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Neurobiological responses to ethanol in mutant mice lacking neuropeptide Y or the Y5 receptor

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Abstract

We have previously shown that voluntary ethanol consumption and resistance are inversely related to neuropeptide Y (NPY) levels in NPY-knockout (NPY −/−) and NPY-overexpressing mice. Here we report that NPY −/− mice on a mixed C57BL/6J × 129/SvEv background showed increased sensitivity to locomotor activation caused by intraperitoneal (ip) injection of 1.5 g/kg of ethanol, and were resistant to sedation caused by a 3.5-g/kg dose of ethanol. In contrast, NPY −/− mice on an inbred 129/SvEv background consumed the same amount of ethanol as wild-type (WT) controls at 3%, 6%, and 10% ethanol, but consumed significantly more of a 20% solution. They exhibited normal locomotor activation following a 1.5-g/kg injection of ethanol, and displayed normal sedation in response to 2.5 and 3.0 g/kg of ethanol, suggesting a genetic background effect. Y5 receptor knockout (Y5 −/−) mice on an inbred 129/SvEv background showed normal ethanol-induced locomotor activity and normal voluntary ethanol consumption, but displayed increased sleep time caused by 2.5 and 3.0 g/kg injection of ethanol. These data extend previous results by showing that NPY −/− mice of a mixed C57BL/6J × 129/SvEv background have increased sensitivity to the locomotor activation effect caused by a low dose of ethanol, and that expression of ethanol-related phenotypes are dependent on the genetic background of NPY −/− mice.

Keywords: Neuropeptide Y; Knockout; Ethanol; Locomotor activity; Sedation; Consumption

Neuropeptide Y (NPY) is a 36-amino acid neuromodulator that is distributed throughout the nervous system [6,13]. Central infusion of exogenous NPY has provided evidence that this peptide can promote feeding behavior [4,25], lower cerebrocortical excitability [33,34], reduce anxiety-like behaviors [20,21], and potentiate the sedative/hypnotic effects of certain drugs [35]. Recent evidence suggests that NPY is involved with the neurobiological effects of ethanol. Quantitative trait loci (QTL) analyses on rats that had been selectively bred for either high (alcohol-preferring, P) or low (alcohol-nonpreferring, NP) ethanol consumption identified a highly significant QTL in a chromosomal region that includes the NPY gene [3]. Furthermore, P rats were found to have significantly lower levels of NPY in several brain regions, including the central nucleus of the amygdala (CeA) and the cortex, than NP rats [14]. More recently, the selectively bred, high alcohol drinking (HAD) rats were also shown to have low NPY in the CeA [23]. NPY and ethanol produce comparable patterns of event-related potentials in rat brain [15], and P and NP rats have opposite event-related potential activity in the CeA following NPY administration [16]. Importantly, mutant mice lacking NPY (NPY −/−) were found to consume greater volumes of solutions containing ethanol, and were less sensitive to the sedative effects of ethanol, as compared with wild-type (WT, NPY +/+) littermate mice. Conversely, transgenic mice that overexpress a marked NPY gene in neurons that normally express it had lower preference for ethanol and were more sensitive to the sedative effects of this drug than controls [31]. Finally, recent evidence suggests that the NPY system may modulate

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ethanol consumption in humans [24]. Collectively, these data make a strong case that NPY modulates voluntary ethanol consumption and the neurobiological effects produced by ethanol.

One goal of the present study was to extend our previous findings with NPY −/− mice by determining if NPY is involved with the locomotor-stimulating effects that are produced by low doses of ethanol. Paradoxically, several sedative drugs like ethanol produce behavioral arousal following administration of subhypnotic doses [9,11,12,30]. It has been suggested that this effect may be related to the euphoric and rewarding properties of ethanol and may be a marker for abuse potential [32]. This argument is strengthened by the observation that ethanol-induced, behavioral arousal is modulated by dopamine pathways that have also been implicated in mediating the positive reinforcing effects of drugs [5]. Interestingly, common genetic factors may influence sensitivity to the sedative and arousal effects of ethanol. Animals selectively bred for low sensitivity to the sedative effects caused by high doses of ethanol are highly sensitive to locomotor stimulation produced by subhypnotic doses [11]. On the other hand, animals selectively bred for high sensitivity to ethanol-induced locomotor activation show resistance to the sedative effects of ethanol [30]. Because NPY −/− mice are resistant to the sedative effects of ethanol, we predicted that NPY −/− mice would manifest enhanced sensitivity to the stimulating effects of ethanol.

The second goal was to determine if the NPY Y5 receptor is involved with modulating voluntary ethanol consumption and neurobiological responses to this drug. NPY acts widely in the brain through modulation of Y1, Y2, and Y5 receptors, all of which couple to heterotrimeric G proteins that inhibit the production of cyclic AMP [18,22]. The Y5 receptor has been identified in the hippocampus and the hypothalamus of the mouse brain [28], brain regions that have been shown to be targets for ethanol and which may be involved with modulating neurobiological effects produced by this drug [29]. We hypothesized that if the Y5 receptor is involved in the modulation of voluntary ethanol consumption and sensitivity, mice lacking the Y5 receptor (Y5 −/−) should exhibit ethanol-related phenotypes similar to those found with NPY −/− mice.

