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ORIGINAL INVESTIGATION

Modulation of ethanol consumption by genetic and pharmacological manipulation of nicotinic acetylcholine receptors in mice

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Abstract
Rationale Alcohol and nicotine are commonly co-abused. Genetic correlations between responses to these drugs have been reported, providing evidence that common genes underlie the response to alcohol and nicotine. Nicotinic acetylcholine receptors (nAChRs) in the mesolimbic dopamine system are important in mediating nicotine response, and several studies suggest that alcohol may also interact with these nAChRs.
Objective The aim of this study was to examine the role of nAChRs containing α7 or β2 subunits in ethanol consumption.
Methods A two-bottle choice paradigm was used to determine ethanol consumption in wild-type and nAChR subunit knockout mice. Challenge studies were performed using the α4β2 nAChR partial agonist varenicline.
Results Mice lacking the β2 subunit consumed a similar amount of ethanol compared to their wild-type siblings in an ethanol-drinking paradigm. In contrast, mice lacking the α7 nAChR receptor subunit consumed significantly less ethanol than wild-type mice but consumed comparable amounts of water, saccharin, and quinine. In C57BL/6J mice, varenicline dose-dependently decreased ethanol consumption with a significant effect of 2 mg/kg, without affecting water or saccharin consumption. This effect of varenicline was not reversed in mice lacking either the α7 or β2 subunit, providing evidence that nAChRs containing one of these subunits are not required for this effect of varenicline.
Conclusions This study provides evidence that α7 nAChRs are involved in ethanol consumption and supports the idea that pharmacological manipulation of nAChRs reduces ethanol intake. Additional nAChRs may also be involved in ethanol intake, and there may be functional redundancy in the nicotinic control of alcohol drinking.

Keywords Alcoholism · Ethanol · Varenicline · Nicotinic acetylcholine receptors · Mice

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Introduction

Alcohol and nicotine are widely abused and commonly used together (Grant et al. 2004; Istvan and Matarazzo 1984; Talcott et al. 1998). In addition, the rate of tobacco use is nearly double in alcohol-dependent individuals compared to the population as a whole (Falk et al. 2006). Similarly, while the incidence of nicotine dependence in the US population is relatively low (12.8%), it is more than three times higher in alcohol-dependent individuals (Falk et al. 2006). Genetic factors account for as much as 40% of the covariance between alcohol and cigarette use (Swan et al. 1997). When clinical diagnoses of nicotine and alcohol dependence were assessed, the covariance was even greater (68%; True et al. 1999). In animal models, there is also evidence of shared genetic determination for alcohol and nicotine traits. Mice selectively bred for extreme sensitivity to the locomotor stimulant properties of ethanol were also more stimulated by nicotine (Bergstrom et al. 2003). Furthermore, there were differences in responses to nicotine

in rats and mice selectively bred for sensitivity to the sedative-hypnotic effects of ethanol (Collins et al. 1993; de Fiebre et al. 1987, 1990, 1991, 2002). Together, these genetic correlations provide evidence that common genes influence alcohol and nicotine behaviors.

Nicotinic acetylcholine receptors (nAChRs) are the primary targets for nicotine in the brain (Picciotto et al. 1998), and it is possible that these receptors may be responsible for a portion of the shared genetic susceptibility to alcohol- and nicotine-related traits. A number of studies have used the broad nAChR antagonist mecamylamine to investigate alcohol drinking, and in most cases, this drug decreased ethanol consumption (Blomqvist et al. 1996; Ericson et al. 1998; Farook et al. 2009; Hendrickson et al. 2009; Kuzmin et al. 2009; Le et al. 2000; Nadal et al. 1998; but see Dyr et al. 1999). Unfortunately, because this drug is nonselective among subtypes, it is unclear which nAChRs are involved in this effect. Recently, varenicline, an α4β2 partial agonist marketed as a smoking cessation aid (Coe et al. 2005; Reus et al. 2007; Rollema et al. 2007a, b), has been used to address this question. Although the effects of varenicline are generally attributed to its actions at α4β2 nAChR, varenicline can interact with other nAChRs, including the α7 homomeric receptor subtype, at higher concentrations. Varenicline can decrease ethanol consumption and seeking in rats (Steensland et al. 2007). Furthermore, this drug blocks elevations in dopamine levels observed after acute ethanol administration in rats (Ericson et al. 2009). Finally, when smokers were given varenicline, they exhibited reduced alcohol consumption and craving compared to placebo (McKee et al. 2009). These studies suggest that targeting nAChRs could be a potential therapy for alcoholism.

The goal of the current study was to evaluate the role of α7 and β2* (where * indicates co-assembled subunits) nAChRs in ethanol consumption using genetic and pharmacologic tools. We hypothesized that mice carrying a null mutation (knockout, KO) for either the α7 or the β2 nAChR subunit might differ from wild-type (WT) mice in ethanol consumption. Moreover, varenicline would decrease ethanol consumption in C57BL/6J mice and that this might be altered in either α7KO or β2KO mice.

Materials and methods

Subjects Mice were singly housed with food (Harlan Teklad #2018) and water available ad libitum. The colony room was maintained at 21±2°C on a 12-h light–dark cycle. Red incandescent lamps were used to aid in measuring fluid levels during the dark cycle. All procedures were approved by the Yale Animal Care and Use Committee and were consistent with the *Guide for the Care and Use of Laboratory Animals* (National Research Council 1996). Male and female C57BL/6J mice were obtained from The Jackson Laboratory (Bar Harbor, ME, USA) and were acclimated to our animal facility for at least 2 weeks prior to testing. α7KO and β2KO mice were generated by homologous recombination (Orr-Urtreger et al. 1997; Picciotto et al. 1995). All KO mice and their WT siblings used in these studies were littermates generated from heterozygous (HET) breeding pairs at Yale University. KO mice had been backcrossed to the C57BL/6J strain for at least 15 (α7) or 37 (β2) generations. Animals were genotyped using genomic DNA extracted from tail biopsy at weaning.

Drugs Ethanol (200 proof) was obtained from Pharmco (Brookfield, CT, USA). Saccharin sodium salt and quinine hemisulfate salt were purchased from Sigma (St. Louis, MO, USA). Ethanol and tastants were diluted in tap water. Varenicline tartrate was from Pfizer (Groton, CT, USA) and was mixed in Dulbecco's phosphate buffered saline (Invitrogen, Grand Island, NY, USA) for i.p. injection. Volume of injection (10 ml/kg) was adjusted to body weight to achieve the desired dose (expressed as varenicline base).

Two-bottle choice ethanol drinking Free-choice ethanol consumption was measured using a standard protocol (Kamens et al. 2006). Mice were singly housed and presented with two 25-ml tubes fitted with drinking spouts filled with water to acclimate them to the testing environment (see Table 1 for the experimental design). Day 1, one water tube was replaced with a tube containing 3% ethanol. Side of ethanol presentation was alternated every 2 days. Every 4 days, ethanol concentration was increased (3%, 6%, 10%, and 20%). For each concentration, the average consumption on days 2 and 4 was used for the analysis. Evaporation/leakage was estimated from tubes placed on empty cages, and mean volume lost was subtracted from individual drinking values. Three dependent values were obtained: ethanol consumption (grams per kilogram), water consumption (milliliters), and total fluid consumption (milliliters).

