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Sedative and motor-impairing effects of neuropeptide Y and ethanol in selectively bred P and NP rats

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Abstract

Past findings suggest a positive association between endogenous neuropeptide Y (NPY) activity and ethanol-induced sedation, and there is evidence for additive effects of administered NPY with sedative-hypnotics. The present investigation examined the effects of intracerebroventricular NPY injection on ethanol-induced sedation and motor impairment in selectively bred alcohol-preferring (P) and -nonpreferring (NP) rats. In Experiment 1, P and NP rats were assessed for loss and recovery of righting reflex (RR) following infusion with either NPY (10.0 μg) or aCSF followed by ethanol injection (2.5 g/kg ip). NPY reduced time to lose RR and increased time to regain RR similarly in P and NP rats. Blood–ethanol levels (BELs) were lower at time of recovery in NPY-treated rats relative to aCSF controls. Thus, NPY enhanced ethanol-induced sedation. In Experiment 2, P and NP rats pretreated with either saline or ethanol (1.0 g/kg ip) were assessed for motor activity following infusion with either NPY (2.5, 5.0, or 10.0 μg) or aCSF. Ethanol alone and NPY alone suppressed motor activity, but there were no additive effects between the two. Taken together, these results provide partial support for past observations of additivity between NPY and drug-induced sedation, and suggest a role for NPY in the neurobehavioral effects of acute ethanol exposure.

Keywords: Neuropeptide Y; Ethanol sedation; Spontaneous motor activity; Alcohol-preferring and -nonpreferring rats

1. Introduction

Characterization of the behavioral effects of neuropeptide Y (NPY) has recently accelerated as increasing numbers of functional roles for the peptide have been delineated. Exogenously administered NPY produces anxiolytic (Heilig et al., 1989a) and orexigenic (Clark et al., 1984; Jolicoeur et al., 1991; Zarjevski et al., 1993) effects. In addition to these well-characterized effects, exogenous NPY can also produce sedation (Heilig et al., 1989b), and alterations of endogenous NPY activity may affect sensitivity to the sedative effects of ethanol (Thiele et al., 1998). Following ethanol injection, transgenic mice that lack NPY ($NPY^{-/-}$) regain the righting reflex (RR) more rapidly than wild-type controls, and those which overexpress NPY (NPY-OX) regain the RR more slowly than wild-type controls (Thiele et al., 1998). Thus, there appears to be a positive association between NPY activity and sensitivity to the sedative effects of ethanol. In addition, $NPY^{-/-}$ mice drink more ethanol than wild-type controls, and NPY-OX mice drink less ethanol than wild-type controls (Thiele et al., 1998), suggesting that there is an inverse relationship between NPY activity and ethanol consumption.

In agreement with the positive association between NPY activity and ethanol-induced sedation, electrophysiological studies have shown that NPY and ethanol have additive effects on the P1 and N3 components of event-related potentials (ERPs) in rats, and the individual effects of NPY and ethanol are nearly identical (Ehlers et al., 1998). Not limited to interactions with ethanol, doses of

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exogenous NPY, which are ineffective when administered alone, prolong loss of RR induced by certain other sedative-hypnotics, such as pentobarbital (PbNa; Yamada et al., 1996). In agreement with the observed inverse relationship between NPY levels and ethanol consumption in transgenic mice (Thiele et al., 1998), intracerebroventricular administration of NPY decreases ethanol consumption in alcohol-preferring (P; Badia-Elder et al., 2001), and in high-alcohol-drinking (HAD; Badia-Elder et al., 2003) rats. However, this effect is not observed in alcohol-nonpreferring (NP), low-alcohol-drinking (LAD), and nonselected rats (Badia-Elder et al., 2001, 2003; Katner et al., 2002; Slawecki et al., 2000). One of the numerous factors that likely contribute to ethanol self-administration behavior could be that sedation places a limit on the amount of ethanol consumed. Thus, a possible explanation for the differential effects of NPY on ethanol drinking between rat lines may be related to the combined sedative effects of NPY and self-administered ethanol. Alcohol-preferring rats may consume sufficient amounts of ethanol to experience these additive, sedative effects, and thus terminate drinking at a smaller ethanol dose than would be consumed in the absence of NPY. Conversely, rats not selectively bred for ethanol drinking may not consume sufficient amounts of ethanol to be affected by this interaction.