1. Methods

1.1. Subjects

NPY −/−, Y5 −/−, and WT mice on an inbred 129/SvEv background, and NPY −/− and WT littermate mice maintained on a mixed 50% C57BL/6J × 50% 129/SvEv background, were described previously [17,26,27]. Experiments were performed on 14- to 30-week old mice. Mice were individually housed in plastic mouse cages with free access to standard rodent chow (Teklad) and water throughout the experiments. The colony room was maintained at ~22°C with a 12:12-h light/dark cycle. All procedures used in the present research were in compliance with National Institutes of Health guidelines, and the protocols were approved by the University of Washington Animal Care Committee.

1.2. Ethanol-induced locomotor activation

For activity testing, mice were placed in transparent Plexiglas cages (40 × 20 × 20 cm) containing standard wood-chip laboratory bedding. Locomotor activity was assessed with a photobeam activity system (San Diego Instruments, San Diego, CA) that consisted of infrared photobeams separated by 8.8 cm. The number of consecutive beam breaks that occurred in each of six 5-min blocks was measured and converted to meters by using the distance between beams (0.088 m) as the conversion factor.

1.2.1. C57BL/6J×129/SvEv mice

This study used NPY −/− (male, $n=6$; female, $n=7$) and NPY +/+ littermate mice (male, $n=8$; female, $n=7$). On the first and second days of the experiment, mice were removed from their home cages and given an intraperitoneal (ip) injection of isotonic saline (7.5 ml/kg). They were then separately placed in the activity apparatus for a 30-min test session, and then returned to their home cages upon completion of the test session. On the third day of the study, mice were removed from their home cages and given an intraperitoneal injection of 1.5 g ethanol/kg (20% (w/v) ethanol mixed in isotonic saline). This dose of ethanol was chosen because it has consistently been reported to promote locomotor activation in mice [9,12,30]. Mice were then separately placed in the activity apparatus for a 30-min test session, and then returned to their home cages. To control for variance caused by individual differences in baseline locomotor activity observed following saline injection, the data are expressed as the change from baseline activity, which is defined as locomotor activity (m) on the day of ethanol injection (Day 3) minus locomotor activity (m) on the second day of saline injection (Day 2). Data obtained during ethanol-induced locomotor activity experiments are commonly expressed in this way [11,30].

1.2.2. 129/SvEv mice

Ethanol-induced locomotor activity tests were performed with NPY −/− (male, $n=10$; female, $n=10$) and WT mice (male, $n=10$; female, $n=9$). A similar study was performed with male Y5 −/− ($n=14$) and WT mice ($n=16$). The procedures used for each study were identical to those described above.

1.3. Ethanol-induced sedation

1.3.1. C57BL/6J×129/SvEv mice

Male NPY −/− ($n=10$) and NPY +/+ littermate mice ($n=12$) were removed from their home cages, body

weights were recorded, and mice were given an intraperitoneal injection of ethanol [3.0 g/kg; 20% (w/v) ethanol mixed in isotonic saline was used for all sedation studies]. At the onset of ethanol-induced sedation, each mouse was placed on its back in a plastic U-shaped trough. The time (min) that elapsed between the ethanol injection and when the mouse could right itself onto all four paws three times within a 30-s interval was used as the index of time to regain the righting reflex. Approximately 2 and 4 weeks after the first injection, the same mice were again given intraperitoneal injections of ethanol (3.5 and 4.0 g/kg, respectively), and the time to regain the righting reflex was assessed. The doses of ethanol used for sedation testing were based on pilot data.

1.3.2. 129/SvEv mice

Similar procedures were used to assess sensitivity to ethanol-induced sedation in male NPY −/− ($n=15$), Y5 −/− ($n=13$), and WT mice ($n=13$) maintained on the 129/SvEv inbred background. Mice were given an intraperitoneal injection of ethanol (2.5 g/kg) and the time to regain the righting reflex was assessed. Approximately 2 weeks later, the same mice were tested with the identical procedures following intraperitoneal injection of 3.0 g/kg of ethanol. A second (Y5 −/− mice, $n=15$; WT mice, $n=17$) and third (Y5 −/− mice, $n=9$; WT mice, $n=8$) sedation study were also performed. Again, the 2.5 and 3.0 g/kg doses of ethanol were used to induce sedation. The doses of ethanol used for sedation testing were based on pilot data. Because the inbred 129/SvEv mice were found to be more sensitive to the sedative effects of ethanol when compared with mice of the mixed C57BL/6 × 129/SvEv background, a lower range of doses were used with these mice.

1.4. Alcohol intake test

Male NPY −/− ($n=16$), Y5 −/− ($n=15$) and WT mice ($n=16$) were habituated in their home cages to drinking from two bottles containing plain water for 6 days (all mice were of the inbred 129/SvEv background). Mice were then given 24 h access to two bottles, one containing plain water and the other containing ethanol in water. The concentration of ethanol (v/v) was increased every 8 days; mice received 3%, 6%, 10%, and finally 20% ethanol over the course of the experiment. To control for position preference, the positions of the bottles were changed every 2 days, at which time consumption measures were collected. To obtain a measure of ethanol consumption that corrected for individual differences in mouse size, grams of ethanol consumed/kg body weight/2 days were calculated for each mouse. As a measure of relative ethanol preference, ethanol preference ratios were calculated at each ethanol concentration by dividing total ethanol solution consumed by total fluid (ethanol plus water) consumed.

