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Neuropeptide Y (NPY) in the central nucleus of the amygdala (CeA) does not affect ethanol-reinforced responding in binge-drinking, nondependent rats

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Abstract

The central nucleus of the amygdala (CeA) has been implicated as having a significant role in mediating alcohol-drinking behavior. Neuropeptide Y (NPY) has been investigated as a potential pharmacotherapeutic due to its ability to attenuate ethanol intake, particularly when administered into the CeA. Previous research suggests, though the evidence is somewhat conflicting, that the efficacy of NPY is contingent upon genetic background and/or prior history of ethanol dependence in rats. However, studies looking at the effects of NPY in nonselected animals lacking a history of ethanol dependence have two factors that could impact the interpretation of the results: ethanol history/selection AND relatively low baseline ethanol intakes as compared to ethanol-dependent and/or genetically selected controls. The purpose of the present study was to generate higher baseline ethanol intakes upon which to examine the effects of NPY on ethanol and sucrose drinking in nonselected rats using a binge drinking model. Long Evans rats were trained to complete a single response requirement resulting in access to either 2% sucrose (Sucrose Group) or 2% sucrose/10% ethanol (Ethanol Group) for a 20-min drinking session. On treatment days, rats were bilaterally microinjected into the CeA with aCSF or one of three doses of NPY (0.25ug, 0.50ug, or 1.00ug/.5uL). Subjects in the Ethanol Group were consuming an average of 1.2 g/kg of ethanol (yielding BELs of ~90mg%) during the 20 minute access period following aCSF treatments. The results revealed that NPY had no effect on either sucrose or ethanol consumption or on appetitive responding (latency to respond). Overall, the findings indicate that even a history of binge-like ethanol consumption is not sufficient to recruit CeA NPY activity, and are consistent with previous studies showing that the role of NPY in regulating ethanol reinforcement in the CeA may be contingent upon a prior history of ethanol dependence.

Keywords

Neuropeptide Y; Central Nucleus of the Amygdala; ethanol consumption; sipper-tube model

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1. Introduction

Neuropeptide Y (NPY), a 36 amino acid polypeptide, has been investigated for its ability to regulating alcohol consumption. Following initial studies in transgenic mice that seemed to suggest an inverse relationship between NPY and alcohol intake (Thiele et al., 1998), subsequent studies evaluated the ability of NPY to attenuate ethanol intake when administered intracerebroventricularly (ICV; Badia-Elder et al., 2001, 2003; Bertholomey et al., 2011; Gilpin et al., 2003, 2005, 2011; Katner et al., 2002b, Slawecki et al., 2000; Thorsell et al., 2005a,b). Results from these studies hinted that the ability of NPY to attenuate ethanol reinforcement might be modulated by the anxiolytic effects of NPY. One likely site of action for these effects could be the central nucleus of the amygdala (CeA), which has been demonstrated as having a role in regulating both alcohol consuming behaviors (for a review, see McBride, 2002) and anxiety (Heilig et al, 1993). Consistent with this, nonselected rats deemed as “anxious” demonstrated a reduction in alcohol intake following the infusion of a NPY vector into the CeA compared to “non-anxious” rats (Primeaux et al., 2006). Subsequent studies revealed that intra-amygdalar administration of NPY was capable of attenuating operant responding and ethanol self-administration in rats selectively bred to prefer high amounts of alcohol (Gilpin et al., 2008b; Pandey et al., 2005; Zhang et al., 2010) and in nonselected lines with a history of ethanol dependency (Gilpin et al., 2008a; Thorsell et al., 2007). However, similar to ICV studies, NPY did not have an effect in nonselected rats lacking a history of ethanol dependence (Katner et al., 2002a; Gilpin et al., 2008a).

These studies would seem to suggest that the efficacy of NPY is contingent upon genetic background and/or a prior history of ethanol dependence. However, the ethanol dependent controls and genetically selected controls consume ethanol at baseline levels of 0.8-1.2 g/kg in these limited access paradigms (Gilpin et al., 2008a; Thorsell et al., 2005a,b). By comparison, many of the studies that focus on the effects of NPY in nonselected rats lacking a history of ethanol dependence report relatively low baseline ethanol intakes (< 0.5 g/kg) in similar limited access models (Katner et al., 2002a; Gilpin et al., 2008a). Such relatively low baseline ethanol intakes could result in the failure to detect measurable effects of NPY either due to a floor effect or by producing intakes insufficient for animals to experience the pharmacological/reinforcing effects of ethanol that may promote problem drinking, initially typified by the onset of binge drinking patterns (Bonomo et al., 2004; Chassin et al., 2002; Hill et al., 2000; Pitkanen et al., 2005). Moreover, the differential baseline ethanol intakes mean that there are two differences between the experimental groups of interest in these studies. Therefore, it would be beneficial to implement the use of a model capable of generating higher ethanol intake comparable to the ethanol-dependent and genetically selected animals in the previous experiments in order to determine whether or not nonselected animals require a history of ethanol dependence induction in order for NPY to be effective, or whether the effects of NPY are simply contingent on the amount of ethanol being consumed.