There appears to be a positive association between endogenous NPY activity and ethanol-induced sedation (Thiele et al., 1998), and there is evidence for additive effects of NPY with sedative-hypnotics (Ehlers et al., 1998; Yamada et al., 1996). Following from this, the present investigation was designed to examine the effects of intracerebroventricular NPY injection on the sedative and motor-impairing effects of ethanol. In Experiment 1, loss and recovery of RR were used because this test has been previously employed to demonstrate additivity between PbNa and NPY (Yamada et al., 1996). In Experiment 2, motor activity was used because this test is more sensitive to the effects of lower doses of ethanol, and these lower doses may approximate the levels of intoxication experienced by animals during ethanol self-administration. P and NP rats were tested for the additive, sedative effects of NPY and ethanol because they have previously been shown to differ in responsiveness to the sedative and motor-impairing effects of ethanol (Kurtz et al., 1996; Waller et al., 1986) and the effects of NPY on ethanol self-administration (Badia-Elder et al., 2001).

2. Experiment 1

2.1. Methods

2.1.1. Subjects

Subjects were 18 P and 17 NP experimentally naïve female rats (School of Medicine, Indiana University) of the 50th generation of selective breeding that weighed between 231 and 352 g on the day of test session. Female rats were tested because of the limited availability of male P and NP rats and because previous studies from this laboratory have shown similar effects of NPY on ethanol drinking in male and female selectively bred rats (Badia-Elder et al., 2001, 2003; Gilpin et al., 2003). Rats were housed in plastic tub-style cages in a vivarium maintained on a 12-h light/12-h dark cycle (lights off at 1400 h). Food and water were available to all rats ad libitum. All testing was conducted at the beginning of the vivarium dark cycle (1400 h). The protocol for this study was approved by the IUPUI School of Science IACUC and was conducted in accordance with NIH guidelines (National Research Council, 1996).

2.2. Stereotaxic surgery

Surgical implantation of intracerebroventricular cannulae was conducted using aseptic procedures. Preceding surgery, rats were injected with a 1-mg/kg dose of ketamine (ketamine/acepromazine/atropine; Phoenix Pharmaceutical, St. Joseph, MO). Rats were implanted with cannulae into either the left or right lateral ventricle. While anesthetized, the incision area of the scalp was shaved and the rat was placed in a Stoelting stereotaxic instrument. A topical analgesic (lidocaine) was applied to the scalp and a sagittal incision (approximately 2 cm long) was made in the midline, which exposed the surface of the skull. A single hole was drilled through the skull targeted above either the left or right lateral ventricle at a site according to the appropriate stereotaxic coordinates, and a guide cannula was implanted. The stereotaxic coordinates were determined according to Paxinos and Watson (1998). The coordinates, measured from the bregma, were (AP -1.0, ML ± 1.5, DV -3.8). Microinjection cannulae components (Plastics One, Roanoke, VA) included a guide cannula (22 gauge), an internal injection cannula (28 gauge), and a dummy cannula (28 gauge). The injection cannula extended 1.0 mm beyond the tip of the guide cannula when inserted. At all times, except when infusions were conducted, the dummy cannula, cut to the same length as the guide cannula, was maintained in the guide cannula. Four stainless steel screws were inserted into the skull at positions around the cannula implant site. Cranioplastic cement was applied over the open surface of the skull which covered the screws and the guide cannula. The incision was closed around the implant and the dummy cannula was inserted. Immediately after surgery, an antibiotic ointment was applied to the surgical wound area, and rats were injected with 0.8 mg/kg dose of atropine (Atropine Sulfate; Elkins–Sinn, Cherry Hill, NJ).

2.3. Procedure

Rats were given between 10 and 17 days to recover from surgery and were monitored closely and weighed daily during the first 7 days of this period. On test day, rats were infused with either aCSF (5.0 μl; Plasma-Lyte [Electrolyte] Solution, Baxter, Deerfield, IL; $n=8$ P rats, 6 NP

rats) or NPY (Porcine; American Peptide, Sunnyvale, CA; $n = 10$ P rats, 11 NP rats) dissolved in aCSF (10.0 μg/5.0 μl). Experimental groups were assigned such that body weights were matched for groups which received NPY and aCSF. This dose of NPY was selected because it reliably decreases ethanol intake and increases sucrose intake in the selectively bred lines (Badia-Elder et al., 2001, 2003), and also falls within the effective dose range used in other behavioral tests (Jolicoeur et al., 1991). Infusions began at 1400 h, coinciding with the start of the dark cycle. A Harvard 33 microinfusion pump was used for NPY and aCSF infusions, which were delivered at a rate of 2.5 μl/min, and the injection cannula was left in the guide cannula for one additional minute to allow for adequate diffusion of solution. Infusions were delivered to the cannula via polyethylene tubing (PE 50) that was connected to a Hamilton 25-μl syringe. Rats were tethered in their home cage and allowed to move about freely during infusions. Rats were then allowed between 5 and 10 min in their home cage before they were injected intraperitoneally with an ethanol dose of 2.5 g/kg body weight (10% w/v; injection volume of 25 ml/kg body weight). This ethanol dose was selected because it is at the lower end of the effective dose range that reliably produces loss of RR. Following injection, each rat was immediately placed on its back in a V-shaped trough (bent at a 90° angle) once every 30 s until it was unable to right itself within a 30-s interval. The time between ethanol injection and onset of the 30-s interval in which the rat was unable to right itself was recorded as the time to lose RR.