1.5. Plasma ethanol concentrations

Plasma ethanol concentrations were determined in male NPY −/−, Y5 −/−, and WT mice maintained on an inbred 129/SvEv background. Mice were given an intraperitoneal injection of ethanol [3.0 g/kg; 20% (w/v) ethanol mixed in isotonic saline] and immediately returned to their home cages. One hour following ethanol injection, half the NPY −/− ($n=8$), Y5 −/− ($n=6$), and WT mice ($n=7$) were rapidly anesthetized with CO_2 and decapitated for blood collection. The remaining NPY −/− ($n=7$), Y5 −/− ($n=6$), and WT mice ($n=7$) were anesthetized and decapitated 3 h following the ethanol injection. Plasma ethanol levels were determined via spectrophotometric methods (Sigma Diagnostics, Enzymatic Determination of Alcohol Test, St. Louis, MO) and reported as mg/dl.

1.6. Data analyses

All values are reported as mean ± S.E.M. All data were analyzed using either repeated measures or multi-factor analysis of variance (ANOVA). To assess genotype differences at each point of the repeated measures variable, planned comparisons were conducted with t tests. In all cases, significance was accepted at $P<.05$ (two-tailed).

2. Results

2.1. C57BL/6J×129/SvEv mice

2.1.1. Ethanol-induced locomotor activation

Ethanol-induced locomotor activity of mice on the mixed background are presented in Fig. 1. Data collected from male and female mice are presented separately because of significant differences between the genders. Examination of these data revealed that both male (Fig. 1A) and female (Fig. 1B) NPY −/− mice displayed significantly greater locomotor activation in response to a 1.5-g/kg dose of ethanol relative to NPY +/+ littermate mice. The NPY +/+ mice did not show any ethanol-induced locomotor activation. The ethanol-induced locomotor activation of NPY −/− females persisted for 20 min, whereas NPY −/− males showed significant locomotor differences only during the first 10 min of the test session. A 2 × 6 (Genotype × Time) repeated measures ANOVA performed on change in activity data [activity following ethanol injection (Day 3) − activity following saline injection (Day2)] collected from male mice yielded a significant effect of genotype [$F(1,12)=8.33$, $P=.014$] and a significant effect of time [$F(5,60)=5.13$, $P=.001$], but the interaction between these variables was not significant. Furthermore, a repeated measures ANOVA performed on baseline activity data from the second day of saline injection (Day 2) revealed no significant differences between male NPY −/− mice (1.13 ± 0.31 m/5 min) and

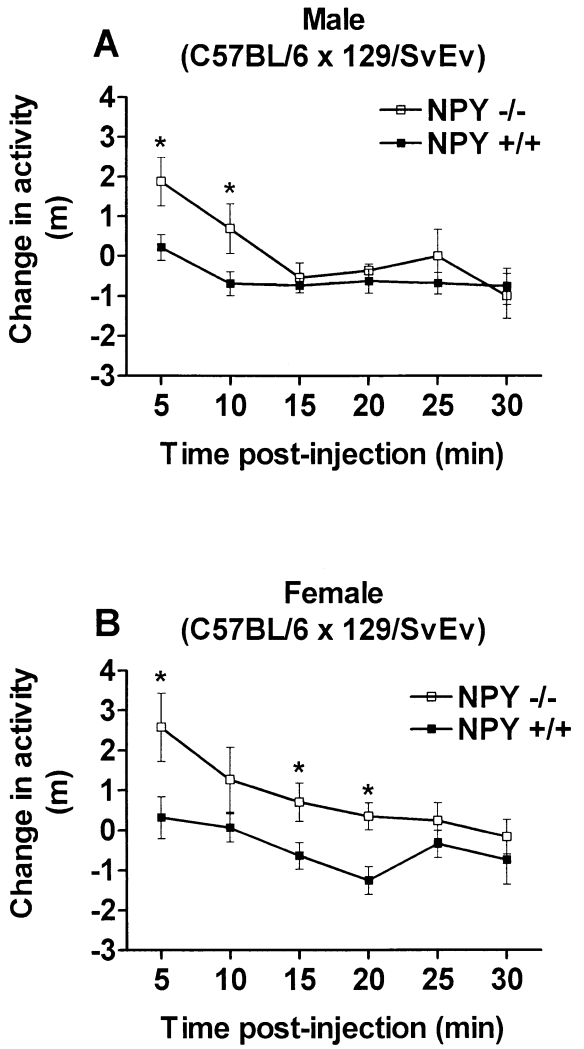

Fig. 1. Mean (±S.E.M.) change in activity (*m*) from (A) male NPY −/− and (B) female NPY −/− mice on the mixed C57BL/6 × 129/SvEv genetic background. Change in activity was calculated as locomotor activity on the day of the ethanol injection (1.5 g/kg, Day 3) minus locomotor activity on the second day of saline injection (Day 2). * $P < .05$ relative to WT (NPY +/+) control mice.

male NPY +/+ mice (1.45 ± 0.50 m/5 min). A repeated measures ANOVA performed on change in activity data collected from female mice also showed a significant effect of genotype [$F(1,12) = 6.91$, $P = .022$] and a significant effect of time [$F(5,60) = 5.89$, $P = .001$], but the interaction effect was not significant. Repeated measures ANOVA showed no significant differences in Day 2 baseline activity between female NPY −/− mice (1.77 ± 0.33 m/5 min) and female NPY +/+ mice (2.45 ± 0.35 m/5 min). Planned comparisons confirmed the conclusions.