One such paradigm capable of producing binge-like drinking levels, defined as attaining a BEL of 80 mg% within a two hour time frame (NIAAA, 2004), is the sipper tube model (Samson et al., 1998; 1999; 2000). This model procedurally separates ethanol seeking and drinking by requiring rats to complete an operant response requirement (RR) in order to gain unrestricted access to ethanol for 20 minutes. Further, by eliminating the need for a contingent operant response that regularly precedes access to a reinforcer at frequent intervals, the sipper tube model avoids the confound of the pharmacological effects of ethanol interfering with the animal’s physical ability to emit a response. Therefore, the purpose of the present study was to use the sipper tube model and the addition of sucrose in the reinforcer solution to: (1) generate higher baseline ethanol intakes and BELs upon which to (2) examine the effects of NPY administered into the CeA on both ethanol and sucrose self-administration in nonselected rats.

2. Methods

2.1 Subjects

Subjects were experimentally naive adult male Long-Evans rats that were obtained from Harlan (Indianapolis, IN) weighing approximately 220 (+ 4.0) g just prior to the start of operant training and averaging 431 (+ 7.7) g on the day of surgery. Three rats used for the BEL timecourse did not undergo surgery (see section 2.7 below). All rats were single housed in standard plastic cages and maintained in a vivarium on a 12:12 hour light/dark cycle (lights on at 700 am). All rats were provided *ad libitum* access to both food and water except where noted. All experimental procedures were done in accordance with NIH guidelines (National Research Council, 1996) and approved by the Indiana University School of Medicine Institutional Animal Care and Use Committee.

2.2 Drugs

The sucrose/ethanol solution was prepared volume/volume in water from 95% (v/v) ethanol and the sucrose solution was used as a solute. Neuropeptide Y (Human, American Peptide Company, Inc., Sunnyvale, CA) was prepared fresh each day just prior to the start of microinjections by dissolving various concentrations in sterile artificial cerebrospinal fluid (aCSF) resulting in a volume of 0.5 μl aCSF containing either 0.0, 0.25, 0.5, or 1.0 μg NPY.

2.3 Stereotaxic Surgeries

Bilateral implantation of cannula aimed at the CeA was performed using aseptic procedures. Rats were anaesthetized via sodium pentobarbital (50 mg/kg intraperitoneally) and placed into a digital stereotaxic apparatus (Benchmark Digital Stereotaxic, myNeurolab, St. Louis, MO) with the incisor bar set at 3.3 mm below the interaural line. Stainless steel guide cannulae (13 mm, 26 gauge) were implanted and terminated 1 mm dorsal to the left and right central amygdaloid nuclei as determined by Paxinos and Watson (1998; coordinates from bregma: anteroposterior −2.1; mediolateral + 4.0; ventral −6.4). The cannulae were secured to the skull using 4 stainless steel screws and cranioplastic cement was applied over the surface of the skull covering both the screws and guide cannula as a means of closing the incision. Removable wire obterators (33 gauge) cut to the same length as the guide cannulae were inserted into the guide cannulae to prevent obstruction and to maintain patency and checked daily. Following surgery, rats were placed on a heating pad until they were awake and alert. All animals resumed training on the following day and were monitored for a week prior to the initiation of any testing to be sure that they resumed their pre-surgery behaviors.

2.4 Operant Training

2.4.1 Apparatus—Operant sessions were conducted in Med-Associates chambers (St. Albans, VT, USA; 30 × 30 × 24.5 cm) equipped with a house light and 2 retractable levers located above the grid floor on the wall opposite of the retractable sipper tube. The sipper tube was a graduated cylinder tube fitted with a rubber stopper and stainless steel spout containing double ball bearings to prevent spillage. Operant chambers were individually housed in sound-attenuated ventilated cubicles to minimize environmental disturbances. Electrical inputs and outputs from each chamber were controlled using Med-Associates software (Med-Associates).

2.4.2 Training Sessions—Following arrival, all subjects were given a week to acclimate during which time they were weighed and handled. Next, to initiate operant responding, all rats were water restricted (~ 20 mLs) in the home cage during the first few days of operant training and initially shaped in a 1-hour operant session on a FR1 schedule allowing for 30 second access to a 10% sucrose solution. Over the next few days, daily training sessions were reduced to 30 minutes and 15 second sipper tube access and the response requirement steadily increased from an FR1 to an FR4 and water restriction was discontinued. To facilitate ethanol intake, a modified sucrose fading procedure (Samson, 1986) was used whereby rats were allowed 1-2 sessions with solutions decreasing in sucrose and increasing in ethanol concentrations. The solutions presented were: 10% sucrose; 10% sucrose/2% ethanol; 10% sucrose/5% ethanol; 10% sucrose/10% ethanol; 5% sucrose/10% ethanol; and 2% sucrose/10% ethanol. The final concentrations of the solution used were 2% sucrose/ 10% ethanol (2S10E) for the ethanol group. A 2% sucrose (2S) sucrose group had identical training but no ethanol exposure and was used to control for leaving the sucrose solution in the ethanol solution. Following acquisition of the FR4 schedule (approximately 3 weeks), subjects were switched to a response requirement (RR) schedule whereby a number of responses were required in order to gain 20 minutes of uninterrupted access to the sipper tube containing either the ethanol or sucrose reinforcer. Gradually, the RR was increased from an RR4 to an RR10. Following approximately 4 weeks of training on the RR schedule, rats underwent bilateral stereotaxic cannulation of the CeA (see above).

