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Modulation of ethanol reward sensitivity by nicotinic acetylcholine receptors containing the $\alpha 6$ subunit

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Abstract

The prevalent co-abuse of nicotine and alcohol suggests a common neural mechanism underlying the actions of the two drugs. Nicotine, the addictive component of tobacco, activates nicotinic acetylcholine receptors (nAChRs) containing the $\alpha 6$ subunit ($\alpha 6^*$ nAChRs) in dopaminergic (DAergic) neurons of the ventral tegmental area (VTA), a region known to be crucial for drug reward. Recent evidence suggests that ethanol may potentiate ACh activation of these receptors as well, although whether $\alpha 6^*$ nAChR expression is necessary for behavioral effects of acute ethanol exposure is unknown. We compared binge-like ethanol consumption and ethanol reward sensitivity between knockout (KO) mice that do not express *chrna6* (the gene encoding the $\alpha 6$ nAChR subunit, the $\alpha 6$ KO line) and wild-type (WT) littermates using the Drinking-in-the-Dark (DID) and Conditioned Place Preference (CPP) assay, respectively. In the DID assay, $\alpha 6$ KO female and male mice consumed ethanol similarly to WT mice at all concentrations tested. In the CPP assay, 2.0-g/kg and 3.0-g/kg, but not 0.5-mg/kg, ethanol conditioned a place preference in WT female and male mice, whereas only 2.0-g/kg ethanol conditioned a place preference in $\alpha 6$ KO mice. Acute challenge with ethanol reduced locomotor activity, an effect that developed tolerance with repeated injections, similarly between genotypes in both female and male mice. Together, these data indicate that expression of $\alpha 6^*$ nAChRs is not required for binge-like ethanol consumption and reward, but modulate sensitivity to the rewarding properties of the drug.

Keywords

alcohol; dopamine; mice; nicotinic receptor; reward

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Introduction

Alcohol-use disorders (AUDs) and the abuse of alcohol lead to 3.3 million deaths annually, and are responsible for 25% of deaths in people age 20–39 (WHO, 2015), making it one of the leading causes of preventable mortality in the world (Mokdad, Marks, Stroup, & Gerberding, 2004). Despite the costs of alcohol use and abuse, both in human life and in the economic burden placed on society (CASA, 2000), there remain few viable treatment options for those suffering from AUDs (Spanagel, 2009), highlighting the need for a better understanding of the mechanism(s) underlying the effects of alcohol on relevant neural circuitry within the brain. The frequent co-abuse of alcohol and nicotine, with an estimated 70–75% of alcoholics also identifying as tobacco-dependent (Miller & Gold, 1998), suggest a common mechanism of the two drugs. Nicotine and alcohol activate dopaminergic (DAergic) neurons of the ventral tegmental area (VTA), ultimately leading to an increase in dopamine (DA) in the nucleus accumbens, which is associated with drug reward and is common for all known drugs of abuse (Hendrickson, Guildford, & Tapper, 2013). Nicotine activates VTA-DAergic neurons directly by binding to and activating nicotinic acetylcholine receptors (nAChRs), pentameric ligand-gated cation channels normally activated by the endogenous neurotransmitter, acetylcholine (ACh). In contrast, the mechanism by which alcohol activates these same neurons is complex and likely involves multiple neurotransmitter systems (Liu, Hendrickson, et al., 2013; Liu, Zhao-Shea, McIntosh, Gardner, & Tapper, 2012; Morikawa & Morrisett, 2010; Pidoplichko, DeBiasi, Williams, & Dani, 1997; Theile, Morikawa, Gonzales, & Morrisett, 2011; Xiao & Ye, 2008).

There are 12 known vertebrate genes, each encoding twelve distinct neuronal nAChR subunits including $\alpha 2$ -10, and $\beta 2$ -4. Five subunits co-assemble to form functional receptors, with the majority of high-affinity nAChRs being heteromeric, consisting of 2–3 α subunits co-assembled with 2–3 β subunits (Moroni, Zwart, Sher, Cassels, & Bermudez, 2006; Nelson, Kuryatov, Choi, Zhou, & Lindstrom, 2003; Zwart & Vijverberg, 1998).