2.1.2. Ethanol-induced sedation

Ethanol-induced sedation data for NPY −/− and NPY +/+ mice of the mixed background are presented in Fig. 2. NPY −/− mice required significantly less time to regain their righting reflex than NPY +/+ mice following injection of the 3.5 g/kg dose of ethanol. A 2 × 3 (Genotype × Dose) repeated measures ANOVA showed a significant effect of genotype [$F(1,20) = 4.34$, $P = .05$] and a significant effect of dose [$F(2,40) = 46.75$, $P = .001$], but the interaction effect was not significant. Planned comparisons revealed that genotypes showed significant differences only following the 3.5 g/kg dose of ethanol.

2.2. 129/SvEv mice

2.2.1. Ethanol-induced locomotor activation

Ethanol-induced locomotor activation data from mice of the inbred 129/SvEv background are presented in Fig. 3. Repeated measures ANOVA performed on change in activity data (Day 3 − Day 2) collected from male NPY −/− and WT mice did not reveal any significant differences (Fig. 3A), nor were there differences between genotypes in baseline activity on Day 2, the second day of saline injection. There were also no significant differences between male Y5 −/− and WT mice (Fig. 3C). There were marginal differences in ethanol-induced locomotor activity seen with the female NPY −/− mice (Fig. 3B). A 2 × 6 (Genotype × Time) repeated measures ANOVA performed on change in activity data showed a significant effect of time [$F(5,85) = 16.37$, $P = .001$], and a significant interaction between the genotype and time variables [$F(5,85) = 2.73$, $P = .024$], but the genotype main effect was not significant. Planned comparisons revealed inconsistent results as female NPY −/− mice showed significant attenuation of activity during the first 5 min of testing, and significant enhancement of activity towards the end of testing, when compared

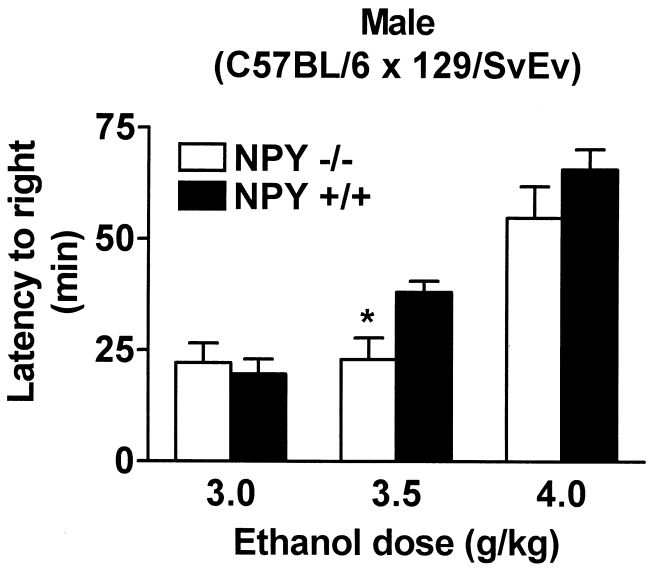

Fig. 2. Mean (±S.E.M.) time to regain the righting reflex (min), as a measurement of sensitivity to the sedative/hypnotic effects of ethanol, from male NPY −/− mice maintained on the mixed C57BL/6 × 129/SvEv genetic background. * $P < .05$ relative to WT (NPY +/+) control mice.