2.4.3 Microinjections and Operant Testing Days—Starting during their recovery week, rats were handled and acclimated to having their obterators adjusted. Following recovery from surgery, rats resumed normal training sessions on Mondays, Tuesdays, Thursdays, and Fridays. Testing/infusion days occurred on Wednesday of each week. On two sequential Wednesdays prior to testing, subjects first underwent a mock infusion and then an aCSF microinjection to habituate them to the microinjection procedures. NPY testing began the following Wednesday during which rats were bilaterally microinjected with one of three doses of NPY (0.25, 0.50, or 1.0 μg) or aCSF in a within-subjects pseudo random Latin square design in a volume of 0.5 μl/side over a 1 minute period. The injectors were left in place for an additional 30 seconds following the microinjection to allow for adequate diffusion. All microinjections were delivered through sterile stainless steel injectors (14mm, 33 gauge) that extended 1 mm beyond the guide cannula connected via PE 50 tubing to 25.0 μl syringes (Hamilton, Reno, NV) using Harvard Apparatus Infusion Pumps (model 22; Harvard Apparatus, Holliston, MA). Following the completion of the microinjection procedure, rats were placed back in the holding cages for 30 minutes after which point they were placed in the operant chambers. NPY doses and pretreatment time interval was based upon Gilpin et al. (2008a) that used the same parameters. Additionally, because the behavior of interest was primarily consumption, on testing days, the response requirement (RR) was lowered from 10 to 1, after which rats had 20 minutes of access to the reinforcer. Intakes (both mLs and g/kg) and response latencies were recorded. At the completion of the balanced drug infusion, during a final week of testing, an aCSF infusion was given on Wednesday and blood collection for BEL determination was conducted on Friday (ethanol group only).

2.5 Blood Ethanol Levels (BELs)

Subjects in the ethanol group underwent one final operant session immediately after which they were gently restrained and blood samples were collected to assess blood ethanol levels. This session was conducted on the Friday following all experimental manipulations, since the sample collection procedures can interfere with operant responding and ethanol intake behaviors. Specifically, to ensure that the collection could occur immediately at the end of the reinforcer access, animals individual start times were staggered (to allow enough time between animals to complete the sample collection) and blood samples were collected in the same room as the behavioral testing. Blood samples (100 μl) were collected from the cut tip of the tail in heparinized capillary tubes and placed on ice during the collection procedures. Immediately after, they were centrifuged and plasma samples (5 μl) were assessed using an Analox AM 1 analyzer (Analox Instruments LTD, Lunenberg, MA) to determined blood ethanol levels (BEL; mg%). Ethanol concentration was determined with an amperometric oxygen electrode that measures oxygen consumption during the enzymatic oxidation of alcohol to acetaldehyde.

2.6 Histological Verification

Immediately following the final operant session, rats were deeply anesthetized (120 mg/kg sodium pentobarbital intraperitoneally) and transcardially perfused with phosphate buffered saline (pH 7.4) followed by 10% formalin. Brains were removed and stored in 10% formalin for a minimum of 10 days. Brains were then cut (30 micron sections) on a cryostat (Bright Instrument Company, St. Louis, MO), mounted, and stained with cresyl violet to determine injection locations using light microscopy.

2.7 BEL Timecourse

For a more thorough determination of BELs resulting from ethanol self-administration in this model, three rats underwent identical operant training as described, but did not undergo any surgical procedures. Blood sampling procedures took place following three weeks of performing on the RR4 schedule with the 2S10E solution. To ensure that the collection procedures did not affect the behaviors of the other subjects, for this timecourse determination only one rat was sampled on any one day, and sampling occurred in a separate room from the operant testing. Over a three week period, each of the three rats had one sample taken at either 10, 15, or 20 minutes after the sipper tube descended into the chamber (i.e., for the 10 and 15 minute samples, animals were removed from the operant chamber earlier than on any “normal” session). Blood was collected and the BEL determined as described above.

2.8 Statistical Analyses

Ethanol (g/body weight kg) and sucrose intakes (mLs) were measured from the sipper tube volumes and daily body weights and were subjected to separate one-way repeated measures analyses of variance (ANOVAs). Latency to lever press and latency to lick were also run in separate one-way repeated measure ANOVAs to determine the effects of NPY on appetitive responding. When appropriate, Bonferroni post hoc analyses were performed. In all cases, the significance level was set at $p < 0.05$. Pearson’s correlation was used to determine the relationship between ethanol intake (g / kg) and BEL (mg%).

3. Results

Only rats with confirmed bilateral cannulae placement in the CeA were included in the final data analyses of NPY’s effects. Of the 42 total animals that began the experiments, 28 were eliminated. The CeA seemed to be a particularly sensitive site in terms of surgery survival, and adverse reactions to microinjector placement (which was confirmed via consultation with other investigators). Twelve animals failed to recover fully in the first hour after successfully completing surgery, and five needed to be sacrificed following adverse reactions and extreme weight loss in the hours following the first microinjector placement (no aCSF). One subject was eliminated prior to surgery for failing to acquire operant responding, and 10 were eliminated due to misplaced cannula. The final group totals were 6 in the ethanol group and 8 in the sucrose group.