Although not a direct agonist of nAChRs, ethanol can activate VTA-DAergic neurons, in part, through increased release of ACh (Larsson, Edström, Svensson, Söderpalm, & Engel, 2005), likely from cholinergic neurons of the lateral dorsal tegmentum (Oakman, Faris, Kerr, Cozzari, & Hartman, 1995), which then leads to increased activation of VTA-DAergic neurons via nAChRs (Blomqvist, Ericson, Engel, & Söderpalm, 1997; Larsson et al., 2005; Liu, Hendrickson, et al., 2013). In addition, ethanol can potentiate ACh-induced nAChR currents (Cardoso et al., 1999; Liu, Hendrickson, et al., 2013; Zuo, Kuryatov, Lindstrom, Yeh, & Narahashi, 2002), although the precise nAChR subtypes critical for ethanol activation of VTA-DAergic neurons are unclear.

Of particular interest, nAChRs containing the $\alpha 6$ subunit ($\alpha 6^*$ nAChR; * denotes that the receptor complex contains additional, non- $\alpha 6$ subunits) are uniquely enriched in catecholaminergic neurons, including midbrain DAergic neurons where they are positioned to influence neuronal activity through cholinergic innervation (Berry, Engle, McIntosh, & Drenan, 2015; Champniaux et al., 2002, 2003; Drenan, Grady, et al., 2008). They also contribute to nicotine dependence-associated behavior, including reward (Gotti et al., 2010; Jackson, McIntosh, Brunzell, Sanjakdar, & Damaj, 2009). Supporting a role for $\alpha 6^*$

nAChRs in ethanol consumption and reward (Lee & Messing, 2011; Liu, Zhao-Shea, McIntosh, & Tapper, 2013; Powers, Broderick, Drenan, & Chester, 2013), VTA infusion of an $\alpha 6$, $\alpha 3$, $\beta 3^*$ antagonist, α -conotoxin MII, reduces ethanol-induced DA release in the nucleus accumbens, reduces ethanol consumption, and reduces reinforcement in rodents (Jerlhag, Grøtli, Luthman, Svensson, & Engel, 2006; Kuzmin, Jerlhag, Liljequist, & Engel, 2009; Larsson, Jerlhag, Svensson, Söderpalm, & Engel, 2004). Additionally, mice expressing mutant $\alpha 6^*$ nAChRs that are hypersensitive to agonists exhibit a place preference to a sub-rewarding dose of ethanol and also consume more ethanol compared to wild-type (WT) littermates (Powers et al., 2013). In contrast, mice lacking expression of *chrna6*, the gene encoding the $\alpha 6$ nAChR subunit ($\alpha 6$ knockout [$\alpha 6$ KO] mice), consume and prefer ethanol similarly to WT mice in a two-bottle free-choice drinking paradigm (Kamens, Hoft, Cox, Miyamoto, & Ehringer, 2012). Thus, it is unclear whether expression of $\alpha 6^*$ nAChRs is necessary for ethanol consumption and reward. To address this question, we compared binge-like ethanol consumption, which may more closely resemble problematic drinking patterns, and the rewarding properties of the drug in the $\alpha 6$ KO mouse line.

Methods and materials

Animals

$\alpha 6$ KO homozygous mice 8–20 weeks old and age-matched WT mice were used in all experiments as indicated. This line was backcrossed to C57BL/6J for at least 10 generations. The genetic engineering of this line has been described previously (Champtiaux et al., 2002). $\alpha 6$ KO and WT mice were generated from homozygous, heterozygous, or WT littermate breeding pairs. In all experiments, KO and WT mice derived from each breeding strategy did not significantly differ in ethanol responses, so they were combined. Animals were housed up to five per cage and kept on a 12-h light cycle prior to the start of each experiment. Mice had access to food and water *ad libitum* except where indicated. Separate cohorts of mice were used for each set of experiments (ethanol consumption, reward, locomotor activity). All experiments were conducted in accordance with the guidelines for care and use of laboratory animals provided by the National Research Council as well as with an approved animal protocol from the Institutional Animal Care and Use Committee of the University of Massachusetts Medical School.