Tastant drinking Two weeks after ethanol consumption, tastant consumption was measured. Saccharin (0.033% and 0.066%) or quinine (0.05 and 0.03 mM) was offered in a two-bottle choice procedure (Kamens et al. 2005). Tastants were presented for 4 days (lower concentration offered first). Dependent variables included tastant (milligrams per kilogram), water (milliliters), and total fluid consumption (milliliters).

Pharmacological manipulation of ethanol and saccharin drinking Mice were challenged with saline or varenicline (0.25, 0.50, 1, and 2 mg/kg) to determine effects on ethanol

Table 1 Experimental design and timeline of procedures

a) Characterization of ethanol consumption in β2 and α7 WT, HET and KO mice.									
Time	**- 4 days -**	**- 4 days -**	**- 4 days -**	**- 4 days -**	**- at least 1 week -**	**- 4 days -**	**- 4 days -**	**- 4 days -**	**- 4 days -**
Drug	Ethanol				Water	Saccharin		Quinine	
	3%	6%	10%	20%		0.033%	0.066%	0.15 mM	0.30 mM

b) Effects of varenicline on ethanol consumption in C57BL/6J, β2 and α7 mice.											
Day	**Until stable**	**1**	**4**	**7**	**10**	**13**	**- at least 1 week -**	**Until stable**	**1**	**4**	**7**
Drug	10% Ethanol						Water	0.033% Saccharin			
Varenicline (mg/kg)		Saline	0.25	0.5	1	2			Saline	1	2
(For both ethanol and saccharin studies, doses of varenicline were randomized and delivered in a Latin square design)											

consumption. Doses were based on prior work examining effects of varenicline on ethanol behaviors (Gulick and Gould 2008; Steensland et al. 2007). Mice were singly housed and presented with two modified 10-ml serological pipettes, one containing 10% ethanol and the other with tap water, for 4 days. The side of ethanol presentation was constant for an individual animal but was counterbalanced across animals. The volume of the tubes was recorded daily 1 h before lights off and food and body weights were recorded. Starting on day 5, mice received daily saline injections 30 min before the dark phase and before presentation of the tubes. Fluid levels were read 3 h later. Varenicline administration started after animals reached stable ethanol consumption (<10% variation over a 3-day period). Each mouse received all doses of varenicline and saline using a Latin square design. Mice were allowed to return to baseline drinking levels between each challenge day (data not shown).

Animals were given 7 days ad libitum access to food and water after ethanol testing before testing saccharin consumption. On day 1, animals received two 25-ml tubes, one filled with water and the other with 0.033% saccharin counterbalanced as described for ethanol. After stable consumption was reached (<20% variation over 3 days), mice were challenged with varenicline (1 or 2 mg/kg) or saline. This study was first conducted in 20 11-week old C57BL/6J mice (ten males/ten females) and then in α7 (nine WT and nine KO) or β2 (13 WT and 14 KO) mice (3–9 months old, matched for age at the start of testing). No interactions of sex and varenicline were seen in C57BL/6J mice, so female mice were used due to availability and higher baseline levels of ethanol intake.

Blood ethanol concentrations To determine if varenicline altered ethanol metabolism, blood ethanol concentrations (BEC) were examined based on published methods (Kamens et al. 2006). Nine male and female C57BL/6J mice were injected with saline or varenicline (0.5, 1, or 2 mg/kg) 30 min prior to 4 g/kg ethanol. At 30, 60, 120, and 180 min following the ethanol injection, a blood sample was taken from the tail vein. Serum ethanol levels were measured by spectrophotometer measurements using the Genzyme Diagnostics (Framingham, MA, USA) ethanol assay.

Statistical analysis Ethanol consumption, water consumption, total fluid consumption, and BEC were used as primary dependent variables. Data were analyzed using factorial analysis of variance (ANOVA) with the alpha level set at 0.05. Strain, sex, dose, time, and concentration were possible variables. Interactions involving multiple factors were examined using successive ANOVAs including fewer factors. Significant two-way interactions were followed up with simple effects analysis and Tukey's post hoc test. Statistica (StatSoft, Tulsa, OK, USA) was used for all analyses.

Results

Effects of deletion of the β2 subunit of the nAChR on ethanol and tastant consumption

β2KO mice consumed a similar amount of ethanol as β2WT mice (Fig. 1a, d). Due to a significant main effect of sex and a significant sex × concentration interaction, male and female mice were analyzed independently. There was a significant main effect of ethanol concentration in male and female mice ($F_{3,\ 51}$=30.3, p<0.001, $F_{3,\ 69}$=31.3, p<0.001, respectively), but no other significant effects. Ethanol consumption increased in a concentration-dependent manner (Fig. 1a, d). A significant strain × concentration interaction ($F_{6,\ 120}$=3.0, p<0.01) was observed for water consumption. When 20% ethanol was available, β2KO mice consumed significantly more water than β2HET mice