Figure 1 shows the total intake of ethanol (g/kg) following microinjection of aCSF across the entire experiment. This includes an initial aCSF administration the week following the mock infusion, the aCSF infusion that occurred within the NPY balanced design treatments (i.e., one to four weeks later), and a final aCSF administration the week following the balanced design infusions (i.e., five weeks after the first aCSF infusion). Notably, ethanol intake was stable and high. Specifically, a one-way repeated measures ANOVA indicated no changes in ethanol intake across the experiment [$F(2,10) = 0.276$, $p = 0.77$], and out of 18 data points, at only one time did one animal consume below 0.8 g/kg ethanol (0.67 g/kg on the first aCSF treatment).

Total ethanol intake (g/kg) following all doses of NPY and the aCSF administration that occurred within the balanced dose design are depicted in Figure 2. Analysis confirms that ethanol intake was consistent across all NPY doses and aCSF infusion, with no significant differences across the treatments [$F(3,23) = 0.62$, $p = 0.61$]. Similarly, Figure 3 shows the intake of sucrose (mLs) following microinjections across all doses of NPY and aCSF, and again there was no effect of NPY on sucrose intake in either mLs [$F(3,31) = 0.15$, $p = 0.93$] or in g/kg [$F(3,31) = 0.12$, $p = 0.95$] *(data not shown)*.

To evaluate the potential effects of NPY on the motivation to seek either an ethanol or sucrose reinforcer, the latencies to first lever press (following lever-extension) and to lick (following response completion and sipper extension) were evaluated. Results revealed that NPY did not affect the latency to first lever press for either ethanol [$F(3,23) = 1.02$, $p = 0.41$] or sucrose [$F(3,31) = 1.08$, $p = 0.38$]. Similarly, NPY did not significantly alter the latency to lick in rats reinforced with ethanol [$F(3,23) = 1.25$, $p = 0.33$] or sucrose [$F(3,31) = 0.85$, $p = 0.48$], indicating that NPY does not differentially affect the motivation to seek ethanol or sucrose in these rats.

Figure 4 shows a scatter plot of the blood ethanol levels (BELs; mg%) and ethanol intakes (g/kg) produced on a single session following the experimental manipulations in the ethanol group. There was a significant correlation between intake and BEL, $r = 0.95$, $p < 0.01$. The average intake of ethanol was 0.88 g/kg and correlated with an average BEL of 64.8 mg%. This was somewhat below the average intake of ethanol following the microinjections of aCSF in these animals (Figure 1: 1.16-1.26 g/kg), which would correspond with a BEL of approximately 90mg%. The data from the timecourse of ethanol intake and BEL measures are shown in Figure 5. Again there was a significant correlation between intake and BEL, $r = 0.90$, $p < 0.001$. At 15 or 20 minutes into a regularly 20-minute session, all animals had consumed ≥0.80 g/kg ethanol, and all animals had BELs measuring ≥80mg% at at least one timepoint.

4. Discussion

Overall, while the use of the sipper-tube model generated binge-like ethanol drinking capable of generating high baseline ethanol intake and BELs in Long Evans rats not selected for ethanol preference, the infusion of NPY into the CeA failed to suppress either ethanol or sucrose intake. These results are consistent with previous reports that have found NPY to have no effect on ethanol intake in nondependent non-selected rats consuming ethanol in the < 0.5 g/kg range with either free (Katner et al., 2002a) or operant access (Gilpin et al., 2008a). The higher baseline ethanol intakes achieved in the present study were comparable to the baseline levels of intake observed in previous studies utilizing ethanol-dependent and genetically selected rats (Gilpin et al., 2008a; Thorsell et al., 2005a,b). Additionally, given that Gilpin and colleagues (2008a) specifically looked at the effects of NPY in the CeA on ethanol-reinforced responding in Wistar rats as a function of ethanol dependence, this experiment utilized identical NPY doses and pretreatment times specifically to assess whether the ability of NPY to attenuate ethanol is a consequence of high ethanol intake or a history of dependency, something the previous study could not conclusively differentiate.

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The findings from the current study uniquely clarify this distinction and suggest that NPY does not modulate ethanol intake in the CeA as a function of amount of intake and provide more evidence that a history of ethanol dependence may be a requisite for NPY to effectively modulate ethanol drinking.

NPY also did not significantly affect the latency to first lever press or to initiate drinking, suggesting that NPY does not affect appetitive responding. This is consistent with the results of Thorsell and colleagues (2005b) that investigated whether or not centrally administered NPY differentially affected the seeking versus consumption of ethanol following nine weeks of ethanol vapor exposure in nonselected Wistar rats. NPY decreased dependence-induced ethanol drinking, but not lever-press responding in that study (Thorsell et al., 2005b). Together with the present findings in nondependent Long Evans rats, the ability of NPY to regulate ethanol reinforcement appears to be specific for ethanol intake as compared to ethanol-seeking, regardless of whether or not rats have a history of ethanol dependence.