Drinking-in-the-Dark (DID)

For the binge-drinking assay, $\alpha 6$ KO and WT mice were moved from their colony room into a reverse light-dark room (lights on at 8:00 PM and lights off at 8:00 AM) to habituate for 2 weeks prior to the start of the experiment. Mice were single-housed 1 week prior to the start of DID. Three days before DID, standard water bottles were replaced with bottles containing sipper tubes with double ball-bearing inserts to prevent leakage. In the DID assay, access to ethanol was restricted to 2 h a day, beginning 2 h into the dark cycle, for 4 days per week (Hendrickson, Zhao-Shea, & Tapper, 2009). Water bottles were removed and replaced with pre-weighed ethanol bottles (50-mL conical tubes outfitted with a stopper and double ball-bearing sipper tube) initially containing 2% ethanol in water (v/v) and increasing to 5%, 10%, and finally 20% over the course of 4 weeks. The ethanol bottles were removed, replaced with water bottles, and weighed after 2 h. Ethanol consumption was measured as

grams of ethanol per kilogram of body weight per mouse. Drip controls were used to monitor for evaporation/spillage, and ethanol consumption was adjusted accordingly. Following the 4-week protocol, mice were given 2 days of 10% sucrose followed by 10-mM saccharin as controls.

Conditioned Place Preference (CPP)

The CPP assay used was a 6-day protocol adapted from Gibb et al. (2011). The CPP apparatus (Med Associates, USA) consisted of three distinct chambers, a small, neutral, gray chamber flanked on each side by a larger conditioning chamber containing a wire-mesh floor and white walls and the other chamber containing a metal-rod floor and black walls. Each compartment contained photobeams to monitor mouse movement in and out of each chamber, and the entire apparatus was contained in a sound-attenuating box with lights and a fan. On day 1 (habituation day), $\alpha 6$ KO and WT mice were placed in the middle chamber and allowed free access into all three chambers for 30 min. Time spent in each chamber was monitored and recorded by Med PC software via photobeam breaks and used to determine any initial bias for either of the conditioning chambers. Mice spending more than 90% of their time in one chamber were excluded from the study. The least-preferred chamber was set as the ethanol-paired chamber. On days 2–5, mice were given an intraperitoneal (i.p.) injection of either saline or ethanol (balanced by sex, dose, and treatment; dose as indicated; 20% ethanol solution v/v), and were immediately confined to the corresponding chamber for 5 min. Mice were then returned to their home cages. Four to six hours later, each mouse received the other injection (ethanol if they had saline in the morning, saline if they had ethanol) and was confined to the other chamber. Day 6 was the test day, and was the same protocol as day 1. Reward was indicated by an increase in time spent in the ethanol-paired chamber on the test day compared to the habituation day.

Locomotor activity

Mice were given pre-injections of saline in their home cage for 2 days prior to initiation of the experiment. Locomotor activity was measured in $\alpha 6$ KO and WT mice using a cage-rack photobeam system (PAS, San Diego Instruments) and the corresponding PAS software. Mice were placed in a novel cage within the locomotor apparatus, and ambulation (locomotion) was measured as the breaking of two distinct beams 10 cm apart. Locomotor activity was recorded on days 0, 1, 4, and 7 for 1 h, at which time mice were injected with saline (Day 0) or 2-g/kg ethanol (Days 1, 4, 7). Activity was recorded for an additional hour immediately following the injection. Mice received home-cage ethanol injections on days 2, 3, 5, and 6.