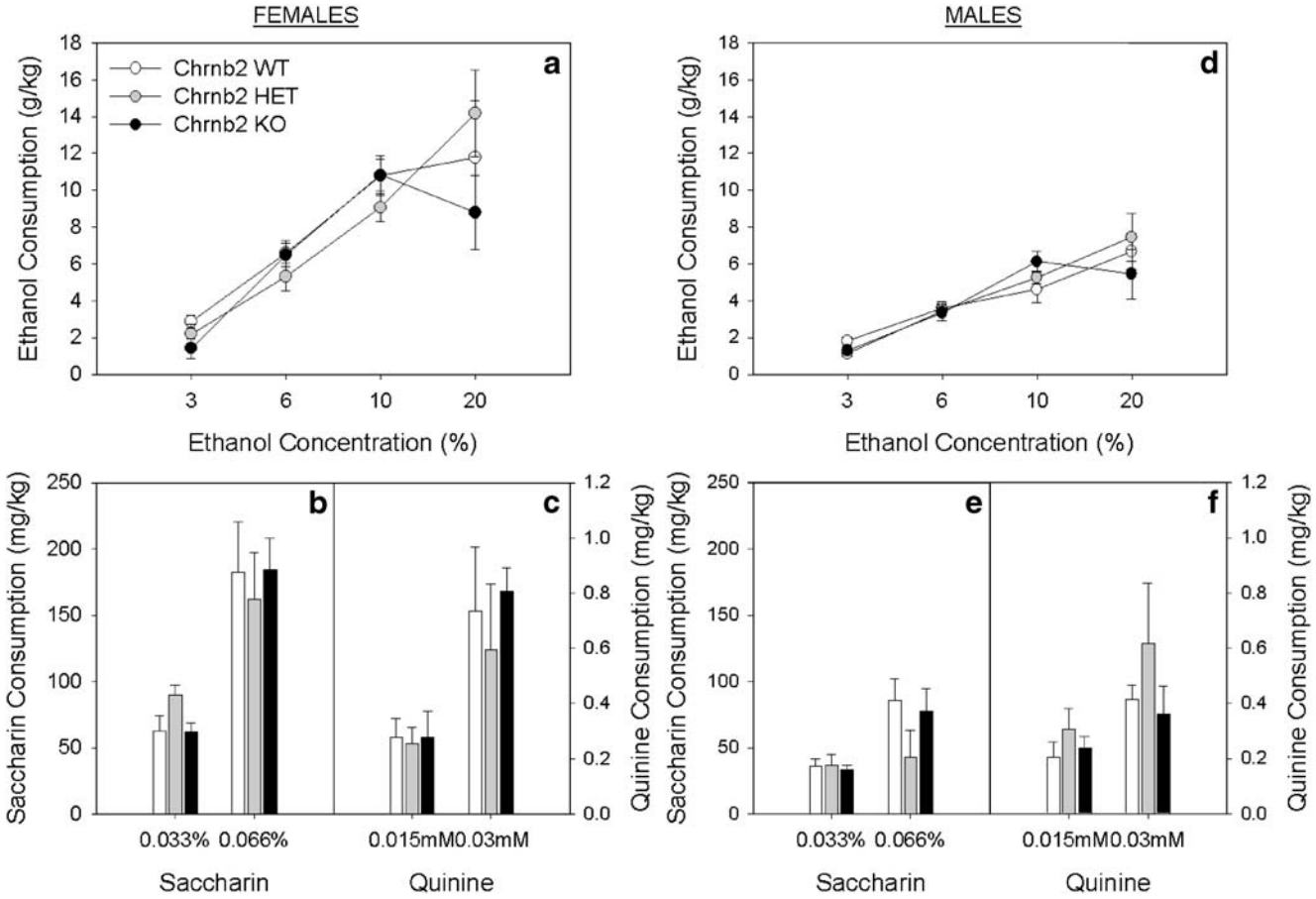

Fig. 1 Deletion of the β2 subunit does not alter ethanol consumption. **a** Ethanol consumption, **b** saccharin consumption, and **c** quinine consumption in female β2WT (Chrnb2 WT), β2HET (Chrnb2 HET), and β2KO (Chrnb2 KO) mice. **d** Ethanol consumption, **e** saccharin consumption, and **f** quinine consumption in male β2WT, β2HET, and β2KO mice. Data are represented as mean ± SEM. N=6–10 per group

(mean ± SEM—2.21±0.25, 1.13±0.28, respectively). Total volume was also analyzed within each sex, due to a significant main effect of sex and significant sex × concentration interaction. In female mice, there was a significant main effect of concentration ($F_{3,\ 51}$=3.6, p< 0.05). Female mice consumed significantly more fluid when 10% ethanol, compared to when 3% ethanol, was presented (4.03±0.13, 3.25±0.24, respectively). In male mice, a significant strain × concentration effect was observed ($F_{6,\ 69}$=2.4, p<0.05). β2WT mice consumed significantly less fluid when 20% ethanol was presented compared to any other concentration (3.48±0.28, 6%—3.56±0.22, 10%—3.57±0.17, 20%—2.52±0.28), while β2HET mice tended to consume more fluid when 6% ethanol was presented compared to 3% (p=0.07) or 10% ethanol (p=0.07).

To determine if differences in ethanol consumption could be attributed to taste sensitivity, we examined consumption of a sweet (saccharin) and bitter (quinine) noncaloric tastant. There were no differences in saccharin consumption in β2KO mice compared to β2WT or β2HET mice (Fig. 1b, e). There was a significant main effect of sex on saccharin consumption, and this interacted with concentration; thus, the sexes were examined independently. Female mice consumed significantly more saccharin when the 0.066% concentration of saccharin was presented compared to when the 0.033% concentration was presented ($F_{1,\ 17}$=28.1, p<0.001; 175.9±18.6 vs. 71.9±5.8, respectively). There was a significant main effect of concentration in male mice as well ($F_{1,\ 23}$=10.5, p<0.01), but no other significant effects. Male mice also consumed more saccharin when the high concentration of saccharin was presented compared to the low concentration (71.1±10.4, 35.1±3.1, respectively). There were no significant effects on water consumption. There was a significant strain × concentration interaction on total fluid consumption ($F_{2,\ 40}$=3.9, p<0.05). β2WT and β2KO mice both consumed more fluid when the 0.066% concentration was presented compared to when the 0.033% concentration was available (β2WT 0.033 vs. 0.066%, 4.61±0.26 vs. 5.71±0.53, respectively; β2KO 0.033 vs. 0.066%, 4.86±0.23 vs. 6.23±0.55, respectively). There were no statistical differences in β2HET mice.

β2KO mice consumed a similar amount of the bitter tastant quinine as β2WT and β2HET animals (Fig. 1c, f). When consumption of quinine was analyzed, there was a significant main effect of concentration ($F_{1, 40}$=22.9, p< 0.001), but no other significant main effects or interactions. More quinine was consumed when the high concentration of quinine (0.03 mM) was presented compared to the low concentration (0.56±0.07 vs. 0.26±0.02, respectively). There were no significant effects observed for water consumption. There was a significant main effect of concentration for total water consumption ($F_{1, 40}$=4.7, p< 0.05). Mice consumed more fluid when the high concentration of quinine was available compared to when the low concentration was presented (3.66±0.14 vs. 3.33±0.09).

Effects of deletion of the α7 subunit of the nAChR on ethanol and tastant consumption

Female α7KO mice consumed significantly less ethanol compared to α7WT mice (Fig. 2a). Ethanol consumption (grams per kilogram) data were analyzed using a three-way ANOVA with strain, sex, and concentration as variables. There was a significant main effect of sex and a significant sex × concentration interaction; thus, we examined each sex independently. Using a two-way ANOVA, there was a significant main effect of strain ($F_{2, 60}$=5.0, p<0.05) and concentration ($F_{3, 60}$=23.7, p<0.001) in female mice, but the interaction was not significant. Female α7KO mice consumed significantly less ethanol than α7WT mice. Ethanol consumption increased in a concentration-dependent manner but leveled off at 10% and 20% ethanol. In male mice, there was a significant main effect of concentration ($F_{3, 63}$= 22.2, p<0.001), but no other significant effects and ethanol consumption increased with concentration, leveling off between 10% and 20% ethanol. There was a significant main effect of concentration on water consumption ($F_{3, 123}$= 22.6, p<0.001), but no other significant effects. Mice consumed more water when 20% ethanol was presented (3%, 6%, 10%, and 20% ethanol—1.27±0.14, 1.18±0.16, 1.24±0.16, 2.67±0.22). There was a significant main effect of sex on total fluid consumption ($F_{1, 123}$=27.6, p<0.001), but no other significant main effects or interactions. Female