The inability of NPY to alter sucrose intake in this study is also consistent with the findings of others that have determined NPY infusion into the CeA has no effect on either intake or responding for food (Gilpin et al. 2008b; Heilig et al., 1993) or other palatable reinforcers (Katner et al., 2002a). This finding also implies that the sucrose component of the ethanol solution did not interfere with the ability of NPY to alter ethanol intake in this study and provides further evidence for the dissociation of the NPY pathways that regulate NPY's potent orexigenic effects and those that modulate ethanol reinforcement. The orexigenic effects of NPY are believed to be mediated through the paraventricular nucleus (PVN) of the hypothalamus. Microinjection of NPY into the PVN has been shown to produce hyperphagia and weight gain in rodents and to increase both feeding and ethanol intake in high and low selected and outbred rats (Gilpin et al., 2004; Kelley et al., 2001; Stanley & Leibowitz, 1984, 1985).

In contrast, the ability of NPY to modulate ethanol intake is believed to be mediated by the anxiolytic effects of NPY, particularly in the amygdala (Heilig et al., 1989, 1992, 1993; Sajdyk et al, 1999). For example, rats that have been selectively bred for high and low alcohol preference (P and NP rats, respectively; Li et al., 1993; Murphy et al., 2002) have been shown to differ from each other not only in alcohol intake but also in basal anxiety levels (Pandey et al., 2005; Salimov et al., 1996; Stewart et al., 1993; Zhang et al., 2010) with P rats demonstrating greater anxiety compared to NP rats. Further research found that both voluntary and injected ethanol decrease anxiety in P but not NP rats in tests of anxiety, including the elevated plus maze (EPM) and light/dark box (Pandey et al., 2005; Zhang et al., 2010) without affecting general activity. Also, compared to the NP rat, P rats have lower NPY protein levels in the CeA which increase with voluntary alcohol administration (Pandey et al., 2005). Finally, microinjection of NPY into the CeA of P rats has also shown to decrease anxiety (as measured by both the EPM and light/dark box) and to attenuate ethanol intake (Pandey et al., 2005; Zhang et al. 2010).

In nonselected Wistar rats, the insertion of a viral vector into the CeA resulting in NPY overexpression decreased anxiety as assessed in the open field test and attenuated a deprivation-induced increase in ethanol intake (Thorsell et al., 2007). Similar use of the viral vector in Long Evans rats resulted in a decrease in ethanol preference of "anxious" but not "nonanxious" rats, while injection of a NPY-antisense vector increased ethanol preference specifically in the "anxious" rats (Primeaux et al., 2006). Currently, research is underway to more definitively understand the neural mechanisms that serve to co-regulate high anxiety and excessive alcohol intake. Molecular approaches suggests a role for the PKA-CREB-NPY signaling cascade within the CeA as potentially mediating differences in the co-expression of high basal anxiety and alcohol preference (Pandey et al., 2005; Zhang et al.,

2010) while electrophysiological data collected from alcohol-dependent and naïve Sprague-Dawley rats suggest a role for NPY in differentially mediating GABAergic transmission in the CeA as a function of dependence (Gilpin et al., 2011).

While the precise mechanisms regulating the role of NPY in alcohol intake and anxiety in the CeA remain unclear, overall, the findings from this study strengthen the theory that putative neuroadaptations related to the association between anxiety and alcohol-dependence may create a role for CeA NPY in ethanol-reinforced responding. In addition, these findings add to the growing literature identifying differences in the efficacy of "treatment approaches" for alcohol use versus dependence (Gilpin, 2008a; Walker et al., 2008, 2011), allowing for better development of targeted pharmacotherapies.

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References

Badia-Elder NE, Stewart RB, Powrozek TA, Murphy JM, Li T-K. 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; 27:894–99. [PubMed: 12824809]

Badia-Elder NE, Stewart RB, Powrozek TA, Roy KF, Murphy JM, Li T-K. Effect of Neuropeptide Y (NPY) on oral ethanol intake in wistar, alcohol-preferring (P), and -nonpreferring (NP) rats. Alcohol Clin Exp Res. 2001; 25:386–90. [PubMed: 11290849]

Bertholomey ML, Henderson AN, Badia-Elder NE, Stewart RB. Neuropeptide Y (NPY) -induced reductions in alcohol intake during continuous access and following alcohol deprivation are not altered by restraint stress in alcohol-preferring (P) rats. Pharmacol Biochem Behav. 2011; 97:453–61. [PubMed: 20937300]

Bonomo YA, Bowes G, Coffey C, Carlin JB, Patton GC. Teenage drinking and the onset of alcohol dependence: a cohort study over seven years. Addiction. 2004; 99:1520–8. [PubMed: 15585043]

Chassin L, Pitts SC, Prost J. Binge drinking trajectories from adolescence to emerging adulthood in a high-risk sample: predictors and substance abuse outcomes. J Consult Clin Psychol. 2000; 70:67–78. [PubMed: 11860058]

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; 69:1091–99. [PubMed: 21459365]

