol intake. These findings support the conclusion that NPY, acting at Y1 receptors in the PVN, is a potent

stimulant of ethanol intake and preference. NPY Y1 receptor antagonists might, therefore, represent viable candidates for the medical management of excessive alcohol consumption seen in alcohol abuse and alcoholism.

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References

1. Beck B, Jhanwar UM, Burlet A, Chapleur Chateau M, Leibowitz SF, Burlet C. Rapid and localized alterations of neuropeptide Y in discrete hypothalamic nuclei with feeding status. *Brain Res.* 1990; 528:245–249. [PubMed: 2271925]
2. Blundell JE. Serotonin manipulations and the structure of feeding behaviour. *Appetite.* 1986; 7:39–56. [PubMed: 3527061]
3. Brown RV, Hutchenson DP. Nutrition and alcohol consumption in Sinclair miniature pig. *Q J Stud Alcohol.* 1973; 34:758–763. [PubMed: 4795454]
4. Carr LG, Foroud T, Bice P, Gobbett T, Ivashina J, Edenberg H, Lumeng L, Li TK. A quantitative trait locus for alcohol consumption in selectively bred rat lines. *Alcohol Clin Exp Res.* 1998; 22:884–887. [PubMed: 9660316]
5. 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; 75–6:335–345.
6. Colditz GA, Giovannucci E, Rimm EB, Stampfer MJ, Rosner B, Speizer FE, Gordis E, Willett WC. Alcohol intake in relation to diet and obesity in women and men. *Am J Clin Nutr.* 1991; 54:49–55. [PubMed: 2058587]
7. Criscione L, Rigollier P, Batzl-Hartmann C, Rüeger H, Stricker-Krongrad A, Wyss P, Brunner L, Whitebread S, Yamaguchi Y, Gerald C, Heurich RO, Walker MW, Chiesi M, Schilling W, Hofbauer KG, Levens N. Food intake in free-feeding and energy-deprived lean rats is mediated by the neuropeptide Y5 receptor. *J Clin Invest.* 1998; 102(12):2136–2145. [PubMed: 9854049]
8. Dube MG, Sahu A, Kalra PS, Kalra SP. Neuropeptide Y release is elevated from the microdissected paraventricular nucleus of food-deprived rats: an in vitro study. *Endocrinology.* 1992; 131:684–688. [PubMed: 1639015]
9. Ehlers CL, Li TK, Lumeng L, Hwang BH, Somes C, Jimenez P, Mathé AA. Neuropeptide Y levels in ethanol-naive alcohol-preferring and nonpreferring rats and in Wistar rats after ethanol exposure. *Alcohol Clin Exp Res.* 1998a; 22:1778–1782. [PubMed: 9835294]
10. Ehlers CL, Somes C, Cloutier D. Are some of the effects of ethanol mediated through NPY? *Psychopharmacology.* 1998b; 139:136–144. [PubMed: 9768551]
11. Farcas ME, Dwyer J. Nutrition education for alcoholic recovery homes. *J Nutr Education.* 1984; 16:123–124.
12. Forsander OA. The interaction between voluntary alcohol consumption and dietary choice. *Alcohol Alcohol.* 1988; 23:143–149. [PubMed: 3390238]
13. Forsander OA, Sinclair JD. Protein, carbohydrate and ethanol consumption: interactions in AA and ANA rats. *Alcohol.* 1988; 5:233–238. [PubMed: 3415768]
14. Gerald C, Walker MW, Criscione L, Gustafson EL, Batzl HC, Smith KE, Vaysse P, Durkin MM, Laz TM, Linemeyer DL, Schaffhauser AO, Whitebread S, Hofbauer KG, Taber RI, Branchek TA, Weinshank RL. A receptor subtype involved in neuropeptide-Y-induced food intake. *Nature.* 1996; 382:168–171. [see comments]. [PubMed: 8700207]
15. Hodge CW, Samson HH, Lewis RS, Erickson HL. Specific decreases in ethanol but not water reinforced responding produced by the 5-HT3 antagonist ICS 205–930. *Alcohol.* 1993; 10:191–196. [PubMed: 8507386]