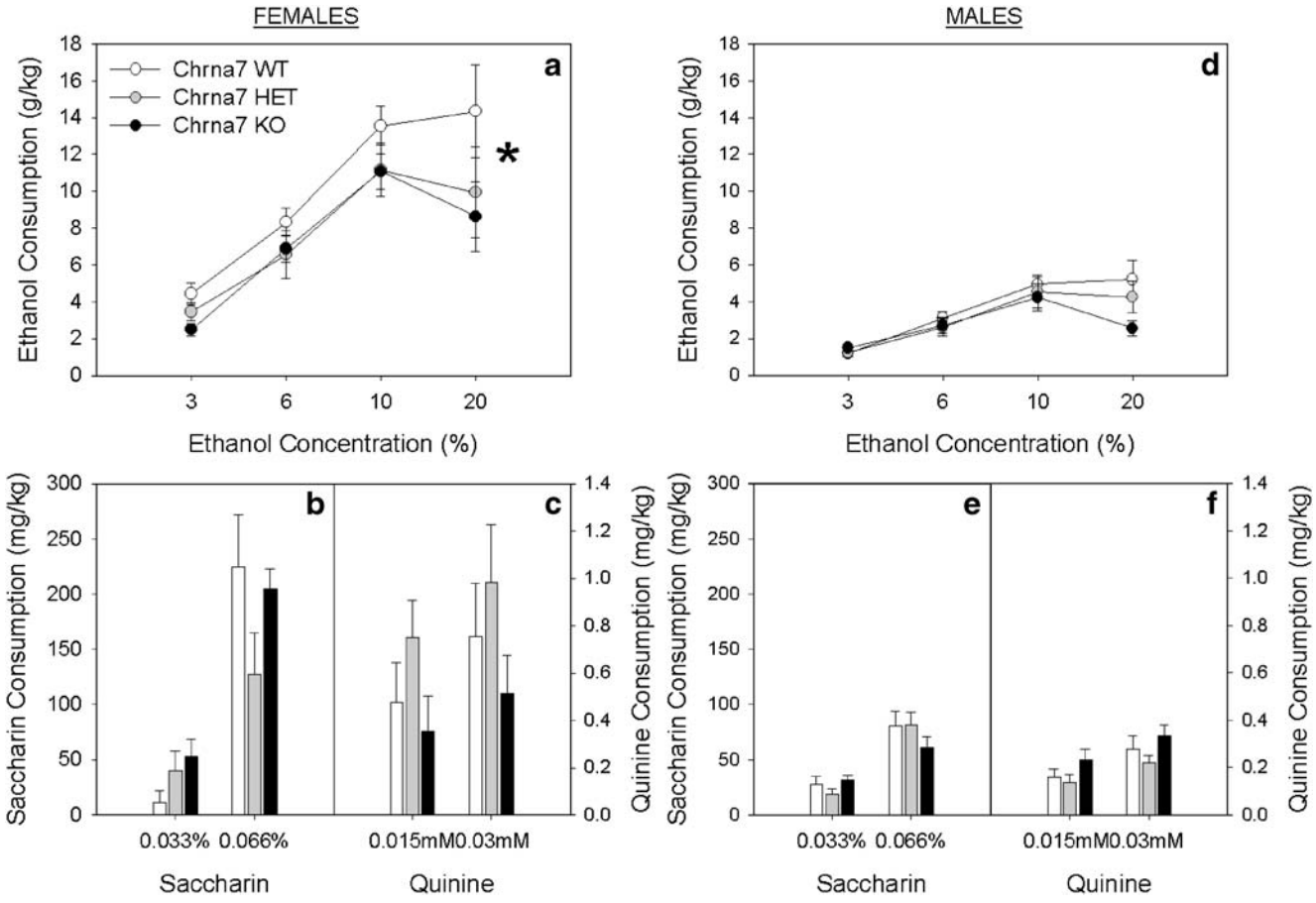

Fig. 2 Deletion of the α7 subunit of the nAChR significantly decreases ethanol consumption. **a** Ethanol consumption, **b** saccharin consumption, and **c** quinine consumption in female α7WT (Chrna7 WT), α7HET (Chrna7 HET), and α7KO (Chrna7 KO) mice. **d** Ethanol consumption, **e** saccharin consumption, and **f** quinine consumption in male α7WT, α7HET, and α7KO mice. Data are represented as mean ± SEM. N=6–9 per group. *p<0.05

mice consumed more fluid than male mice (4.25±0.09 vs. 3.50±0.07, respectively).

α7KO mice did not differ when saccharin consumption (milligrams per kilogram) was examined using a three-way ANOVA with strain, sex, and concentration as factors (Fig. 2b, e). There was a significant main effect of sex and a significant sex × concentration interaction; therefore, data were analyzed independently by sex. There was a significant main effect of concentration in female mice ($F_{1, 20}$= 44.8, p<0.001) with more saccharin consumed when the high concentration of the saccharin was presented compared to the low concentration (187.5±20.5 vs. 36.4±9.0, respectively). In male mice, there was a main effect of concentration ($F_{1, 21}$=53.5, p<0.001) with more saccharin consumed when the high concentration was presented compared to the low concentration (73.8±6.4 vs. 26.0± 3.0, respectively). Water consumption was also analyzed using three-way ANOVA. There was a significant main effect of sex as well as a significant interaction between concentration and sex, so each sex was analyzed separately. In female mice, there was a significant main effect of concentration ($F_{1, 20}$=14.4, p<0.01) with significantly more water consumption when the lower concentration of saccharin was present compared to the higher concentration (2.51±0.45 vs. 0.58±0.24, respectively). In male mice, there was a significant strain×concentration interaction ($F_{2, 21}$=3.9, p<0.05). α7HET mice consumed significantly more water when the low concentration was presented compared to the high concentration (1.62±0.41 vs. 0.26± 0.14, respectively). There were no significant differences in water consumption in α7WT or α7KO mice. There was a significant main effect of sex and a sex × concentration interaction on total fluid consumption; thus, data were examined independently by sex. In female mice, there was a significant main effect of concentration ($F_{1, 20}$=9.6, p< 0.001) with more fluid consumed when the high concentration of saccharin was presented compared to the low concentration of saccharin (7.32±0.66 vs. 5.07±0.31). In males, there was a significant main effect of concentration ($F_{1, 21}$=14.5, p<0.01), but no other significant effects. Male mice consumed more fluid when the high concentration of saccharin was present (0.033% vs. 0.066%; 4.05± 0.19 vs. 4.76±0.24, respectively).

α7KO mice consumed a similar amount of quinine as α7WT and α7HET mice (Fig. 2c, f). There was a significant main effect of sex and interaction with other factors on quinine consumption; thus, we examined female and male mice independently. In female mice, there were no significant main effects or interactions. In male mice, a significant main effect of concentration was observed ($F_{1, 21}$=13.0, p< 0.01). Male mice consumed significantly less quinine at the lower concentration of quinine compared to the higher concentration (0.18±0.02 vs. 0.29±0.03, respectively). There was a significant main effect of strain and a significant strain × sex interaction on water intake. In female mice, there was a significant main effect of strain ($F_{1, 41}$=4.7, p<0.05), such that α7KO and α7WT mice consumed more water than α7HET mice (α7WT—3.12±0.26, α7HET—1.82±0.32, α7KO—2.98±0.26). There were no significant effects in male mice. When total fluid consumption was examined using a three-way ANOVA, the only significant effect was a main effect of sex ($F_{1, 41}$=23.3, p<0.001). Females consumed significantly more fluid than males (4.40±0.17 vs. 3.23±0.08, respectively).

Effects of varenicline on ethanol and tastant consumption in C57BL/6J mice

Varenicline decreased ethanol consumption in C57BL/6J mice during the first 3 h following varenicline administration as evidenced by a significant main effect of dose ($F_{4, 72}$=4.6, p<0.01; Fig. 3a). Overall, female C57BL/6J mice consumed significantly more ethanol than male mice ($F_{1, 72}$=13.7, p< 0.01; 2.0±0.1, 1.1±0.1, respectively), but this factor did not interact with dose. At 3 h post varenicline administration, there was no significant main effect of dose on water consumption (Table 2), but there was a significant main effect of sex ($F_{1, 72}$=8.5, p<0.01). Male mice consumed significantly more water than female mice (0.34±0.03, 0.17±0.02, respectively). There was a significant main effect of dose on total volume consumed ($F_{4, 72}$=3.8, p<0.01). There was an overall reduction in fluid intake when the mice received 2 mg/kg varenicline compared to saline (Table 2). By 23 h after drug administration, the effects of varenicline on ethanol consumption were no longer apparent (Table 2). Female mice consumed significantly more ethanol than male mice ($F_{1, 72}$=45.5, p<0.001; 12.5±0.4, 6.1±0.4, respectively), but there were no other significant effects. Similar to the 3 h time point, at 23 h after varenicline administration, male mice consumed significantly more water than female mice ($F_{1, 72}$=13.4, p<0.01; 1.61±0.11, 0.73±0.07, respectively). There were no significant differences in total fluid intake. Treatment with varenicline had no significant effects on food consumption (data not shown), but there was a significant main effect of sex ($F_{1, 72}$=15.0, p<0.01). Male mice consumed more food than female mice (3.42±0.08, 2.84± 0.08, respectively).