Following loss of RR, the rat was placed in its home cage and left undisturbed for 15 min. At this time, the first righting trial was conducted. A righting trial begins by placing a rat on its back in the V-shaped trough. Righting is defined as the rat achieving an orientation in which at least three feet are under its body and not visible from above. Recovery of RR was defined as the ability of the rat to right itself in two consecutive 15-s trials. If the rat was unable to right itself in the first 15-s trial (conducted 15 min after loss of RR), it was left undisturbed in its home cage for an additional 15 min. Each rat was tested in this way every 15 min until it recovered the RR, at which point, a blood sample (0.2 ml) was collected from the cut tip of the tail.

Six rats failed to lose the RR within a 20-min period on test day. This was attributed to a poor ethanol injection and these rats were tested a second time at least 1 week later. All of these rats lost the RR when tested a second time, indicating that failure to lose the RR during the first test was likely the result of inadequate ethanol injections together with the threshold ethanol dose, rather than low sensitivity of these rats to the effects of ethanol. Furthermore, these rats were distributed across both selectively bred lines and both treatment conditions, which implies that there was no single combination of treatment levels that affected failure of rats to lose the RR. Only the data from their respective second trials are presented here.

Blood–ethanol levels (BELs) were determined with an alko-analyzer (AM1 series, Analox Instruments, Massachusetts) and blood alcohol reagent (400 ml for 8X70 analyser cycles, GMRD-110J, Analox Instruments). At the end of the study, all rats were sacrificed and anatomic localization was verified histologically. A 1% solution of bromphenol blue dye in aCSF was injected for histological verification of cannulae placement.

2.4. Data analysis

Time to lose RR, time to regain RR, and BELs were analyzed separately using two-way ANOVAs. In all cases, significance was determined at $P < .05$.

2.5. Results

One rat (NPY-treated P rat) did not lose the RR until 20 min postinjection. Further analysis of its datum with the Geigy Extreme Test later revealed it to be a statistical outlier ($P < .01$) and it was thus eliminated from the study.

A two-way ANOVA revealed a significant effect of treatment on time to lose RR across all rats, $F(1,30) = 5.18$, $P = .030$. There was no significant effect of line or interaction. Fig. 1 shows that rats pretreated with NPY lost the RR more rapidly than those pretreated with aCSF. A two-way ANOVA yielded a significant effect of treatment on time to regain RR across all rats, $F(1,30) = 22.99$, $P < .001$. There was no significant effect of line or interaction on time to regain RR. Fig. 2 (left panel) shows that time

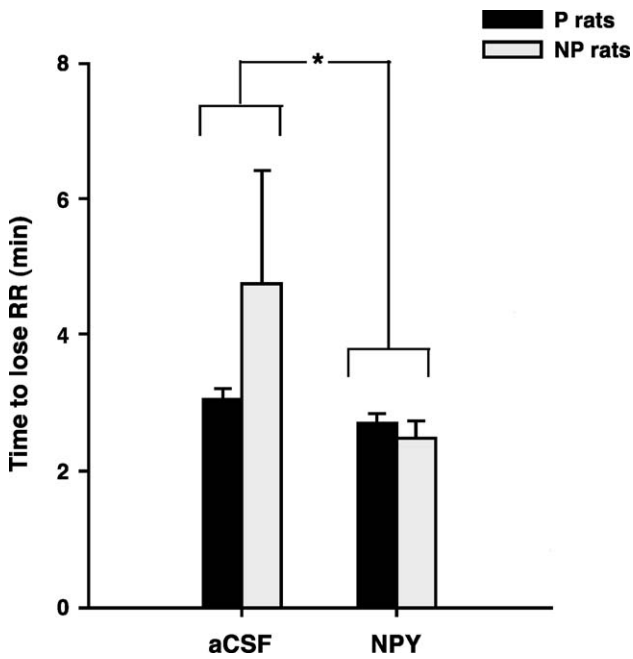

Fig. 1. Mean (± S.E.M.) time to lose RR (min; following 2.5 g/kg ethanol injection) for P rats (solid bars) and NP rats (open bars). * $P < .05$ significant difference from aCSF group across all rats.