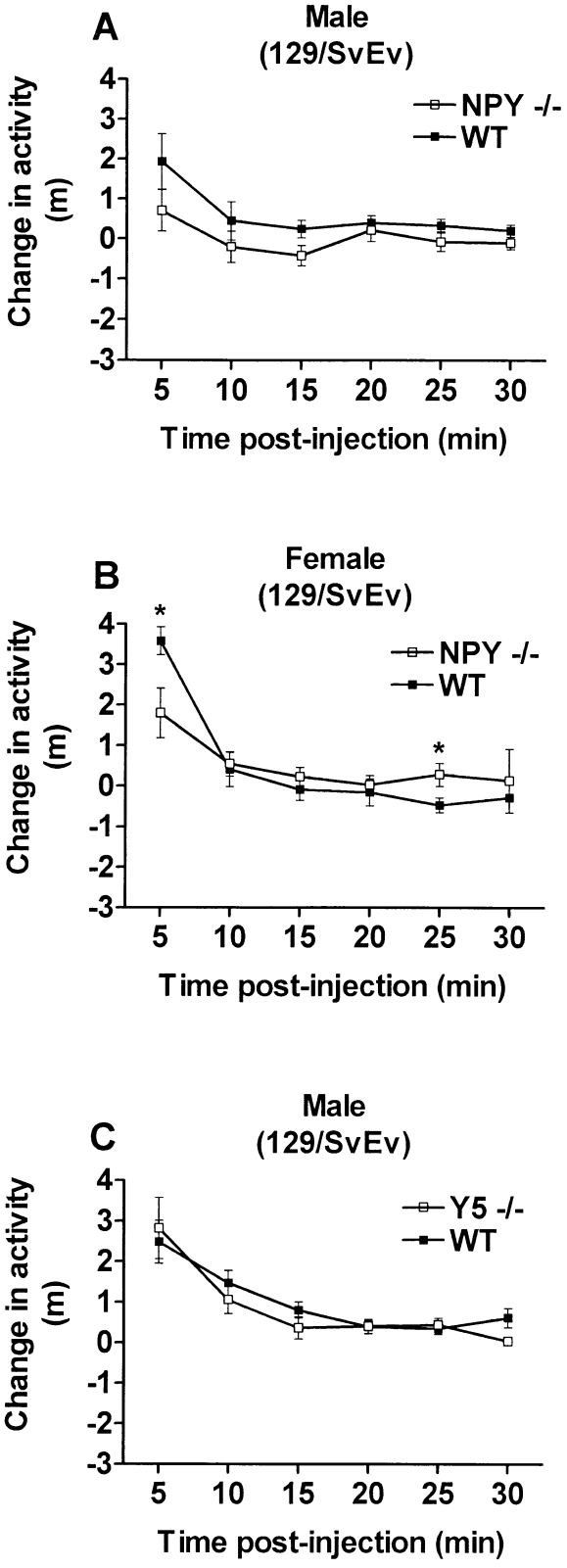

Fig. 3. Mean (±S.E.M.) change in activity (m) from (A) male NPY −/− and (B) female NPY −/− mice of the inbred 129/SvEv genetic background, and (C) male Y5 −/− mice of the inbred 129/SvEv background. Change in activity was calculated as locomotor activity on the day of the ethanol (1.5 g/kg) injection (Day 3) minus locomotor activity on the second day of saline injection (Day 2). * $P < .05$ relative to WT control mice.

with the female NPY +/+ mice. There were no significant differences in Day 2 baseline activity between female NPY −/− mice (0.79 ± 0.10 m/5 min) and female NPY +/+ mice (0.80 ± 0.22 m/5 min). It is worth noting that in each case, ethanol stimulated locomotor activity in both knockout and WT mice (activity scores above zero) during the first 10 min of the test session.

2.2.2. Ethanol-induced sedation

Data from the ethanol-induced sedation tests with NPY −/− mice are presented in Fig. 4A. There were no difference between NPY −/− and WT mice of the inbred background. However, the Y5 −/− mice showed a significant increase in sedation in response to the 3.0 g/kg dose when compared to the same controls (Fig. 4B). A 3 × 2 (Genotype × Dose) repeated measures ANOVA yielded a significant dose effect [$F(1,39) = 151.24$, $P = .001$] and a significant interaction between the genotype and dose variables [$F(2,39) = 7.31$, $P = .002$], but the genotype main effect was not significant. Planned comparisons confirmed that the Y5 −/− mice differed significantly from WT mice only following the 3.0-g ethanol/kg dose. Because we expected the Y5 −/− mice to exhibit a phenotype similar to the NPY −/− mice and manifest low, rather than high, sensitivity to the sedative effects of ethanol, two additional sedation studies were performed with Y5 −/− mice. Data from the first replicate study are presented in Fig. 4C. The Y5 −/− mice required significantly more time to regain their righting reflex when compared with WT mice following both doses of ethanol. The same pattern of results was obtained in the second replicate study (Fig. 4D). A 2 × 2 (Genotype × Dose) repeated measures ANOVA performed on data from the first replicate study showed a signifi-cant effect of genotype [$F(1,30) = 15.60$, $P = .001$] and a significant effect of dose [$F(1,30) = 49.34$, $P = .001$], but the interaction effect was not significant. A repeated measures ANOVA performed on the second replicate study also revealed a significant effect of genotype [$F(1,15) = 12.13$, $P = .003$] and a significant dose effect [$F(1,15) = 30.07$, $P = .001$], but the interaction effect was not significant.

2.2.3. Alcohol intake test

Average consumption of solutions containing ethanol in NPY −/−, Y5 −/−, and WT mice maintained on the inbred 129/SvEv background are presented in Fig. 5. Relative to WT mice, NPY −/− mice drank significantly more of the 20% ethanol but not the lower concentrations (Fig. 5A); however, Y5 −/− mice did not differ significantly in consumption of ethanol at any of the concentrations when compared with WT mice. A 3 × 4 (Genotype × Concentration) repeated measures ANOVA performed on these data yielded a significant genotype effect [$F(2,44) = 4.06$, $P = .024$], a significant concentration effect [$F(3,132) = 151.49$, $P = .001$], and a