Gilpin NW, Misra K, Koob GF. Neuropeptide Y in the central nucleus of the amygdala suppresses dependence-induced increase in alcohol drinking. Pharmacol Biochem Behav. 2008a; 90:475–80. [PubMed: 18501411]

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. 2008b; 90:470–74. [PubMed: 18499241]

Gilpin NW, Stewart RB, Murphy JM, Badia-Elder NE. Neuropeptide Y in the paraventricular nucleus of the hypothalamus increases ethanol in high- and low-alcohol drinking rats. Alcohol Clin Exp Res. 2004; 28:1492–98. [PubMed: 15597081]

Gilpin NW, Stewart RB, Murphy JM, Badia-Elder NE. Sensitized effects of neuropeptide Y on multiple ingestive behaviors in P rats following ethanol abstinence. Pharmacol Biochem Behav. 2005; 81:740–49. [PubMed: 16023707]

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; 27:787–94. [PubMed: 12766623]

Heilig M, McLeod S, Brot M, Heinrichs SC, Menzaghi F, Koob GF, et al. Anxiolytic-like actions of neuropeptide Y: mediation by Y1 receptors in amygdala and dissociation from food intake effects. Neuropsychopharmacology. 1993; 8:357–63. [PubMed: 8099792]

Heilig M, McLeod S, Koob GK, Britton KT. Anxiolytic-like effect of neuropeptide Y (NPY), but not other peptide in an operant conflict test. Reg Peptides. 1992; 41:61–9.

Heilig M, Söderpalm B, Engel JA, Widerlöv E. Centrally administered neuropeptide Y (NPY) produces anxiolytic-like effects in animal anxiety models. Psychopharmacology. 1989; 98:524–29. [PubMed: 2570434]

Hill KG, White HR, Chung IJ, Hawkins JD, Catalano RF. Early adult outcomes of adolescent binge drinking: person- and variable-centered analyses of binge drinking trajectories. Alcohol Clin Exp Res. 2000; 24:892–901. [PubMed: 10888080]

Katner SN, Slawecki CJ, Ehlers CL. Neuropeptide Y administration into the amygdala does not affect ethanol consumption. Alcohol. 2002a; 28:29–38. [PubMed: 12377358]

Katner SN, Slawecki CJ, Ehlers CL. Neuropeptide Y administration into the third ventricle does not increase sucrose or ethanol self-administration but does affect the cortical EEF and increases food intake. Psychopharmacology. 2002b; 160:146–54. [PubMed: 11875632]

Kelley SP, Nannini MA, Bratt AM, Hodge CW. Neuropeptide Y in the paraventricular nucleus increases ethanol self-administration. Peptides. 2001; 22:515–522. [PubMed: 11287109]

Li T-K, Lumeng L, Doolittle DP. Selective breeding for alcohol preference and associated responses. Behav Genet. 1993; 23:163–70. [PubMed: 8099788]

McBride WJ. Central nucleus of the amygdala and the effects of alcohol and alcohol-drinking behavior in rodents. Pharmacol Biochem Behav. 2002; 71:509–15. [PubMed: 11830185]

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; 32:363–88. [PubMed: 12405517]

National Research Council. Guide for the care and use of laboratory animals. National Research Council; Washington, D.C.: 1996.

NIAAA. 2004 Winter;

Pandey SC, Zhang H, Roy A, Xu T. Deficits in amygdaloid camp-responsive element-binding protein signaling play a role in genetic predisposition to anxiety and alcoholism. J Clin Invest. 2005; 115:2762–73. [PubMed: 16200210]

Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. 4nd ed. Academic Press; San Diego CA: 1998.

Pitkanen T, Lyyra AL, Pulkkinen L. Age of onset of drinking and the use of alcohol in adulthood: a follow-up study from age 8-42 for females and males. Addiction. 2005; 100:652–61. [PubMed: 15847623]

Primeaux SD, Wilson SP, Bray GA, York DA, Wilson MA. Overexpression of Neuropeptide Y in the central nucleus of the amygdala decreases ethanol self-administration in “anxious” rats. Alcohol Clin Exp Res. 2006; 30:791–801. [PubMed: 16634847]

Sajdyk TJ, Vandergriff MF, Gehlert DR. Amygdalar neuropeptide Y Y1 receptors mediate the anxiolytic-like actions of neuropeptide Y in the social interaction test. Eur J Pharmacol. 1999; 386:143–7. [PubMed: 10193650]

Salimov RM, McBride WJ, Sinclair JD, Lumeng L, Li T-K. Performance in the cross-maze and slip funnel tests of four pairs of rat lines selectively bred for divergent alcohol drinking behavior. Addict Biol. 1996; 1:273–80. [PubMed: 12893466]

Samson HH. Initiation of ethanol reinforcement using a sucrose-substitution procedure in food- and water-sated rats. Alcohol Clin Exp Res. 1986; 10:436–42. [PubMed: 3530023]

Samson HH, Czachowski CL, Slawecki CJ. A new assessment of the ability of oral ethanol to function as a reinforcing stimulus. Alcohol Clin Exp Res. 2000; 24:766–73. [PubMed: 10888063]