16. Hodge CW, Slawecki CJ, Aiken A. Norepinephrine and serotonin receptors in the paraventricular nucleus of the hypothalamus interactively modulate ethanol consumption. *Alcohol Clin Exp Res*. 1996; 20:1669–1674. [PubMed: 8986220]
17. Hu YH, Bloomquist BT, Cornfield LJ, Decarr LB, Floresriveros JR, Friedman L, Jiang PL, Lewishiggins L, Sadlowski Y, Schaefer J, Velazquez N, McCaleb ML. Identification of a novel hypothalamic neuropeptide Y receptor associated with feeding behavior. *J Biol Chem*. 1996; 271:26315–26319. [PubMed: 8824284]
18. 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(6):1023–1030. [PubMed: 10397286]
19. Kalra SP, Dube MG, Pu S, Xu B, Horvath TL, Kalra PS. Interacting appetite-regulating pathways in the hypothalamic regulation of body weight. *Endocr Rev*. 1999; 20:68–100. [PubMed: 10047974]
20. Kalra SP, Dube MG, Sahu A, Phelps CP, Kalra PS. Neuropeptide Y secretion increases in the paraventricular nucleus in association with increased appetite for food. *Proc Natl Acad Sci USA*. 1991; 88:10931–10935. [PubMed: 1961764]
21. Kulkosky PJ. Brain-gut neuropeptides and the limitation of alcohol consumption. *Neurosci Biobehav Rev*. 1985; 9:179–190. [PubMed: 3892378]
22. Lambert PD, Wilding JP, al-Dokhayel AA, Bohuon C, Comoy E, Gilbey SG, Bloom SR. A role for neuropeptide-Y, dynorphin, and noradrenaline in the central control of food intake after food deprivation. *Endocrinology*. 1993; 133:29–32. [PubMed: 8100519]
23. Lambert PD, Wilding JP, Turton MD, Ghatei MA, Bloom SR. Effect of food deprivation and streptozotocin-induced diabetes on hypothalamic neuropeptide Y release as measured by a radioimmunoassay-linked microdialysis procedure. *Brain Res*. 1994; 656:135–140. [PubMed: 7804826]
24. Leibowitz SF, Shor-Posner G. Brain serotonin and eating behavior. *Appetite*. 1986; 7(Suppl 1–14)
25. Morley JE, Flood JF. The effect of neuropeptide Y on drinking in mice. *Brain Res*. 1989; 494:129–137. [PubMed: 2765911]
26. O'Shea D, Morgan DG, Meeran K, Edwards CM, Turton MD, Choi SJ, Heath MM, Gunn I, Taylor GM, Howard JK, Bloom CI, Small CJ, Haddo O, Ma JJ, Callinan W, Smith DM, Ghatei MA, Bloom SR. Neuropeptide Y induced feeding in the rat is mediated by a novel receptor. *Endocrinology*. 1997; 138:196–202. [PubMed: 8977404]
27. O'Shea RD, Gundlach AL. NPY mRNA and peptide immunoreactivity in the arcuate nucleus are increased by osmotic stimuli: correlation with dehydration anorexia. *Peptides*. 1995; 16:1117–1125. [PubMed: 8532596]
28. Pau MY, Pau KY, Spies HG. Characterization of central actions of neuropeptide Y on food and water intake in rabbits. *Physiol Behav*. 1988; 44:797–802. [PubMed: 3249755]
29. Paxinos, G.; Watson, C. *The rat brain in stereotaxic coordinates*. Sydney: Academic Press; 1982.
30. Pekkanen L, Eriksson K, Sihvonen ML. Dietary-induced changes in voluntary alcohol consumption and ethanol metabolism in the rat. *Br J Nutr*. 1978; 40:103–113. [PubMed: 666993]
31. Richter CP. Alcohol, beer, and wine as foods. *Q J Stud Alcohol*. 1953; 14:525–539. [PubMed: 13121226]
32. Rosenfield S, Stevenson JS. Perception of daily stress and oral coping behaviors in normal, overweight, and recovering alcoholic women. *Res Nurs Health*. 1988; 11:165–174. [PubMed: 3399697]
33. Samson, HH.; Hodge, CW. Neurobehavioral regulation of ethanol intake. In: Deitrich, RA.; Erwin, VG., editors. *Pharmacological Effects of Ethanol on the Nervous System*. Boca Raton: CRC Press; 1996. p. 203–226.
34. Samson HH. Initiation of ethanol reinforcement using a sucrose-substitution procedure in food- and water-sated rats. *Alcohol Clin Exp Res*. 1986; 10:436–442. [PubMed: 3530023]
35. Schwartz MW, Sipols AJ, Grubin CE, Baskin DG. Differential effect of fasting on hypothalamic expression of genes encoding neuropeptide Y, galanin, and glutamic acid decarboxylase. *Brain Res Bull*. 1993; 31:361–367. [PubMed: 7683962]