Data analysis

Data were analyzed using one- or two-way ANOVA followed by Bonferroni *post hoc* tests or two-tailed *t* tests as indicated. Results were considered significant at $p < 0.05$. All data are expressed as means $\pm$ SEM.

Results

Expression of $\alpha 6^*$ nAChRs is not necessary for binge-like consumption of alcohol

We used a restricted-access alcohol consumption assay, drinking-in-the-dark (DID) (Rhodes, Best, Belknap, Finn, & Crabbe, 2005; Rhodes et al., 2007) to test whether $\alpha 6^*$ nAChRs play a role in binge-like alcohol consumption of 2%, 5%, 10%, or 20% ethanol (see Methods, Fig. 1). A two-way ANOVA revealed a significant interaction between sex and ethanol concentration on consumption [main effect of sex: $F(1,156) = 54.72, p < 0.0001$; concentration: $F(3,156) = 380.7, p < 0.0001$; interaction: $F(3,156) = 7.341, p < 0.0001$], so male and female data were separated and analyzed independently. We observed no significant difference in ethanol consumption between WT and $\alpha 6$ KO female or male mice at any ethanol concentration (Fig. 1A, B). In both female and male mice, two-way ANOVA revealed a significant main effect of ethanol concentration [$F(3,96) = 323.7, p < 0.0001$, and $F(3,78) = 198.6, p < 0.0001$ respectively], but not genotype [$F(1,32) = 0.1407, p = 0.71014$ and $F(1,26) = 0.1747, p = 0.6794$, respectively], with no significant interaction (Fig. 1C, D). In addition, neither group exhibited a significant difference in sucrose or saccharin consumption (Fig. 1E, F). The absence of any effect of genotype on ethanol consumption suggests that expression of $\alpha 6^*$ nAChRs is not necessary for binge-like ethanol drinking.

$\alpha 6^*$ nAChRs modulate reward response to high-dose ethanol

To test the hypothesis that expression of $\alpha 6^*$ nAChRs is necessary for the rewarding effects of ethanol, we tested the ability of the drug to condition a place preference in female and male WT and $\alpha 6$ KO mice using the CPP assay at three distinct doses, 0.5-g/kg, 2.0-g/kg, and 3.0-g/kg ethanol (Fig. 2A, Table 1). An additional group of control animals received saline in the “ethanol-paired” chamber. A two-way ANOVA with sex and ethanol dose as main factors indicated a significant effect of dose [$F(3,121) = 14.40, p < 0.0001$] on time spent in the ethanol-paired chamber, but no effect of sex [$F(1,121) = 1.205, p = 1.205$] and no significant interaction, so sexes were combined for further analysis. A two-way ANOVA of ethanol dose and time of injection (i.e., morning vs. afternoon) as main factors (ignoring the saline-control group, which received saline at both times) revealed a significant effect of dose [$F(2,104) = 9.29, p = 0.0002$], but not time of injection [$F(1,104) = 0.0261, p = 0.872$] nor interaction. A two-way ANOVA with genotype and ethanol dose as main factors revealed a significant effect of dose on time spent in the ethanol-paired chamber [$F(3,127) = 12.56, p < 0.0001$] and a significant dose $\times$ genotype interaction [$F(3,127) = 3.142, p = 0.0276$]. In WT mice, *post hoc* analysis indicated a statistically significant increase in time spent in the ethanol-paired chamber in response to 2.0-g/kg and 3.0-g/kg ethanol compared to saline, whereas in $\alpha 6$ KO mice, time spent in the ethanol-paired chamber was increased at the 2.0-g/kg dose compared to saline, but not the 3.0-g/kg dose. In addition, time spent in the ethanol-paired chamber was significantly decreased in $\alpha 6$ KO mice compared to WT mice at the 3.0-g/kg ethanol dose. Together, these data indicate that expression of $\alpha 6^*$ nAChRs is not required for ethanol reward, but modulates reward sensitivity of the drug.