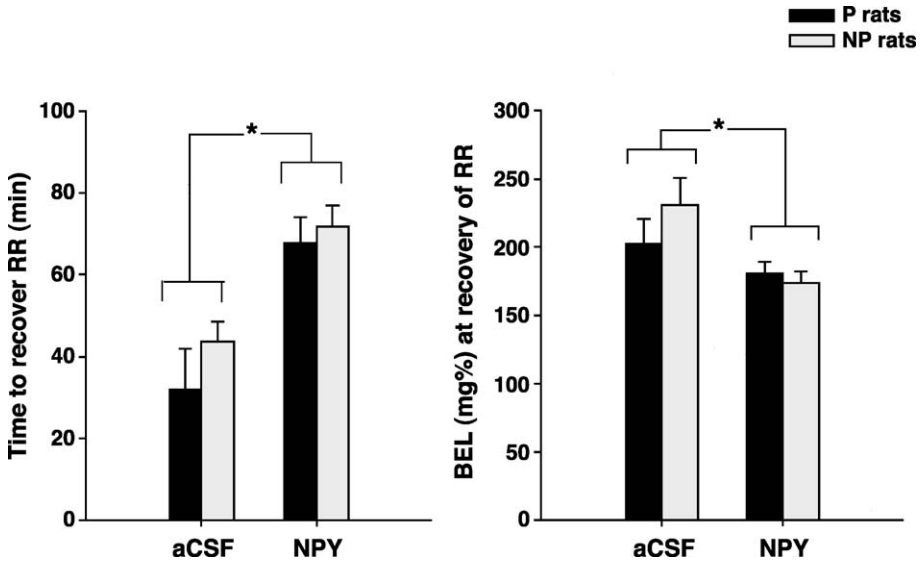

Fig. 2. Left panel: mean (± S.E.M.) time to regain RR (min; measured from time of loss of RR) for P rats (solid bars) and NP rats (open bars). Right panel: mean (± S.E.M.) BELs (mg %) at time of recovery of RR. * $P < .05$ significant difference from aCSF group across all rats.

to regain RR was longer in rats pretreated with NPY than in rats pretreated with aCSF.

Fig. 2 (right panel) shows BELs measured at time of recovery of RR were lower in rats pretreated with NPY than in rats pretreated with aCSF. A two-way ANOVA yielded a significant effect of treatment on BEL at time of recovery across all rats, $F(1,30) = 9.846$, $P = .004$. There was no significant effect of line or interaction.

3. Experiment 2

3.1. Subjects

Subjects were 24 P and 23 NP experimentally naïve female rats (School of Medicine, Indiana University) of the 51st generation that were 9 months old and weighed between 261 and 348 g on the first day of testing. Housing conditions were similar to those in Experiment 1 except that the vivarium 12-h light/12-h dark cycle was on a different schedule (lights off at 1900 h). Food and water were available to all rats ad libitum. All testing was conducted near the end of the vivarium light cycle (testing began at approximately 1500 h). The protocol for this study was approved by the IUPUI School of Science IACUC and was conducted in accordance with NIH guidelines (National Research Council, 1996).

3.2. Apparatus

Rats were assessed for motor activity in Digiscan animal activity monitors (Model RXYZCM[16]CDD; Accuscan Instruments, Columbus, OH) with dimensions 42 × 42 × 30 cm. There were 32 beams to detect horizontal activity or ambulation (16 front-to-back, 16 side-to-side) and, above these, 16 beams (side-to-side) to detect vertical activity or rearing. Beam spacing for all sensors was 2.5 cm. All walls of the activity chambers were composed of clear sheet acrylic. Activity chambers were connected to the Digiscan analyzer (Model CDA-8) for relay of movement data to the Digipro software system (version 1.50). During test trials, activity chambers were cleaned thoroughly between animals.

3.3. Stereotaxic surgery

Surgical implantation of intracerebroventricular cannulae was conducted using the same aseptic procedures described in Experiment 1. In Experiment 2, however, rats were anesthetized before and during surgery via inhalation of isoflourane (IsoFlo, Abbott Laboratories, North Chicago, IL).

3.4. Procedure

Preceding stereotaxic surgery, P and NP rats were separated into two groups which were matched for body weight. Within each line of rats, one group was injected with ethanol and one group with saline prior to activity test sessions. All testing was immediately preceded by five consecutive days of mock infusions in which rats were acclimated to the infusion procedure and test room environment.