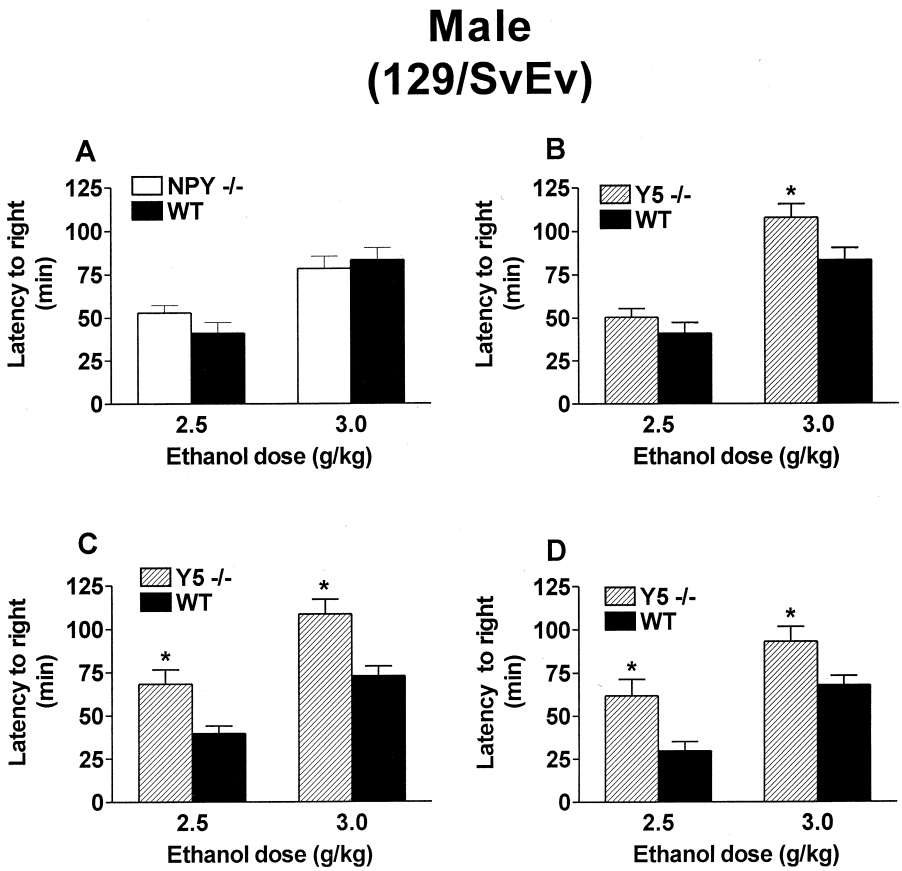

Fig. 4. Mean (±S.E.M.) time to regain the righting reflex (min), as a measurement of sensitivity to the sedative/hypnotic effects of ethanol, from (A) male NPY −/− and (B), (C), and (D) male Y5 −/− mice maintained on the inbred 129/SvEv genetic background. Note that the same WT data are represented in (A) and (B). *$P<.05$ relative to WT control mice.

significant interaction between these variables [$F(6, 132)=4.34$, $P=.001$]. Planned comparisons confirmed that NPY −/− mice differed from WT mice only during consumption of the 20% ethanol solution. When consumption of 20% ethanol was examined at each of the 2-day trials, NPY −/− mice were again found to drink significantly more ethanol than WT mice (Fig. 5B), particularly during the first 6 days of the 8-day session. Again, there were no significant differences between Y5 −/− mice and WT mice. A 3 × 4 (Genotype × Trials) repeated measures ANOVA performed on 20% ethanol consumption data showed a significant effect of genotype [$F(2,44)=4.49$, $P=.017$] and a significant effect of trials [$F(3,132)=9.18$, $P=.001$], but the interaction effect was not significant. Planned comparisons confirmed that above conclusion. Fig. 5C shows consumption of ethanol during access to the 20% solution expressed as ethanol preference ratios. All groups showed preference ratios of less than 0.5, indicating that each group preferred water over the 20% ethanol solution. However, during the second and third trials, the NPY −/− mice showed significantly greater preference for ethanol than WT mice. Y5 −/− mice did not differ significantly from WT mice. A 3 × 4 (Genotype × Trials) repeated measures ANOVA performed on the 20% ethanol preference ratio data showed a significant effect of test trial [$F(3, 132)=6.49$, $P=.001$], and a significant interaction between the genotype and test trial variables [$F(6, 132)=2.19$, $P=.047$], but the genotype main effect was not significant. Planned comparisons confirmed that NPY −/− mice differed significantly from WT mice during the second and third trials.

2.2.4. Plasma ethanol concentrations

Plasma ethanol concentrations from NPY −/−, Y5 −/−, and WT mice of the inbred 129/SvEv background are presented in Fig. 6. When compared with WT mice, Y5 −/− mice showed significantly higher plasma ethanol levels both at 1 and 3 h following injection of a 3.0-g/kg dose of ethanol. Consistent with previous reports, the Y5 −/− mice were mildly obese, weighing 35.37±1.87 g, compared to 32.10±3.20 g for the WT mice. NPY −/− and WT mice did not differ significantly in plasma ethanol levels at either time point. A 3 × 2 (Genotype × Hour) multi-factor ANOVA performed on the data showed a significant effect of genotype [$F(2,35)=9.67$, $P=.001$], and a significant