$\alpha 6^*$ nAChRs are not necessary for ethanol-induced hypolocomotion

To test whether acute ethanol-induced locomotor impairment and subsequent tolerance differed between genotypes, we challenged female and male WT and $\alpha 6$ KO mice with an

acute injection of saline (control) followed by single daily injections of 2.0-g/kg ethanol for 7 days. Locomotor activity was measured immediately after ethanol challenge on days 1, 4, and 7 (Fig. 2B, Table 2). Two-way ANOVA with sex and injection day as main factors indicated a significant effect of ethanol injection [$F(3,69) = 10.49, p < 0.0001$] but not sex [$F(1,23) = 0.2175, p = 0.6453$], with no injection day $\times$ sex interaction, so male and female data were combined. Subsequent two-way ANOVA, analyzing genotype and injection day, again revealed a significant main effect of ethanol injection [$F(3,69) = 10.97, p < 0.0001$], but not genotype [$F(1,23) = 0.0875, p = 0.7701$] or an interaction between the two. Overall, ethanol decreased locomotor activity upon first injection with tolerance developing over subsequent ethanol exposures in both WT and $\alpha 6$ KO mice. These data suggest no significant role of $\alpha 6^*$ nAChRs in the locomotor response to ethanol in males or females.

Discussion

The present study analyzed the contribution of $\alpha 6^*$ nAChRs in binge-like ethanol consumption and ethanol reward using a loss of function mouse model, the $\alpha 6$ KO line. Previously, this same mouse line was used to determine whether $\alpha 6^*$ nAChR expression is involved in ethanol preference and consumption (Kamens et al., 2012). Using a 24-h two-bottle choice paradigm, Kamens et al. found that $\alpha 6$ KO mice consume and prefer ethanol similarly to their WT littermates. However, binge-like ethanol intake and reward was not analyzed. Using the DID assay, we also did not detect significant differences in ethanol consumption between $\alpha 6$ KO mice and their WT littermates at any ethanol dose tested in male or female mice. In addition, ethanol was rewarding in $\alpha 6$ KO mice, in that it could condition a place preference in these animals, but only at one dose, while a higher dose of ethanol failed to condition a place preference. Thus, our data indicate that expression of $\alpha 6^*$ nAChRs is not necessary for ethanol consumption or reward *per se*, but these receptors do contribute to reward sensitivity at high concentrations of ethanol.

Interestingly, Powers et al. analyzed ethanol consumption and reward in a “gain-of-function” bacterial artificial chromosome transgenic mouse line expressing $\alpha 6$ subunits harboring a point mutation (a leucine residue at the 9' position mutated to a serine residue, the $\alpha 6$ L9'S line) that renders $\alpha 6^*$ nAChRs hypersensitive to ACh (Drenan, Grady, et al., 2008; Powers et al., 2013). These mice displayed an increase in ethanol consumption in both a two-bottle choice test and the DID assay, compared to WT littermates. In addition, mutant mice were more sensitive to the rewarding effects of ethanol (i.e., a sub-rewarding dose of ethanol conditioned a place preference in the mutant mice). Because $\alpha 6$ L9'S mice are exquisitely sensitive to agonist, the increased ethanol consumption and leftward shift in ethanol reward sensitivity of these mice should be interpreted with caution. While the results obtained using the $\alpha 6$ L9'S mice implicate $\alpha 6^*$ nAChRs in regulating ethanol consumption and reward sensitivity, the endogenous impact of these receptors on ethanol behaviors in WT mice may be overestimated. Similarly, there are caveats to our interpretations of ethanol behaviors in $\alpha 6$ KO mice. In particular, compensatory expression of non- $\alpha 6$ nAChR subunits may occur in these animals in the absence of *chrna6* expression, thereby minimizing any potential differences in ethanol consumption and reward between genotypes. While previous work by Champniaux et al. did not detect compensatory changes in nAChR subunit gene expression in $\alpha 6$ KO mice, mRNA transcript analysis was not quantified nor was protein expression