Varenicline had no effect on saccharin consumption in C57BL/6J mice (Fig. 3b). There were no significant main effects or interactions on saccharin or water consumption 3 h after varenicline administration. There was a significant main effect of sex ($F_{1, 36}$=12.7, p<0.01) and dose ($F_{2, 36}$=3.3, p< 0.05) on total fluid consumption 3 h after varenicline administration, but the interaction was not significant. Males consumed significantly more fluid than female mice (1.43± 0.11, 0.87±0.08, respectively). The 2 mg/kg dose of

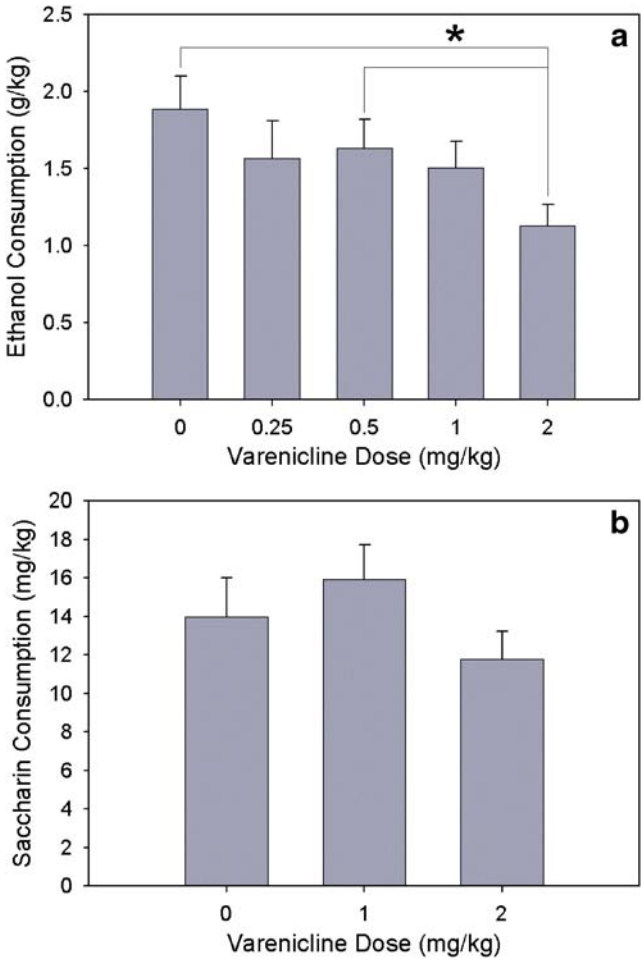

Fig. 3 Pretreatment with varenicline decreases ethanol consumption, but not saccharin consumption in C57BL/6J mice. The effect of pretreatment with varenicline on **a** ethanol consumption and **b** saccharin consumption. Data are represented as mean ± SEM for consumption during the first 3 h of choice administration. N=20 per group. *p<0.05

varenicline tended to decrease total fluid consumption compared to saline (p=0.08) or 1 mg/kg varenicline (p=0.07); 23 h after varenicline administration, there were no significant main effects or interactions with any variable (Table 3).

Effects of varenicline on ethanol and tastant consumption in mice lacking the β2 subunit of the nAChR

The highest dose of varenicline (2 mg/kg) decreased ethanol consumption in β2WT and β2KO mice (Fig. 4a). Varenicline decreased ethanol consumption 3 h after administration in both β2WT and β2KO mice as indicated by a significant main effect of dose ($F_{4,\ 100}$=3.9, p<0.01) that did not interact with strain. Varenicline had no effect on water consumption, but there was a significant main effect of dose on total fluid consumption ($F_{4,\ 100}$=2.6, p<0.05). Mice given 2 mg/kg varenicline consumed significantly less fluid than mice given 1 mg/kg varenicline (0.46±0.04 vs. 0.60±0.06, respectively).

At 23 h after varenicline administration, there was no significant effect of varenicline treatment on ethanol consumption (Table 4). There was a significant strain × dose interaction on water consumption ($F_{4,\ 100}$=2.6, p< 0.05). β2KO and β2WT mice did not differ in water consumption across doses, but water consumption in the β2KO mice differed across dose. β2KO mice receiving 0.5 mg/kg varenicline consumed significantly less water than mice receiving a saline injection (p<0.05; 1.14±0.28 vs. 1.78±0.35, respectively). There was no difference in water consumption across doses in the β2WT mice. There was a significant strain × dose interaction ($F_{4,\ 100}$=4.3, p< 0.01) on total fluid consumption 23 h after varenicline administration. β2KO mice consumed significantly less fluid than β2WT mice (p<0.05; 3.60±0.24 vs. 4.31±0.17, respectively) when given 0.5 mg/kg varenicline. While the dose of varenicline had no effect on total fluid consumption in β2KO mice, β2WT mice receiving 0.25 or 2 mg/kg varenicline consumed significantly less fluid than β2WT mice receiving 0.5 mg/kg varenicline (p<0.05; 3.88±0.13 or 3.80±0.20 vs. 4.31±0.17, respectively).

At 3 h after varenicline administration, the 2 mg/kg dose of varenicline tended to reduce saccharin consumption compared to the 1 mg/kg dose of varenicline (Fig. 4b). When saccharin consumption was examined 3 h after varenicline administration using a two-way ANOVA with

Table 2 Ethanol, water, and total fluid intake 3 and 23 h after varenicline administration in C57BL/6J mice

Varenicline (mg/kg)	Hours of drinking				
	3h		23h		
	Water (ml)	Total fluid (ml)	Ethanol (g/kg)	Water (ml)	Total fluid (ml)
Saline	0.27±0.04	0.78±0.05	10.0±1.0	1.17±0.18	3.90±0.18
0.25	0.27±0.04	0.69±0.06	9.2±0.9	1.19±0.17	3.72±0.17
0.5	0.24±0.04	0.68±0.05	9.1±1.0	1.26±0.19	3.75±0.16
1	0.23±0.04	0.65±0.04	9.6±0.9	1.00±0.15	3.62±0.11
2	0.28±0.06	0.59±0.06[a]	8.6±0.9	1.24±0.22	3.57±0.17

[a] Represents significantly less consumption than saline (p<0.05)

Table 3 Saccharin, water, and total fluid intake 3 and 23 h after varenicline administration in C57BL/6J mice

Varenicline (mg/kg)	Hours of drinking				
	3h		23h		
	Water (ml)	Total fluid (ml)	Saccharin (mg/kg)	Water (ml)	Total (ml)
Saline	0.22±0.07	1.26±0.15	52.2±6.7	0.92±0.25	4.67±0.27
1	0.08±0.26	1.27±0.16	59.6±4.1	0.29±0.14	4.62±0.26
2	0.35±0.11	0.93±0.09[a]	51.1±5.5	0.58±0.23	4.22±0.21