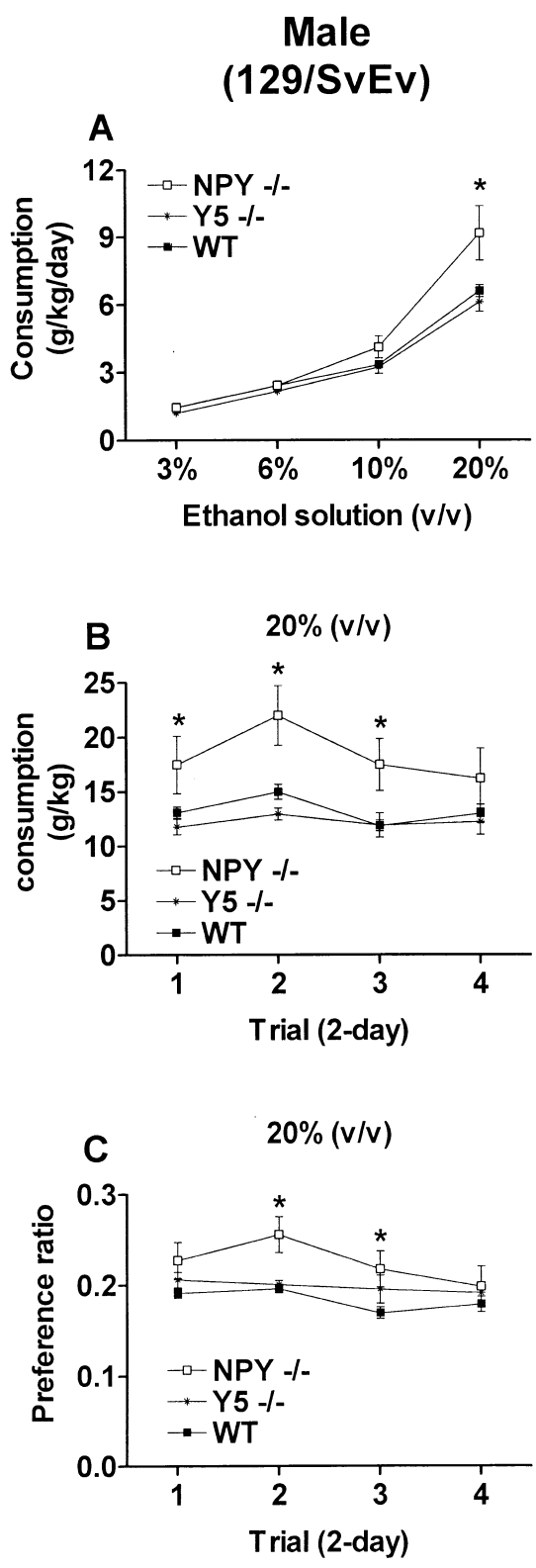

Fig. 5. Mean (±S.E.M.) voluntary consumption of ethanol from male NPY −/− and male Y5 −/− mice maintained on the inbred 129/SvEv genetic background. (A) consumption (g/kg/day) of each ethanol solution (8-day average). (B) Consumption (g/kg/2-day) of 20% ethanol. (C) Ethanol-preference ratios (volume of ethanol consumed/total fluid consumption) during access to the 20% ethanol solution. *$P<.05$ relative to WT control mice.

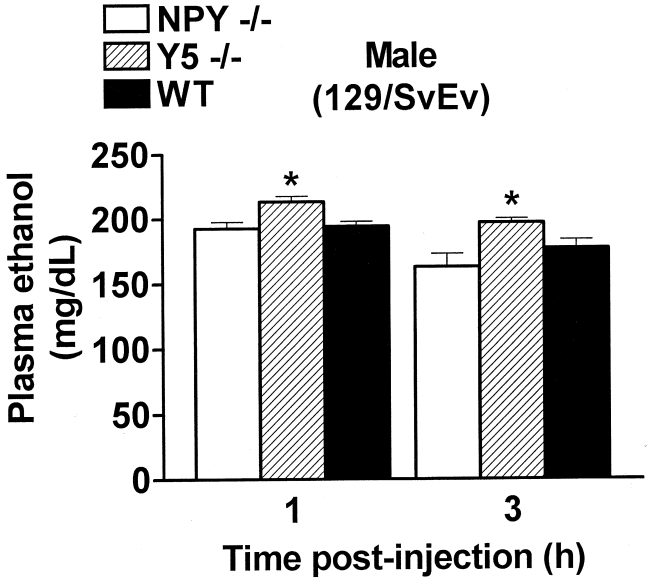

Fig. 6. Mean (±S.E.M.) plasma ethanol concentration (mg/dl) 1 and 3 h after ethanol injection (3.0 g/kg) from male NPY −/− and male Y5 −/− mice maintained on the inbred 129/SvEv genetic background. *$P<.05$ relative to WT control mice.

hour effect [$F(1,35)=17.13$, $P=.001$], but the interaction effect was not significant.

3. Discussion

A summary of results from NPY −/− and Y5 −/− mice can be found in Table 1. These results are from the present study and from previous research [31]. The present data are consistent with previous studies and show that subhypnotic doses of ethanol promote behavioral arousal in mice [9,11,12,30]. We showed that NPY −/− mice maintained on the C57BL/6J × 129/SvEv background displayed significantly greater locomotor activity following a 1.5-g/kg dose of ethanol than NPY +/+ littermate mice (Fig. 1). This is the first direct evidence that NPY is involved in the locomotor activation effects produced by subhypnotic doses of ethanol. Additionally, NPY −/− mice maintained on the C57BL/6J × 129/SvEv background showed reduced sensitivity to the sedative effects produced by a 3.5-g/kg dose of

Table 1
Response to ethanol of knockout mice relative to WT mice

Phenotype	NPY −/− (B6 × 129)	NPY −/− (129)	Y5 −/− (129)
Ethanol intake	High	High[a]	Normal
Ethanol-induced locomotion	High	Normal	Normal
Ethanol-induced sedation	Low	Normal	High
Plasma ethanol levels	Normal	Normal	High

B6 × 129 = C57BL/6 × 129/SvEv; 129 = 129/SvEv.
[a] High intake observed only during access to a 20% (v/v) ethanol solution.

ethanol, as evidenced by their shorter time to regain the righting reflex relative to controls (Fig. 2). This is consistent with previous research and suggests that NPY is also involved with the sedative effects of ethanol [31]. Together, the locomotor activation and sedation data suggest that NPY normally dampens the stimulatory effect of low doses of ethanol and promotes sedation at higher doses. This reasoning is consistent with NPY acting as an inhibitory neuropeptide on neuronal circuits involved in ethanol responsiveness.