36. Stanley BG, Daniel DR, Chin AS, Leibowitz SF. Paraventricular nucleus injections of peptide YY and neuropeptide YY preferentially enhance carbohydrate ingestion. *Peptides*. 1985; 6:1205–1211. [PubMed: 3841735]
37. Stanley BG, Kyrkouli SE, Lampert S, Leibowitz SF. Neuropeptide Y chronically injected into the hypothalamus: a powerful neurochemical inducer of hyperphagia and obesity. *Peptides*. 1986; 7:1189–1192. [PubMed: 3470711]
38. Stanley BG, Leibowitz SF. Neuropeptide Y injected in the paraventricular hypothalamus: a powerful stimulant of feeding behavior. *Proc Natl Acad Sci USA*. 1985; 82:3940–3943. [PubMed: 3858854]
39. Stanley BG, Leibowitz SF. Neuropeptide Y: stimulation of feeding and drinking by injection into the paraventricular nucleus. *Life Sci*. 1984; 35:2635–2642. [PubMed: 6549039]
40. Stanley BG, Magdalin W, Seirafi A, Thomas WJ, Leibowitz SF. The perifornical area: the major focus of (a) patchily distributed hypothalamic neuropeptide Y-sensitive feeding system(s). *Brain Res*. 1993; 604:304–317. [PubMed: 8457858]
41. Stanley G, Magdalin W, Seirafi A, Nguyen MM, Leibowitz SF. Evidence for neuropeptide Y mediation of eating produced by food deprivation and for a variant of the Y1 receptor mediating this peptide's effect. *Peptides*. 1992; 13:581–587. [PubMed: 1326105]
42. Thiele TE, Marsh DJ, Ste. Marie L, Bernstein IL, Palmiter RD. Ethanol consumption and resistance are inversely related to neuropeptide Y levels. *Nature*. 1998; 396:366–369. [PubMed: 9845072]

Fig. 1. Histological representation of microinjection sites in the paraventricular nucleus (PVN) of the hypothalamus. (A) Injection sites in animals trained to self-administer ethanol via the sucrose-fading procedure. ●—injector location in animals that received NPY and D-NPY infusion; □—indicates injector location in animals that received NPY and BIBP 3226. (B) Injector sites in control animals that received no long-term history of ethanol self-administration. (C) Photomicrograph showing cannula and injector tract. No significant

tissue damage or gliosis was observed beyond the guide cannula or injector tracks. Numbers between the sections represent distance from bregma in mm.

Fig. 2.

Effects of NPY or NPY + D-NPY infused in the PVN immediately before 1-h test sessions on (A) ethanol intake, (B) preference, and (C) water intake. Drug doses were administered in random order. Data are plotted as Mean $\pm$ SEM of 11 rats. *—significantly different from vehicle control, Dunnett's t-test ($p < 0.05$). †—significantly different from NPY alone, paired-t-test with Bonferroni correction ($p < 0.05$).

Fig. 3. Effects of NPY or NPY + D-NPY infused in the PVN on (A) body weight, (B) food intake, and (C) water intake measured in the home cage 24-h after infusion. Drug doses were administered in random order. Measurements were taken on the same days as data shown in Fig. 2. Data are plotted as Mean $\pm$ SEM of 11 rats. *—significantly different from vehicle control, Tukey $p < 0.05$.

Fig. 4.

Effects of NPY, BIBP 3226, or NPY + BIB 3226 infused in the PVN immediately before 1-h test sessions on (A) ethanol intake, (B) preference, and (C) water intake. Drug doses were administered in random order. Data are plotted as Mean $\pm$ SEM of 9 rats. *—significantly different from vehicle control, paired-t-test $p < 0.05$.

Table 1

Effects of NPY on rats with limited history of ethanol self-administration. Data values are mean $\pm$ SEM. Drug doses were administered in random order. No differences were significant.

	NPY (fmol)		
	0.0	10.0	100.0
ETOH Intake (g/kg/hr)	0.071 $\pm$ 0.026	0.0330 $\pm$ 0.024	0.0550 $\pm$ 0.03
ETOH Preference (%)	43.33 $\pm$ 15.755	20.0 $\pm$ 13.333	30 $\pm$ 15.275
Water Intake (ml/hr)	0.1667 $\pm$ 0.118	0 $\pm$ 0	0.3 $\pm$ 0.133