[a] Represents a trend toward less consumption compared to saline (p=0.08) or 1 mg/kg (p=0.07)

strain and dose as factors, there was a significant main effect of dose ($F_{2,\ 48}$=3.3, p<0.05) that did not interact with strain. Tukey's post hoc tests revealed that 2 mg/kg varenicline decreased saccharin consumption when compared to 1 mg/kg varenicline (p=0.05), but no other significant effects were observed. There were no significant main effects or interactions on water consumption (Table 5). There was a significant strain × dose interaction ($F_{2,\ 48}$= 3.2, p<0.05) when total fluid consumption was examined 3 h after varenicline administration. β2WT mice that received a saline injection consumed significantly more total fluid than β2KO mice (p<0.05; 1.55±0.15 vs. 1.00± 0.08, respectively). Total fluid consumption was also dependent on dose in both β2WT and β2KO mice. β2WT mice receiving 2 mg/kg consumed significantly less than mice receiving saline (p<0.05; 1.02±0.15 vs. 1.55± 0.15, respectively). β2KO mice receiving 2 mg/kg varenicline consumed significantly less than those receiving 1 mg/kg varenicline (p<0.05; 0.83±0.15 vs. 1.25±0.14, respectively). When saccharin consumption, water consumption, and total fluid consumption were examined 23 h after varenicline administration, there were no significant main effects or interactions observed (Table 5).

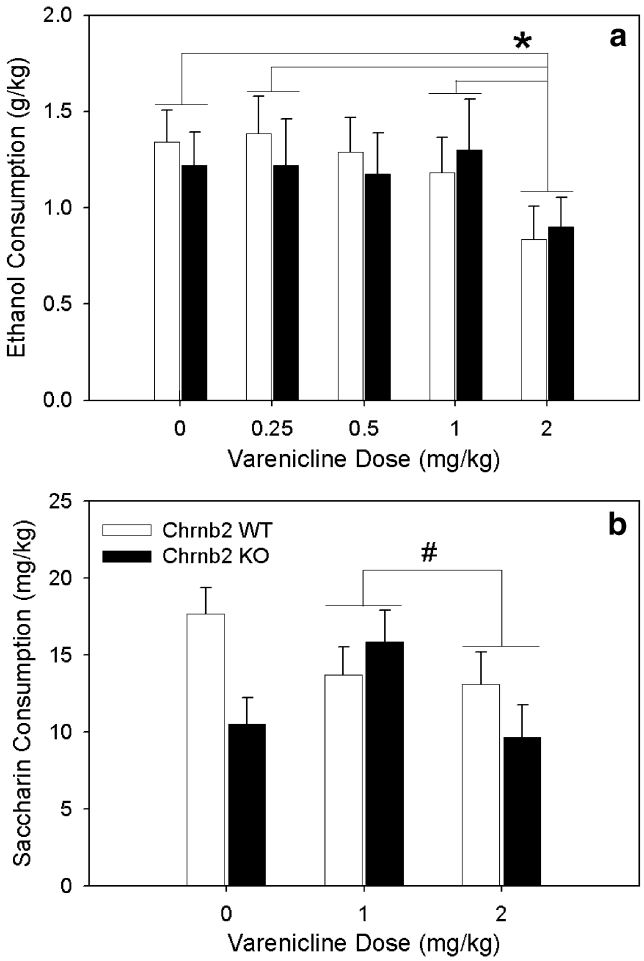

Fig. 4 Pretreatment with varenicline decreases ethanol consumption in β2WT (Chrnb2 WT) and β2KO (Chrnb2 KO) mice. The effect of pretreatment with varenicline on **a** ethanol consumption and **b** saccharin consumption. Data are represented as mean ± SEM for consumption during the first 3 h of choice administration. N=13–14 per group. *p<0.05; #p=0.05

Effects of varenicline on ethanol and tastant consumption in mice lacking the α7 subunit of the nAChR

Varenicline decreased ethanol consumption in both α7WT and α7KO mice (Fig. 5a). During the first 3 h of ethanol choice, there was a main effect of dose ($F_{4,\ 64}$=4.7, p< 0.01) that did not interact with strain, indicating that while varenicline decreases ethanol consumption, the α7 genotype does not modulate this effect. During the first 3 h of choice consumption, there were no significant main effects or interactions on water consumption, but there was a significant main effect of dose ($F_{4,\ 64}$=3.4, p<0.05) on total fluid intake (Table 6). Mice receiving 2 mg/kg varenicline consumed significantly less fluid compared to mice receiving saline (0.37±0.04 vs. 0.56±0.05, respectively) independent of genotype. At 23 h after varenicline administration, there were no significant main effects or interaction with any variable (grams per kilogram of ethanol consumption, water consumption, or total fluid intake; Table 6).

Administration of varenicline had no effect on saccharin consumption (Fig. 5b; Table 7). There were no significant main effects or interactions observed on saccharin consumption, water consumption, or total fluid consumption when monitored 3 or 23 h after varenicline administration.

Table 4 Ethanol, water, and total fluid intake 3 and 23 h after varenicline administration in β2WT and β2KO mice

Varenicline (mg/kg)	Hours of drinking				
	3h		23h		
	Water (ml)	Total fluid (ml)	Ethanol (g/kg)	Water (ml)	Total fluid (ml)
β2WT—saline	0.23±0.05	0.62±0.05	10.10±0.93	1.50±0.32	4.12±0.20
β2WT—0.25	0.12±0.04	0.55±0.06	10.46±0.93	1.18±0.26	3.88±0.13[b]
β2WT—0.5	0.20±0.05	0.56±0.06	9.91±0.93	1.73±0.31	4.31±0.17
β2WT—1	0.27±0.08	0.61±0.10	9.80±0.70	1.56±0.24	4.13±0.13
β2WT—2	0.24±0.04	0.49±0.06	8.44±0.63	1.59±0.27	3.80±0.20[b]
β2KO—saline	0.22±0.05	0.55±0.03	8.81±1.21	1.78±0.35	4.06±0.17
β2KO—0.25	0.17±0.05	0.52±0.05	9.58±1.45	1.38±0.32	3.88±0.14
β2KO—0.5	0.11±0.02	0.46±0.06	9.58±1.31	1.14±0.28[a]	3.60±0.24[b]
β2KO—1	0.22±0.04	0.59±0.07	10.28±1.37	1.34±0.30	3.98±0.19
β2KO—2	0.18±0.05	0.44±0.05	9.60±1.26	1.46±0.30	3.95±0.14

[a] Represents significantly less than β2 KO saline ($p<0.05$)

[b] Represents significantly less than β2 WT 0.5 mg/kg varenicline ($p<0.05$)

Varenicline does not alter ethanol metabolism

Pretreatment with varenicline did not alter the clearance of 4 g/kg ethanol (Fig. 6). A three-way repeated measures ANOVA revealed a significant main effect of sex ($F_{1,\ 84}=4.3$, $p<0.05$) and time ($F_{3,\ 84}=60.1$, $p<0.001$), but not other significant effects. Overall, male mice had a higher BEC compared to female mice (3.32±0.09 vs. 3.06±0.08, respectively). As expected, blood ethanol levels decreased in the 3 h following ethanol injection.

Discussion

Numerous reports have shown that the nAChR antagonist mecamylamine decreases ethanol consumption (Blomqvist et al. 1996; Ericson et al. 1998; Farook et al. 2009; Hendrickson et al. 2009; Kuzmin et al. 2009; Le et al. 2000; Nadal et al. 1998), but the nAChR subtypes through which this occurs are not known. We used genetic and pharmacological approaches to demonstrate a role for α7, but not β2*, nAChRs in baseline ethanol consumption. Furthermore, varenicline, a partial agonist of α4β2* and a less potent full agonist of α7 nAChRs, decreased ethanol consumption in mice, but this did not require the expression of either nAChR subunit perhaps because of functional redundancy among subtypes. Moreover, varenicline had no effect on ethanol metabolism.