analyzed (Champtiaux et al., 2002). In addition, $\alpha 6$ nAChR subunits cannot form homomeric receptors; rather, they require at least one additional β nAChR subunit (in particular $\beta 2$ and $\beta 3$) and also can co-assemble with the $\alpha 4$ subunit, especially in VTA-DAergic neurons (Cui et al., 2003; Drenan, Nashmi, et al., 2008; Engle, Shih, McIntosh, & Drenan, 2013; Liu et al., 2012; Zhao-Shea et al., 2011). Thus, in the absence of *chrna6* expression, VTA-DAergic neurons may express a greater number of nAChRs containing $\alpha 4$ and $\beta 2$ subunits compared to WT mice. Because we have previously identified a role for $\alpha 4^*$ nAChRs in ethanol behaviors including reward and acute ethanol consumption, increased $\alpha 4^*$ nAChRs in $\alpha 6$ KO mice may partially mask the contribution of $\alpha 6^*$ nAChRs in an $\alpha 6$ KO mouse background (Hendrickson, Gardner, & Tapper, 2011; Hendrickson, Zhao-Shea, Pang, Gardner, & Tapper, 2010; Liu, Hendrickson, et al., 2013).

Despite the caveats described above, we did identify a loss of ethanol reward sensitivity in $\alpha 6$ KO mice when challenged with a high ethanol dose. This is consistent with previous data showing that ethanol-induced activation of VTA-DAergic neurons is reduced in *ex vivo* midbrain slices from $\alpha 6$ KO mice compared to WT slices (Liu, Zhao-Shea, et al., 2013). Although we did not measure ethanol clearance in $\alpha 6$ KO and WT mice, Kamens et al. demonstrated that $\alpha 6$ KO mice have similar blood ethanol concentrations after a 3.0-g/kg ethanol challenge, suggesting that a difference in ethanol metabolism between genotypes is unlikely to underlie the difference in ethanol reward that we have observed (Kamens et al., 2012). In addition, although $\alpha 6$ KO mice are modestly more sensitive to the sedative effects of ethanol (Kamens et al., 2012), we did not observe significant differences in the hypolocomotor response to 2-g/kg ethanol or tolerance to this effect in $\alpha 6$ KO mice compared to WT mice, suggesting that differences in the locomotor-impairing effects of ethanol do not underlie differences in ethanol reward between genotypes. Future studies using alternative approaches such as viral-mediated shRNA knockdown of individual nAChR subunits in discrete brain regions of adult animals should be done to test contributions of nAChR subtypes in ethanol consumption and reward, as well as to determine whether the key nAChRs involved in these behaviors are expressed in subpopulations of VTA neurons or other brain regions.

In conclusion, our data indicate that expression of $\alpha 6^*$ nAChRs is not necessary for binge-like ethanol consumption, but does modulate ethanol reward sensitivity at high doses.

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Highlights

- Expression of $\alpha 6^*$ -nicotinic receptors is not necessary for ethanol consumption.
- $\alpha 6^*$ -nicotinic receptor expression is not required for ethanol reward.
- $\alpha 6^*$ -nicotinic receptors contribute to high- but not low-dose ethanol reward.

Fig. 1. Expression of $\alpha 6^*$ nAChRs are not necessary for binge-like ethanol consumption in the drinking-in-the-dark assay
 Daily consumption of 2–20% ethanol during the DID binge-drinking assay in A) female and B) male WT and $\alpha 6$ KO mice. Average daily ethanol intake for each ethanol concentration for C) female ($n = 19$ WT, $n = 5$ KO) and D) male mice ($n = 14$ WT, $n = 14$ KO) of each genotype. Average sucrose and saccharin consumption in E) female and F) male mice of each genotype.