Rats were given between 8 and 18 days to recover from stereotaxic surgery. To allow rats to acclimate to their respective test chambers, rats underwent five daily 15-min habituation trials. Habituation trials were followed by a series of 30-min test trials, each separated by a period of 1 week. Each rat was tested in the same activity chamber on all test trials and in the same activity chamber to which it had previously been habituated. For each test trial, rats were infused with either aCSF (5.0 μl) or one of three doses of

Table 1
Mean (± S.E.M.) transformed (square root) vertical movement data for rats that received either saline (7 P, 9 NP) or ethanol (8 P, 9 NP) injections preceded by intracerebroventricular infusion of one of four doses of NPY

		aCSF	2.5 μg	5.0 μg	10.0 μg
P	Saline	24.80 (2.62)	16.61 (2.81)	19.51 (2.94)	16.62 (4.39)
	Ethanol	11.83 (2.84)	11.03 (1.94)	10.89 (2.13)	11.69 (2.71)
NP	Saline	23.73 (1.99)	15.07 (2.72)	13.70 (2.02)	13.84 (2.84)
	Ethanol	3.84 (0.64)	2.23 (1.15)	3.01 (1.93)	2.29 (1.13)

NPY (2.5, 5.0, or 10.0 μg). For approximately half of the animals ($n = 12$ P rats, 12 NP rats), intracerebroventricular infusions were always immediately followed by intraperitoneal injections of 1.0 g ethanol/kg body weight. Five to ten minutes following intraperitoneal injection, rats were placed in activity chambers. On the first test trial, rats were infused with aCSF; on the second, third, and fourth test trials, rats were infused with each of the three doses of NPY in either ascending or descending order; on the fifth and final test trial, rats were once again infused with aCSF. Remaining animals ($n = 12$ P rats, 11 NP rats) were injected with saline on all test trials, but were otherwise treated identically to rats which received ethanol.

3.5. Data analysis

Total vertical movements, and total horizontal movements were analyzed separately using three-way (Line × NPY Dose × EtOH Treatment) ANOVAs. In all cases, significance was determined at $P < .05$.

3.6. Results

Of the 47 rats which were originally implanted, only rats which maintained viable cannulae for the duration of the study and had histologically verified placements were used in data analysis. The distribution of rats which were used for data analysis across treatment groups was as follows: ethanol-treated P rats ($n = 8$); saline-treated P rats ($n = 7$); ethanol-treated NP rats ($n = 9$); and saline-treated NP rats ($n = 9$).

There were no significant differences in horizontal or vertical activity between aCSF Tests 1 and 2 (the first and last test trials). As a result, the average of the data from aCSF Tests 1 and 2 for each animal was used in all subsequent data analysis. Further analysis yielded no significant effects of order of administration of NPY doses on horizontal or vertical activity. Therefore, order of NPY dose administration was not included as a variable in data analysis.

Analysis revealed lack of normality (Anderson–Darling Normality Test) and unequal error variances (Modified Levene Test) in both vertical and horizontal movement data. Because these tests indicated that the data might belong to an exponential distribution, the square root transformation was performed on the movement data (see Tables 1 and 2).

A three-way ANOVA on vertical movements revealed a significant effect of line, $F(1,116) = 33.31$, $P < .001$, a significant effect of NPY, $F(3,116) = 4.52$, $P < .01$, and a significant effect of ethanol, $F(1,116) = 106.94$, $P < .001$. There was a significant Line × Ethanol interaction effect, $F(1,116) = 6.29$, $P = .013$. Tukey post hoc pairwise comparisons revealed that P rats injected with ethanol were significantly less active than P rats injected with saline ($P < .001$; Fig. 3 left panel and Table 1). Likewise, NP rats injected with ethanol were significantly less active than NP rats injected with saline ($P < .001$). Furthermore, ethanol-treated NP rats were significantly less active than ethanol-treated P rats ($P < .001$). However, there was no significant difference