Previous studies using nAChR subunit knockout mice have implicated both β2* and α7 nAChRs in ethanol-related behaviors, but neither subunit alone has been shown to alter ethanol consumption. We found for the first time that α7KO mice consume significantly less ethanol than α7WT mice. These data are consistent with the observation

Table 5 Saccharin, water, and total fluid intake 3 and 23 h after varenicline administration in β2WT and β2KO mice

Varenicline (mg/kg)	Hours of drinking				
	3h		23h		
	Water (ml)	Total fluid (ml)	Saccharin (mg/kg)	Water (ml)	Total fluid (ml)
β2WT—saline	0.23±0.05	1.55±0.15	104.72±7.03	0.47±0.08	7.29±0.54
β2WT—1	0.23±0.10	1.25±0.10	94.35±10.15	0.61±0.31	6.76±0.51
β2WT—2	0.14±0.05	1.02±0.15[a]	88.50±8.40	0.43±0.21	6.12±0.48
β2KO—saline	0.18±0.07	1.00±0.08[b]	83.21±4.74	0.53±0.25	5.91±0.50
β2KO—1	0.07±0.03	1.25±0.14	87.55±5.76	0.23±0.10	5.94±0.43
β2KO—2	0.12±0.09	0.83±0.15[c]	73.33±12.34	1.07±0.34	5.78±0.64

[a] Represents significantly less than β2 WT—saline ($p<0.05$)

[b] Represents significantly less than β2 WT—saline ($p<0.05$)

[c] Represents significantly less than β2 KO—1 mg/kg varenicline ($p<0.05$)

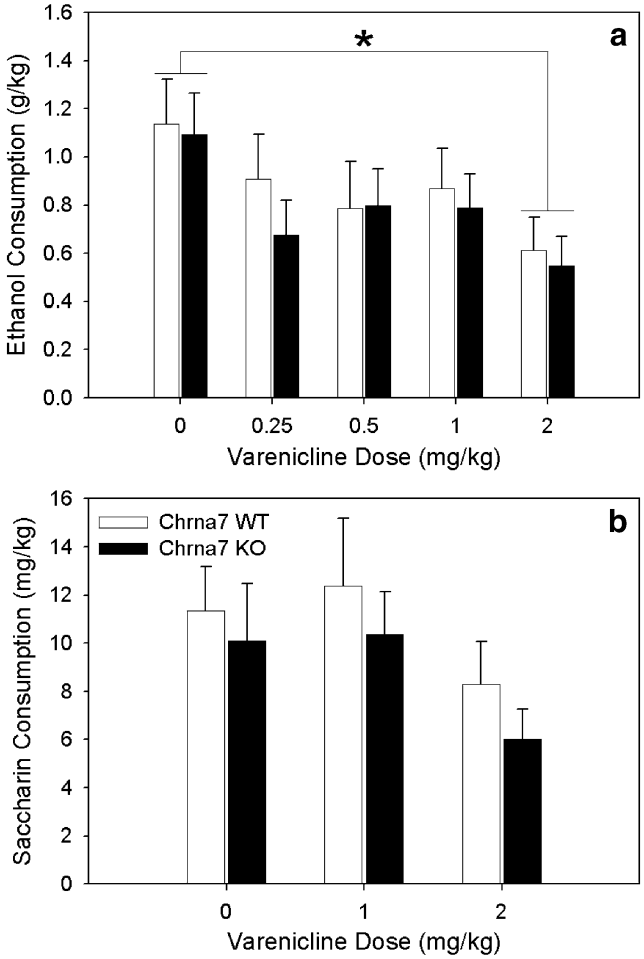

Fig. 5 Pretreatment with varenicline decreases ethanol consumption in α7WT (Chrna7 WT) and α7KO (Chrna7 KO) mice. The effect of pretreatment with varenicline on **a** ethanol consumption and **b** saccharin consumption. Data are represented as mean ± SEM for consumption during the first 3 h of choice administration. $N=9$ per group. $^{*}p<0.05$

that α7KO mice are more sensitive to ethanol-induced locomotor stimulation, hypothermia, and loss of righting reflex effects compared to α7WT mice (Bowers et al. 2005). α7KO mice were also less sensitive to memory impairing effects of ethanol compared to α7WT animals in a contextual fear paradigm (Wehner et al. 2004). These data provide support that α7 nAChRs are involved in modulation of ethanol responses. Interestingly, two studies have used the α7 antagonist, methyllycaconitine (MLA), and have shown no effect on ethanol consumption (Hendrickson et al. 2009; Kuzmin et al. 2009). A number of factors could account for the difference in findings between pharmacologic manipulation and knockout technology. Specifically, different ethanol consumption methods were used in this study compared to the pharmacologic studies. Furthermore, our α7KO mice lack the receptor throughout development while pharmacologic manipulation alters receptor availability at a specific point in time. Finally, MLA can have poor penetration into the brain and may not reach brain areas important for consumption. We also note that the effects of mecamylamine on ethanol intake may be the result of simultaneous blockade of multiple nAChRs, so a large effect on ethanol drinking may only be seen when multiple nAChR subtypes are antagonized or when there is some alteration in the nicotinic system as a result of genetic deletion.

We found no evidence for a significant involvement of β2* receptors in baseline ethanol consumption, which agrees with prior studies that showed no effect of the α4β2 antagonist dihydro-β-eryhroidine on ethanol consumption (Hendrickson et al. 2009; Kuzmin et al. 2009). nAChRs containing this subunit have been implicated in other behavioral effects of ethanol. β2KO mice are less sensitive to ethanol withdrawal (Butt et al. 2004) and ethanol-induced modulation of the acoustic startle response

Table 6 Ethanol, water, and total fluid intake 3 and 23 h after varenicline administration in α7WT and α7KO mice

Varenicline (mg/kg)	Hours of drinking				
	3h		23h		
	Water (ml)	Total fluid (ml)	Ethanol (g/kg)	Water (ml)	Total fluid (ml)
α7WT—saline	0.18±0.05	0.53±0.08	9.04±0.65	1.53±0.39	3.99±0.21
α7WT—0.25	0.23±0.06	0.50±0.08	9.03±1.27	1.65±0.44	4.06±0.32
α7WT—0.5	0.21±0.07	0.48±0.06	9.73±1.51	1.64±0.47	4.31±0.25
α7WT—1	0.17±0.07	0.45±0.07	8.51±0.84	1.64±0.39	3.98±0.21
α7WT—2	0.26±0.06	0.40±0.08	8.62±0.92	1.27±0.31	3.64±0.23
α7KO—saline	0.26±0.07	0.59±0.06	9.78±1.41	1.35±0.29	4.06±0.20
α7KO—0.25	0.30±0.09	0.49±0.10	8.45±1.00	1.49±0.28	3.97±0.31
α7KO—0.5	0.13±0.02	0.39±0.05	8.72±0.86	1.30±0.29	3.81±0.12
α7KO—1	0.20±0.07	0.48±0.07	8.85±0.83	1.28±0.23	3.86±0.14
α7KO—2	0.18±0.05	0.34±0.05	8.18±0.95	1.31±0.29	3.67±0.23