Samson HH, Sharpe AL, Denning C. Initiation of ethanol self-administration in the rat using sucrose substitution in a sipper-tube procedure. Psychopharmacology. 1999; 147:274–79. [PubMed: 10639685]

Samson HH, Slawecki CJ, Sharpe AL, Chappell A. Appetitive and consummatory behaviors in the control of ethanol consumption: A measure of ethanol seeking behavior. Alcohol Clin Exp Res. 1998; 22:1783–87. [PubMed: 9835295]

Slawecki CJ, Betancourt M, Walpole T, Ehlers CL. Increases in sucrose consumption but not ethanol consumption following ICV NPY administration. Pharmacol Biochem Behav. 2000; 66:591–94. [PubMed: 10899375]

Stanley BG, Leibowitz SF. Neuropeptide Y: Stimulation of feeding and drinking by injection into the paraventricular nucleus. Life Sci. 1984; 35:2635–42. [PubMed: 6549039]

Stanley BG, Leibowitz SF. Neuropeptide Y injected in the paraventricular hypothalamus: A powerful stimulant of feeding behavior. Nat Acad Sci Prod USA. 1985; 82:3940–43.

Stewart RB, Gatto GJ, Lumeng L, Li T-K, Hwang BH. Comparison of alcohol-preferring (P) and non-preferring (NP) rats on tests of anxiety and for the anxiolytic effects of ethanol. Alcohol. 1993; 10:1–10. [PubMed: 8095393]

Thiele TE, Marsh DJ, Ste Marie L, Bernstein IL, Palmiter RD. Ethanol consumption and resistance are inversely related to neuropeptide Y levels. Nature. 1998; 396:366–69. [PubMed: 9845072]

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; 130:1330–37. [PubMed: 17405766]

Thorsell A, Slawecki CJ, Ehlers CL. Effects of neuropeptide Y and corticotropin-releasing factor on ethanol intake in wistar rats: Interaction with chronic ethanol exposure. Behav Brain Res. 2005a; 161:133–40. [PubMed: 15904720]

Thorsell A, Slawecki CJ, Ehlers CL. Effects of neuropeptide Y on appetitive and consummatory behaviors associated with alcohol drinking in wistar rats with a history of ethanol exposure. Alcohol Clin Exp Res. 2005b; 29:584–90. [PubMed: 15834223]

Walker BM, Rasmussen DD, Raskind MA, Koob GF. alpha1-noradrenergic receptor antagonism blocks dependence-induced increases in responding for ethanol. Alcohol. 2008; 42:91–7. [PubMed: 18358987]

Walker BM, Zorrilla EP, Koob GF. Systemic κ-opioid receptor antagonism by nor-binaltorphimine reduces dependence-induced excessive alcohol self-administration in rats. Addict Biol. 2011; 16:116–9. [PubMed: 20579007]

Zhang H, Sakharkar AJ, Shi G, Ugale R, Prakash A, Pandey SC. Neuropeptide Y signaling in the central nucleus of amygdala regulates alcohol-drinking and anxiety-like behaviors in alcohol-preferring rats. Alcohol Clin Exp Res. 2010; 34:451–61. [PubMed: 20028368]

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Highlights

- At baseline, the paradigm yielded EtOH intake in the pharmacologically relevant range (~90mg%)
- CeA NPY did not affect ethanol-reinforced behavior, despite its role in ethanol-dependent rats
- Binge-like ethanol consumption is not sufficient to recruit CeA NPY regulation of seeking/intake

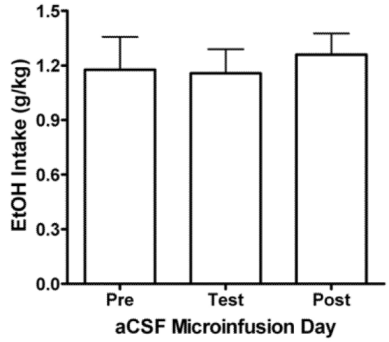

Figure 1.
In the ethanol group, mean (+ SEM) total ethanol intake (g/kg) during the 20 minutes of reinforcer access following completion of a single response requirement across the three aCSF treatments (before, during, and after the administration of NPY in a balanced design) spanning the six-week experiment.