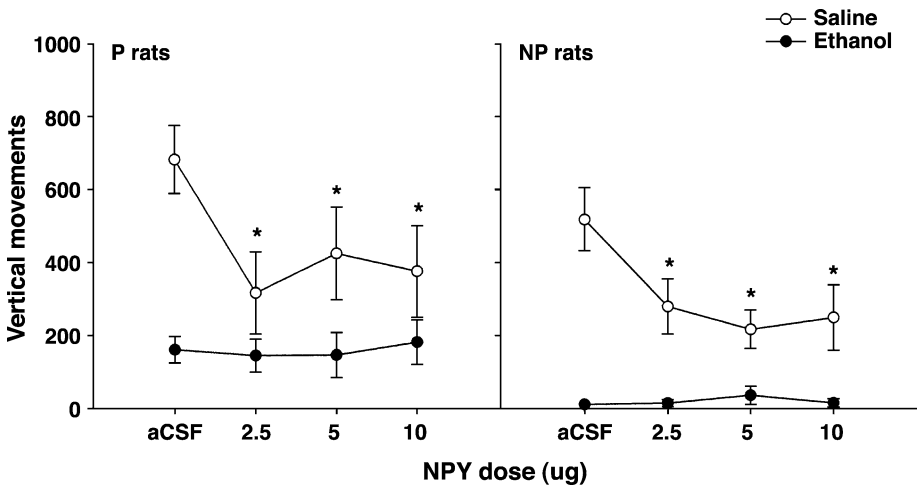

Fig. 3. Left panel: mean (± S.E.M.) vertical movements (i.e., rearing) measured in ethanol-treated (●) and saline-treated (○) P rats during a 30-min activity session following intracerebroventricular infusion of NPY or aCSF. Right panel: mean (± S.E.M.) vertical movements measured in ethanol-treated (●) and saline-treated (○) NP rats during a 30-min activity session following intracerebroventricular infusion of NPY or aCSF. * $P < .05$ significant difference from aCSF baseline.

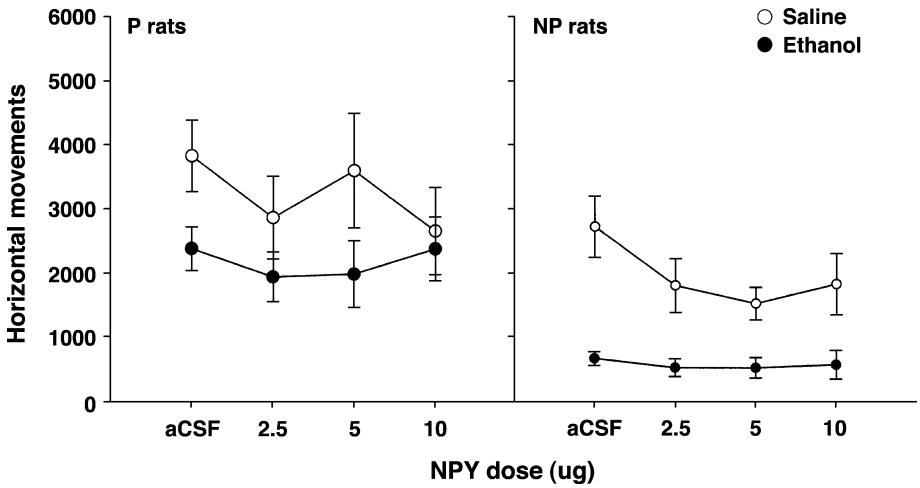

Fig. 4. Left panel: mean (± S.E.M.) horizontal movements measured in ethanol-treated (●) and saline-treated (○) P rats during a 30-min activity session following intracerebroventricular infusion of NPY or aCSF. Right panel: mean (± S.E.M.) horizontal movements measured in ethanol-treated (●) and saline-treated (○) NP rats during a 30-min activity session following intracerebroventricular infusion of NPY or aCSF.

in vertical motor activity between saline-treated P and NP rats. There was also a significant NPY × Ethanol interaction effect, $F(3,116) = 3.16$, $P = .027$. Across saline-treated rats in both selected lines, pretreatment with all three NPY doses reduced vertical motor activity relative to pretreatment with aCSF ($P < .05$ in all cases). Among ethanol-treated rats, vertical motor activity did not significantly differ following any dose of NPY relative to aCSF trials.

A three-way ANOVA on horizontal movements revealed a significant effect of line, $F(1,116) = 54.78$, $P < .001$, and a significant effect of ethanol, $F(1,116) = 40.30$, $P < .001$. There was a nonsignificant tendency toward an effect of NPY on horizontal movement ($P = .081$). There was a significant Line × Ethanol interaction effect, $F(1,116) = 4.34$, $P = .039$. Tukey post hoc pairwise comparisons revealed that P rats injected with ethanol were significantly less active than P rats injected with saline ($P = .024$; Fig. 4 left panel and Table 2). Likewise, NP rats injected with ethanol were significantly less active than NP rats injected with saline ($P < .001$; Fig. 4 right panel and Table 2). Furthermore, ethanol-treated NP rats were significantly less active than ethanol-treated P rats ($P < .001$). In contrast to results with vertical motor activity, saline-treated NP rats exhibited significantly less horizontal motor activity than saline-treated P rats ($P = .002$). NPY did not significantly suppress horizontal motor activity in saline-treated or ethanol-treated rats of either selected line.