Table 7 Saccharin, water, and total fluid intake 3 and 23 h after varenicline administration in α7WT and α7KO mice

Varenicline (mg/kg)	Hours of drinking				
	3h		23h		
	Water (ml)	Total fluid (ml)	Saccharin (mg/kg)	Water (ml)	Total fluid (ml)
α7WT—saline	0.10±0.05	0.93±0.18	71.01±14.68	0.78±0.37	5.64±0.67
α7WT—1	0.11±0.04	1.13±0.24	96.46±15.82	0.69±0.40	7.16±0.76
α7WT—2	0.10±0.05	0.71±0.16	76.64±10.63	0.44±0.21	5.58±0.53
α7KO—saline	0.10±0.05	0.96±0.18	73.38±15.24	0.46±0.29	5.57±0.65
α7KO—1	0.16±0.08	0.93±0.16	72.98±12.25	0.60±0.22	5.80±0.67
α7KO—2	0.25±0.11	0.75±0.10	62.63±12.72	1.36±0.55	5.90±0.60

(Owens et al. 2003) compared to β2WT mice. Interestingly, the impairment of contextual recall by ethanol was not affected by β2KO (Wehner et al. 2004). A polymorphism in the β2 gene (CHRNB2) has also been associated with the initial subjective response to alcohol in human subjects (Ehringer et al. 2007). Thus, although the β2 subunit of the nAChRs may not be critical for ethanol consumption, it appears to be involved in other behavioral effects of ethanol.

Varenicline has been shown to be an efficacious treatment for nicotine dependence (Cahill et al. 2008). Varenicline decreases nicotine consumption and nicotine's effects on extracellular mesolimbic dopamine levels in preclinical models (Coe et al. 2005; Rollema et al. 2007a, b). Interestingly, varenicline also decreased ethanol consumption and seeking in rats (Steensland et al. 2007) and decreased self-administration in a human clinical study (McKee et al. 2009). We show here that varenicline selectively decreases ethanol consumption in C57BL/6J mice at doses that do not affect saccharin or water consumption. Varenicline can block elevations in dopamine levels observed after acute ethanol administration in rats (Ericson et al. 2009); therefore, varenicline may decrease ethanol intake by modulating activity of endogenous ACh at nAChRs, potentially leading to a reduction in activity of dopaminergic neurons and thus reducing ethanol reinforcement.

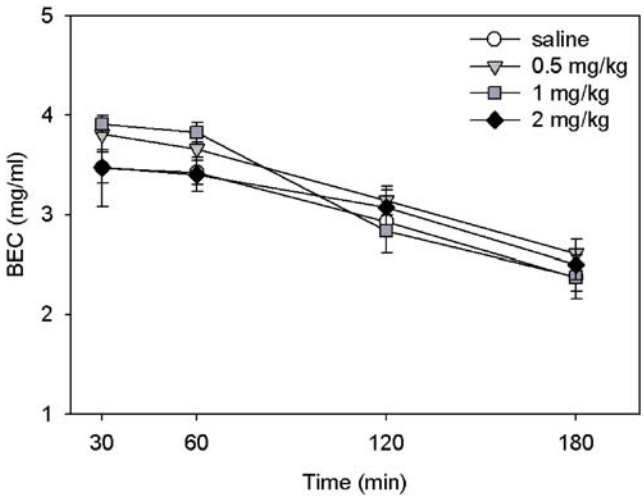

Fig. 6 Pretreatment with varenicline does not alter ethanol metabolism in C57BL/6J mice. Data (mean ± SEM) represent blood ethanol concentration taken following a 4 g/kg ethanol injection. N=9 per group

The subtype(s) of nAChR involved in varenicline's ability to decrease ethanol consumption are unclear. Varenicline is a selective α4β2 nAChR partial agonist (Coe et al. 2005; Obach et al. 2006; Rollema et al. 2007a) but can also activate other nAChRs, including the α7 receptor (Mihalak et al. 2006). Surprisingly, knocking out either the α7 or β2 subunits of the nAChR did not reverse the effect of varenicline on ethanol consumption. Together, these data suggest that nAChRs containing either the β2 or α7 subunit alone are not required for varenicline's observed reduction in ethanol consumption.

It should be noted that in the study of β2KO mice and their WT siblings, varenicline did reduce saccharin consumption at the 2 mg/kg dose compared to the 1 mg/kg dose (p=0.05). The reduction in saccharin consumption was small, however, and was not significantly different from saline. Moreover, there was no significant effect of varenicline on saccharin consumption in C57BL/6J mice or α7WT and α7KO mice. We therefore believe that the effect of varenicline on ethanol intake is not likely to be explained by a nonspecific effect on overall fluid intake or intake of other tastants.

The data presented here suggest that the nAChRs containing the β2 or α7 subunits are not solely responsible for varenicline's ability to decrease ethanol consumption. It is possible that varenicline normally influences ethanol consumption through a combination of nAChRs and that knocking out a single subunit is not sufficient to disrupt its overall effect. Varenicline is known to interact with other nAChR subunits in vitro, namely $\alpha_3\beta_2$, $\alpha_3\beta_4$, and α6*

receptors (Mihalak et al. 2006; Rollema et al. 2007b), but has substantially lower affinity compared to $\alpha_4\beta_2$ nAChRs (Coe et al. 2005; Rollema et al. 2007b). However, at the highest varenicline doses used in this study, the brain exposure of varenicline has been estimated to be high enough (Rollema et al. 2009) to affect several of the nAChR mentioned above. Pharmacological studies in mice have shown that nAChRs containing the $\alpha_3\beta_2$* or β_3* subunits are involved in the locomotor stimulatory and dopamine enhancing effects of ethanol. Administration of the $\alpha_3\beta_2$*-, α_6*-, and β_3*-selective antagonist α-conotoxin MII, but not the α_6*-selective antagonist α-CtxPIA, significantly decreased ethanol-induced locomotor stimulation and ethanol-induced dopamine increases in the nucleus accumbens (Jerlhag et al. 2006; Larsson et al. 2004). Behavioral genetic studies have suggested that the α_3 subunit of the nAChR may be a candidate gene in a region known to modulate ethanol-induced locomotor activity (Kamens et al. 2009). Furthermore, 18-methoxycoronaridine, a α3β4 selective antagonist, can also decrease ethanol consumption (Rezvani et al. 1997). Human genetic studies have also provided evidence for associations between alcohol phenotypes and polymorphisms in the CHRNA5/A3/B4 gene cluster as well as in the genes encoding the α6 (CHRNA6) and β3 (CHRNB3) nAChR subunits (Hoft et al. 2009; Schlaepfer et al. 2008; Wang et al. 2009). Thus, ACh acting through nAChRs may be a critical modulator of ethanol intake, but individual subunits of the nAChR may not be absolutely required for the nicotinic modulator varenicline to have effects on ethanol consumption.

In conclusion, our results provide supporting evidence for the involvement of nAChRs in ethanol consumption in mice. Surprisingly, we have identified a novel role for α7 nAChRs in baseline ethanol drinking. In addition, the smoking cessation aid varenicline is effective in decreasing ethanol intake in mice, which supports the idea that it could be a useful pharmacotherapy for alcohol use disorders. Studies using mice with a constitutive knockout of either the α7 or β2 subunit suggest that neither subunit is absolutely required for the effect of varenicline on ethanol intake. Further studies identifying the set of nAChRs involved in this effect could be useful in developing more targeted therapies for alcohol abuse.

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