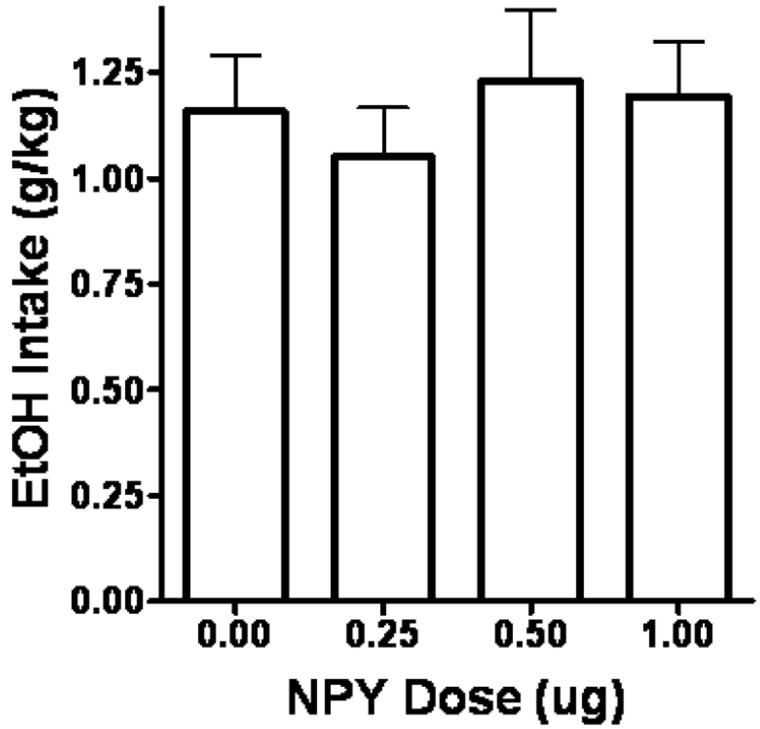

Figure 2.

In the ethanol group, mean (+ SEM) total ethanol intake (g/kg) during the 20 minutes of reinforcer access following completion of a single response requirement. Microinjections of aCSF or NPY were administered bilaterally into the CeA (-30 min; doses are total per animal) over a four week period.

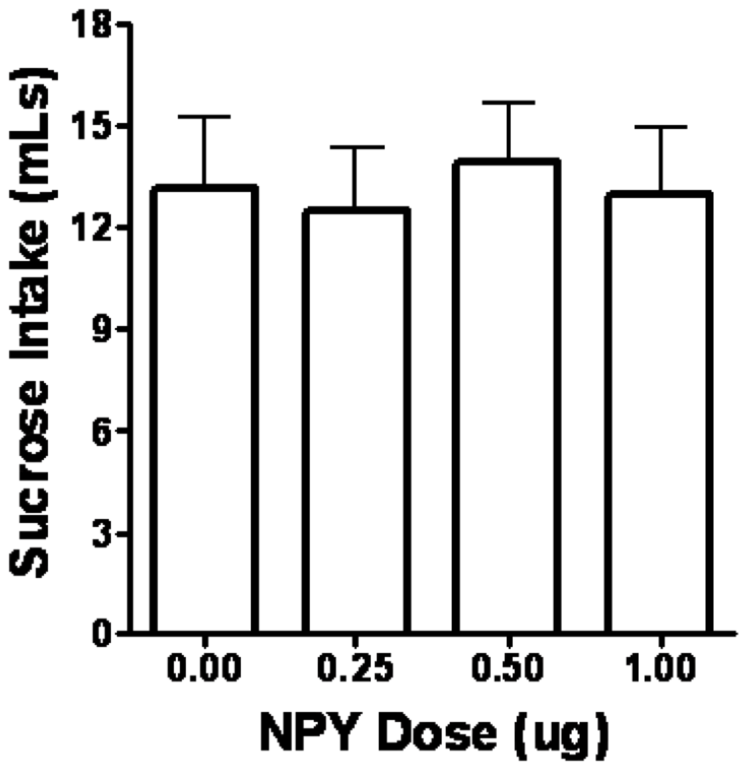

Figure 3.
In the sucrose group, mean (+ SEM) total sucrose intake (mLs) during the 20 minutes of reinforcer access following completion of a single response requirement. Microinjections of aCSF or NPY were administered bilaterally into the CeA (-30 min; doses are total per animal) over a four week period.

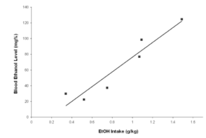

Figure 4.

Scatter plot of total g/kg ethanol intake as a function of blood ethanol levels (BELs) during a single session that followed the completion of all experimental manipulations in the ethanol group. Blood samples were taken at the end of 20 minutes of reinforcer access to 10% alcohol in 2% sucrose and water following completion of a single response requirement. There was a significant correlation (r = 0.95) between alcohol intake and BELs, p < 0.01.

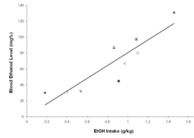

Figure 5.

Scatter plot of g/kg ethanol intake as a function of blood ethanol levels (BELs) taken at three different time points (10 min = diamonds; 15 min = triangles; 20 min = squares) from three different subjects (designated by open, open/shadow, and closed symbols). Note that when removed from the chambers at either 15 or 20 minutes into a regularly 20-minute session, all animals had consumed ≥0.80 g/kg ethanol, and all animals had BELs measuring ≥80mg% at at least one timepoint. There was a significant correlation ($r = 0.90$) between alcohol intake and BELs, $p < 0.001$.

Table 1

		Dose of NPY (μg)		
	aCSF	**2.5 μg**	**5.0 μg**	**10.0μg**
Latency to Lever Press (s)				
EtOH	28.9(12.6)	78.9(33.4)	32.1(16.8)	47.2(24.1)
Sucrose	22.6(8.7)	25.2(14.0)	44.1(22.1)	19.7(11.8)
Latency to Lick (s)				
EtOH	1.5(0.1)	2.9(1.0)	2.1(0.5)	1.8(0.2)
Sucrose	4.3(3.1)	1.4(0.1)	1.7(0.3)	1.5(0.2)