Alterations in sensitivity to the sedative and locomotor activating effects of ethanol in NPY −/− mice are dependent on the genetic background of this model. The NPY −/− mice maintained on the inbred 129/SvEv background did not show alterations in ethanol-induced locomotor activity (Fig. 3A,B), nor did they show alterations in ethanol-induced sedation (Fig. 4A). Additionally, NPY −/− mice of this background showed increased consumption of ethanol only during access to the 20% solution (Fig. 5). These contrasting results demonstrated the dependence of phenotype on the genetic background of the knockout model, and are consistent with several examples in the literature, including studies that have examined ethanol-related phenotypes [2,10,19].

We have found that NPY −/− mice maintained on the C57BL/6J × 129/SvEv background (but not the inbred 129/SvEv background) are resistant to the sedative effects of ethanol, but show increased sensitivity to ethanol's locomotor-activation effect following low doses. This relationship has been described previously. Short-sleep (SS) and long-sleep (LS) mice were selectively bred for differential sensitivity to the sedative effects of ethanol as measured by recovery of the righting reflex. SS mice, which are resistant to the sedative effects of ethanol, show greater locomotor activity in response to low doses of ethanol than LS mice [11]. Similarly, FAST and SLOW mice were selected for differences in their response to the stimulating effects of ethanol. FAST mice, which are highly sensitive to ethanol-induced behavioral arousal, were found to be resistant to ethanol-induced sedation [30]. Thus, common genetic factors may influence sensitivity to the sedative and stimulating effects of ethanol. It is tempting to speculate that NPY signaling may be involved with sensitivity to ethanol-induced sedation and arousal in these selectively bred mouse models. However, it should be noted that this relationship between ethanol-induced sedation and arousal does not always hold when comparing different inbred strains of mice [7,8].

It has been suggested that the locomotor-activation effect caused by low doses of ethanol may be related to the euphoric and rewarding properties of this drug and may be a marker for abuse potential [32]. This argument is strengthened by the observation that ethanol-induced behavioral arousal is modulated by dopamine pathways that have also been implicated in mediating the positive reinforcing effects of drugs. Selective dopamine antagonists directed at either the D1 or D2/D3 dopamine receptor subtypes have been found to block behavioral arousal caused by ethanol without influencing baseline behavior [5]. An interesting possibility is that NPY −/− mice of the C57BL/6J × 129/SvEv background have increased sensitivity to the stimulating effects of ethanol because of alterations in dopamine signaling. Consistent with this hypothesis, NPY has been found to regulate dopamine levels in the nucleus accumbens [1].

Given that the ethanol-related phenotypes were less obvious in NPY −/− mice maintained on the 129/SvEv background, we did not expect large differences with Y5 −/− mice of this same genetic background. Y5 −/− mice did not show alterations in ethanol-induced locomotor activity when compared with WT mice (Fig. 3C), nor did the Y5 −/− mice show alterations in voluntary consumption of ethanol (Fig. 5). However, before ruling out a contribution of the Y5 receptor to voluntary ethanol consumption and ethanol-induced arousal, it will be important to evaluate Y5 −/− mice maintained on other genetic backgrounds. Surprisingly, Y5 −/− mice showed increased, rather than decreased, sensitivity to ethanol-induced sedation (Fig. 4B–D). We hypothesized that if the Y5 receptor is involved with sensitivity to the intoxicating effects of ethanol, mice lacking Y5 receptor should exhibit ethanol-related phenotypes similar to those found with NPY −/− mice. There are several possible explanations for these data. One explanation is based on our observation that the Y5 −/− mice had slight but significantly higher plasma ethanol levels following a 3.0-g/kg dose of ethanol (Fig. 6). High plasma ethanol levels could prolong the sedative effects produced by ethanol in Y5 −/− mice. Second, it is possible that elimination of the Y5 receptor causes up-regulation of other postsynaptic NPY receptors. For example, an increase in Y1 receptor population could augment NPY signaling. As such, the Y5 −/− mice would be similar to NPY overexpressing mice, which also show increased sensitivity to ethanol-induced sedation [31]. However, whole brain analyses with RT–PCR failed to reveal alterations of Y1 receptor expression in Y5 −/− mice [27]. Finally, it is possible that in neuronal pathways involved with the neurobiological actions of ethanol, the Y5 receptor functions as a presynaptic autoreceptor. As such, the Y5 −/− mice would lack presynaptic inhibition of NPY release, thus augmenting NPY signaling and rendering the Y5 −/− mice similar to NPY over-expressing mice.

In summary, we have extended previous results by showing that NPY −/− mice of the C57BL/6 × 129/SvEv background demonstrate increased sensitivity to the locomotor activation effects of subhypnotic doses of ethanol. This phenotype, as well as voluntary ethanol consumption and ethanol-induced sedation, are dependent on the genetic background of the mouse. Additionally, we have shown that Y5 −/− mice, of an inbred 129/SvEv

background, exhibit normal ethanol consumption and ethanol-induced locomotor activity, yet display increased ethanol-induced sleep time that may be related to high plasma ethanol levels. Assessing ethanol-associated phenotypes in Y5 −/− mice of other genetic backgrounds will be necessary to better understand the role of this receptor in regulating voluntary ethanol consumption and neurobiological responses to ethanol.

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