Table 2
Mean (± S.E.M.) transformed (square root) horizontal movement data for rats that received either saline (7 P, 9 NP) or ethanol (8 P, 9 NP) injections preceded by intracerebroventricular infusion of one of four doses of NPY

		aCSF	2.5 μg	5.0 μg	10.0 μg
P	Saline	60.47 (5.97)	51.57 (6.37)	57.62 (7.47)	48.22 (8.17)
	Ethanol	49.58 (4.86)	42.35 (4.98)	41.46 (6.70)	47.24 (5.11)
NP	Saline	53.23 (3.69)	40.44 (5.18)	38.39 (3.20)	39.64 (5.86)
	Ethanol	27.05 (2.06)	22.19 (2.72)	21.59 (3.31)	22.42 (3.61)

4. Discussion

In Experiment 1 of the present investigation, experimentally naïve P and NP rats received one infusion of either NPY or aCSF followed by acute intraperitoneal injection of a moderately sedative dose of ethanol (2.5 g/kg). NPY pretreatment decreased time to lose the RR and increased the time to regain the RR; that is, NPY enhanced the sedative effects of ethanol. There were no differences between P and NP rats. BELs at time of recovery of RR were significantly lower in rats infused with NPY than in rats infused with aCSF, which is consistent with the extended time to regain RR in rats infused with NPY. This indicates that differences in ethanol metabolism cannot account for the observed differences in time to regain RR.

In Experiment 2, experimentally naïve P and NP rats received a series of five infusions (three NPY doses and two aCSF infusions), each separated by a period of 1 week, and each followed by intraperitoneal injection of either 1.0 g ethanol/kg body weight or saline. Ethanol suppressed vertical and horizontal motor activities in all rats, and produced a greater suppression of motor activity in NP rats than in P rats. All doses of NPY produced a significant suppression of vertical, but not horizontal, motor activity in saline-treated rats of the two selected lines. This is consistent with past results in which similar doses of NPY administered intracerebroventricularly suppressed motor activity in nonselected rats (Heilig and Murison, 1987; Heilig et al., 1989a). Because NPY reduced vertical motor activity in saline-treated rats, but did not enhance reductions in motor activity in ethanol-treated rats, there was no evidence for additive effects of ethanol and NPY on motor activity. The strong

suppression of motor activity, especially in NP rats, produced by ethanol, may have created a floor effect that prevented the observation of any effect of NPY in ethanol-treated rats.

Previous investigations have reported that P rats are less sensitive to ethanol than NP rats in tests of loss and recovery of RR (Kurtz et al., 1996) and motor activity (Waller et al., 1986). In Experiment 1, P and NP rats pretreated with aCSF did not exhibit differences in sensitivity to the sedative effects of ethanol as measured by time to lose and time to regain RR. This discrepancy could be accounted for by differences in gender (female rats in present study, male rats in Kurtz et al., 1996). In addition, the dose of ethanol employed in the present study was lower than that used by Kurtz et al. (1996). However, in Experiment 2, P rats were less sensitive to the motor-impairing effects of ethanol than NP rats on aCSF test trials. This replicates previous findings (Waller et al., 1986) in which the same ethanol dose was administered to female P and NP rats prior to spontaneous motor activity testing.

The observation that NPY potentiates ethanol-induced sedation, as measured by loss and recovery of RR, is consistent with previous findings that NPY and ethanol have additive effects on electrophysiological measures in rats (Ehlers et al., 1998). This observation is also consistent with previous results that indicate a positive association between NPY activity and duration of ethanol-induced loss of RR in NPY transgenic mice (Thiele et al., 1998). It is of interest to determine which anatomical and functional pathways mediate this relationship between NPY activity and the neurobehavioral effects of ethanol. Y_1 receptor (Y_1R) knockout mice exhibit behavioral responses to ethanol that are similar to those seen in NPY knockout mice, i.e., prolonged duration of ethanol-induced loss of RR (Thiele et al., 2002). This implies a role for Y_1 receptors in mediating the effects of NPY on ethanol-induced sedation.