Fig. 2. Expression of $\alpha 6^*$ nAChRs modulate sensitivity to ethanol reward

A) Time spent in the ethanol-paired chamber on the test day. Mice were conditioned with 0-g/kg (saline), 0.5-g/kg, 2.0-g/kg, or 3.0-g/kg ethanol. $n = 12\text{--}22$ mice/genotype (see Table 1 for specific n values). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ compared to intra-group saline, $^{\wedge}p < 0.05$ between groups at the indicated dose. Two-way ANOVA, Bonferroni *post hoc*. B) Summed average ambulation 60 min post i.p. saline (day 0) or 2.0-g/kg ethanol (Days 1, 4, 7). $n = 12$ WT, $n = 13$ $\alpha 6$ KO.

Table 1

Time spent in saline- and ethanol-paired CPP chambers. Average time spent in each chamber

Gender/Genotype	Dose EtOH (g/kg)	SAL (Hab, s)	SAL (Test, s)	EtOH (Hab, s)	EtOH (Test, s)
Male WT (n=6)	0	681.1 ± 40.6	389.6 ± 41.7	576.7 ± 25.8	452.3 ± 73.7
(n=9)	0.5	650.8 ± 51.8	343.6 ± 34.9	494.5 ± 25.7	483.7 ± 85.9
(n=10)	2.0	696.2 ± 37.5	403.5 ± 72.7	513.1 ± 28.9	722.4 ± 78.53 *
(n=9)	3.0	654.1 ± 33.4	397.5 ± 54.9	488.0 ± 24.8	785.6 ± 86.5 *
Male KO (n=7)	0	647.6 ± 63.2	608.4 ± 43.3	414.5 ± 40.4	486.6 ± 56.9
(n=6)	0.5	573.5 ± 53.7	499.0 ± 96.1	504.3 ± 32.8	450.6 ± 87.3
(n=9)	2.0	661.3 ± 29.8	414.3 ± 79.5	439.8 ± 19.3	820.5 ± 106.2 *
(n=8)	3.0	676.2 ± 34.3	484.8 ± 68.5	425.9 ± 38.7	580.8 ± 96.3
Female WT (n=9)	0	630.6 ± 41.8	472.1 ± 65.8	542.7 ± 32.9	441.0 ± 81.3
(n=10)	0.5	640.8 ± 36.2	402.1 ± 49.5	460.4 ± 34.3	438.3 ± 79.0
(n=10)	2.0	709.1 ± 49.1	361.0 ± 58.5	540.6 ± 22.8	855.5 ± 115.6 *
(n=7)	3.0	670.3 ± 35.5	407.6 ± 139.8	495.9 ± 28.9	907.5 ± 146.1 *
Female KO (n=5)	0	571.5 ± 39.7	533.6 ± 46.0	595.0 ± 65.6	391.4 ± 57.4
(n=11)	0.5	626.8 ± 32.1	480.7 ± 37.2	447.9 ± 30.5	599.8 ± 45.0
(n=13)	2.0	683.9 ± 28.3	516.4 ± 49.3	518.7 ± 27.7	724.4 ± 57.0 **
(n=7)	3.0	627.0 ± 34.1	761.9 ± 107.9	600.9 ± 42.0	494.6 ± 76.3

(SAL = saline-paired, EtOH = ethanol-paired, Hab = habituation day) presented in seconds with SEM.

* $p < 0.05$,

** $p < 0.01$, one-way ANOVA, compared to saline

Table 2

Summed average ambulation after saline (Day 0) or 2.0-g/kg ethanol injection.

Sex/Genotype	Day 0	Day 1	Day 4	Day 7
Male WT	91.7 ± 26.6	38.5 ± 5.0	72.0 ± 9.3	59.1 ± 4.2
Male $\alpha 6$ KO	91.4 ± 13.0	41.4 ± 9.8	86.9 ± 25.8	107.2 ± 31.1
Female WT	118.3 ± 34.8	37.7 ± 5.9	62.1 ± 16.0	113.7 ± 29.4
Female $\alpha 6$ KO	111.1 ± 7.4	39.3 ± 8.3	81.0 ± 10.1	77.2 ± 19.2