At the neurochemical level, ethanol is known to potentiate the actions of both GABA agonists and NMDA antagonists (Carboni et al., 1993; Mehta and Ticku, 1988). Y_1R KO mice are less sensitive than wild-type mice to the sedative effects of GABA agonists but not NMDA antagonists (Naveilhan et al., 2001a), whereas Y_2R KO mice are more sensitive than wide-type mice to sedation induced by GABA agonists (Naveilhan et al., 2001b). Similarly, intracerebroventricular infusion of either NPY or [Leu 31, Pro 34]-NPY (a Y_1R/Y_5R agonist) potentiates sedation induced by a GABA agonist or an NMDA antagonist (Naveilhan et al., 2001a). However, the ability of NPY to potentiate sedation caused by either of these two compounds is diminished significantly in Y_1R KO mice (Naveilhan et al., 2001a). Furthermore, these two receptor types likely compensate for each other in knockout mice, although Y_1 receptors likely play the dominant role in mediating such sedation and Y_2 receptors likely play a modulatory role (Naveilhan et al., 2001b). In addition, the mediator role of Y_1 receptors in NPY potentiation of PbNa-induced sedation has been isolated to the posterior hypothalamus (Naveilhan et al., 2001b). Taken together, these studies indicate that Y_1 receptors are involved in mediating the effects of NPY on drug-induced sedation, although additional Y receptors are likely associated with sedation induced by NMDA antagonists.

The sedative effects of NPY might be explained, at least in part, by its interaction with alpha-2 adrenoreceptors. Decreases in norepinephrine (NE) release in the rat hypothalamus following infusion of NPY parallel those seen following administration of an alpha-2 adrenoreceptor agonist (Tsuda et al., 1995). Alpha-2 adrenergic receptor agonists increase anesthesia-induced sleep time (Kushikata et al., 2002). Accordingly, the ability of NPY to suppress locomotor activity in rats appears to be dependent on the ability of the peptide to up-regulate numbers of alpha-2 adrenoreceptors (Heilig et al., 1989b). In fact, lines of rats in which NPY does not up-regulate alpha-2 adrenergic binding sites exhibit *increases* in locomotor activity following central administration of NPY (Heilig et al., 1989b). This suggests that the sedative effects of NPY might be explained in terms of its cooperation with NE systems.

It has previously been observed that intracerebroventricularly administered NPY decreases ethanol intake in alcohol-preferring rats (Badia-Elder et al., 2001, 2003). Because the present study shows that NPY can enhance the sedative effects of ethanol, it is possible that the ability of NPY to decrease ethanol intake is due, at least in part, to the combined sedative effects of NPY and ethanol. However, this explanation does not adequately account for findings from a recent study in which P rats were given chronic free-choice continuous access to ethanol followed by either a period of imposed ethanol abstinence or uninterrupted access to ethanol. In this study, a single intracerebroventricular infusion of NPY effectively suppressed ethanol intake in both groups; however, this suppression was enhanced in magnitude and duration when NPY treatment immediately preceded ethanol reinstatement in the abstinence group (Gilpin et al., 2003). The combined sedative effects of NPY with self-administered ethanol cannot account for the enhanced effect of NPY on ethanol intake following a period of ethanol abstinence. It has been suggested that NPY systems are compromised and corticotrophin-releasing factor (CRF) systems are concurrently activated during ethanol withdrawal (Koob, 2003). The resultant altered states of NPY and CRF systems may lead to abnormal interactions between the two, and these interactions may contribute to the enhanced ability of NPY to suppress ethanol intake following a period of ethanol abstinence (Gilpin et al., 2003). In addition, corticosterone dose-dependently suppresses ethanol-induced sleep time in mice (Sze, 1993). Thus, it is conceivable that glucocorticoid systems are involved in the

ability of NPY to potentiate ethanol-induced sleep time. In rats, NPY reverses the ability of CRF and stress to reduce PbNa-induced sleep time (Yamada et al., 1996). Electrophysiological measures have revealed that intracerebroventricular administration of CRF causes a decrease in nonrapid eye movement (NREM) sleep and an increase in latency to sleep onset in rats, and both of these effects are dose-dependently reversed by NPY (Ehlers et al., 1997). In addition, intravenous injection of NPY in humans causes decreases in nocturnal ACTH and cortisol secretion (Antonijevic et al., 2000). It would be of interest to further elucidate the relationship between NPY and CRF, and to examine the role of their interaction in the neurobehavioral effects of acute ethanol exposure and in chronic ethanol-seeking behavior to determine whether there is any connection between their roles in each.

In summary, intracerebroventricular administration of NPY enhances the sedative effects of ethanol in P and NP rats as measured by loss and recovery of RR. However, NPY did not augment ethanol-induced reductions in motor activity despite the observation that, in the absence of ethanol, NPY reduced vertical motor activity. It remains to be determined whether NPY and ethanol might produce additive effects on motor activity under different parameters (e.g., lower ethanol doses). These results are in partial agreement with past findings which have shown the peptide to have moderately sedative effects that are exaggerated in the presence of ethanol. Although discussion of the anatomical pathways mediating the sedative effects of NPY are speculative based on the data presented here, past findings indicate that Y_1 receptors, specifically those in the hypothalamus, play an important role in the effect.

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