Substance Abuse: Research and Treatment

ORIGINAL RESEARCH

Trace Amine Associated Receptor 1 Modulates Behavioral Effects of Ethanol

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Abstract

Background: Few treatment options for alcohol use disorders (AUDs) exist and more are critically needed. Here, we assessed whether trace amine associated receptor 1 (TAAR1), a modulator of brain monoamine systems, is involved in the behavioral and reinforcement-related effects of ethanol and whether it could potentially serve as a therapeutic target.

Methods: Wild-type (WT) and TAAR1 knockout (KO) mice (75% C57J/BL6 and 25% 129S1/Sv background) were compared in tests of ethanol consumption (two-bottle choice [TBC]), motor impairment (loss of righting reflex, [LORR], locomotor activity) and ethanol clearance (blood ethanol level [BEL]).

Results: As compared with WT mice, KO mice displayed (1) significantly greater preference for and consumption of ethanol in a TBC paradigm (3%–11% vol/vol escalating over 10 weeks), with no significant difference observed in TBC with sucrose (1%–3%); (2) significantly greater sedative-like effects of acute ethanol (2.0 or 2.5 g/kg, intraperitoneal [i.p.]) manifested as LORR observed at a lower dose and for longer time, with similar BELs and rates of ethanol clearance; and (3) lower cumulative locomotor activity over 60 minutes in response to an acute ethanol challenge (1.0–2.5 g/kg, i.p.).

Conclusions: The present findings are the first to implicate TAAR1 in the behavioral and reinforcement-related effects of ethanol and raise the question of whether specific drugs that target TAAR1 could potentially reduce alcohol consumption in humans with AUDs.

Keywords: alcoholism, treatment, reward, addiction, dopamine

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Introduction

Along with cognitive, behavioral, and spiritual approaches, medications are used to assist in reducing alcohol consumption or craving during abstinence. Three medications are approved in the United States for treating alcoholism. These are: (1) disulfiram, an acetaldehyde dehydrogenase blocker that causes an adverse reaction to alcohol due to accumulation of acetaldehyde; (2) naltrexone, a substituted oxymorphone that functions as an opioid receptor antagonist. Naltrexone reduces the desire for alcohol and helps some motivated problem drinkers stay sober; and (3) acamprosate, a drug that alters excitatory activity of NMDA receptors. Acamprosate reduces the physical distress and emotional discomfort of craving. Each of these drugs have variable effectiveness and acceptance levels within populations and are used by a minority of people harboring a medical diagnosis of alcoholism. Additionally, currently available medications are prescribed to individuals who have already stopped drinking and are trying to maintain alcohol abstinence. There are no medications that are prescribed for people who are actively drinking alcohol yet want to stop drinking. Accordingly, more and better treatment options for alcoholism and prevention of AUDs is a major objective.

Trace amine associated receptor 1 (TAAR1) is a G protein–coupled receptor that is expressed in monoaminergic brain regions implicated in the reward pathway, including the nucleus accumbens, ventral tegmental area, and substantia nigra.[1–3] TAAR1 is activated by a wide spectrum of endogenous ligands including classic biogenic amines, trace amines, and thyronamines and is also a direct target of psychostimulant drugs of abuse including amphetamine, methamphetamine, and 3,4-methylenedioxy-N-methamphetamine.[3–5] Receptor activation results in cAMP/PKA and Ca^{++}/PKC signaling and phosphorylation of PKA and PKC.[1,4,6,7] In monoaminergic synaptosomes, cellular signaling pathways elicited from TAAR1 activation modulate dopamine transporter (DAT), norepinephrine transporter (NET), and serotonin transporter (SERT) kinetic activity (inhibit uptake and promote efflux) as well as DAT internalization.[7–10] In brain slices of the ventral tegmental area and dorsal raphe nucleus, TAAR1 activation inhibits the firing frequency of dopaminergic and serotonergic neurons, respectively.[11,12]

Given the ability of TAAR1 to modulate dopamine as well as other monoamines[8,13] and the involvement of dopamine as well as other monoamines in alcohol abuse,[14,15] this study tested the hypothesis that TAAR1 is involved in the behavioral and reinforcement-related effects of ethanol. The effects of ethanol in wild-type (WT, 75% C57J/BL6 and 25% 129S1/Sv) and congenic TAAR1 knockout (KO) mice were compared in tests of ethanol consumption (2-bottle choice [TBC]), motor impairment (loss of righting reflex [LORR]), locomotor activity and the ethanol clearance (blood ethanol levels [BEL]). The findings provide the first evidence that the TAAR1 receptor is a modulator of ethanol responsiveness and ethanol-related behaviors and suggest that TAAR1-targeted drugs be investigated as potential therapeutics for combating AUDs.

Materials and Methods

Subjects

Homozygous male and female WT (+/+) and KO (–/–) mice (strain background: 75% C57BL/6J and 25% 129S1/Sv) were maintained on a 12-hour light/dark lighting schedule at a room temperature of 22 °C ± 1 °C with food and water available ad libitum. All experiments were conducted during the middle hours of the light cycle. The KO mice were derived from heterozygous breeding pairs, originally gifted from Lundbeck Research USA, Inc (Paramus, NJ). Their origination has been previously described.[16] Briefly, KO mice lack the coding sequence of the TAAR1 gene (which is a single exon) as well as 1.0 kilobase (kb) of upstream sequence and 1.3 kb of downstream sequence. The deleted sequence was replaced with a neo cassette in reverse orientation. Accordingly, the KO mice do not express TAAR1 protein. Mice were generally 8 to 12 weeks old at the time of study, with the exception of LORR and BEL measurements that used some mice that were 24 weeks old at the time of study. Due to funding limitations, we used available populations of mice for the different behavioral testing paradigms, which were performed sequentially over time. In this regard, we controlled for age and sex within each behavioral test to the best of our abilities. All animal care was in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, National Academy Press, 1996) and all procedures were conducted in accordance with the Animal Experimentation Protocol #04184 approved by the Harvard Medical Area Standing Committee on Animals.

Solutions

For 2-bottle choice, 200-proof ethanol (Pharmco-AAPER, Brookfield, CT) was diluted into tap water at concentrations ranging from 3% to11% (vol/vol). Sugar (Office Snax, Hinsdale, IL) was dissolved into tap water at concentrations of either 1% or 3% (wt/vol), and 200-proof ethanol was diluted into saline at 20% vol/vol for i.p. injections.

Equipment

For 2-bottle choice, large breeding cages (10 × 18 in) were equipped with tops that were custom modified to allow access to two 50 mL drinking tubes equipped with Lixit valves (Med Associates Inc., St. Albans, VT). For LORR assessments, a custom-designed trough was made of metal shaped in an upside down W with a sheet of Plexiglas covering it. Locomotor assessments were conducted in ventilated, sound-attenuated chambers with white background noise (MED Associates, St. Albans, VT). The square open-field plexiglas arena (11 cm × 11 cm) within each chamber was equipped with 3 rows of infrared light-sensitive photocells mounted at 40, 115, and 195 mm above the floor. All locomotor assessments were performed under illuminated conditions. An Analox AM1 series analyzer (Analox Instruments USA Inc., Lunenburg, MA) was used to determine BELs. Each apparatus was sanitized between animals.

The 2-bottle choice: ethanol versus water

The 2-bottle choice procedures allow for measurement of ethanol preference and intake under conditions of voluntary consumption. In our studies, a counterbalanced procedure was employed. Naïve female mice (approximately 20–24 g) were pair housed and were 8 to 12 weeks old at study initiation (n = 9 cages per genotype). Mice were allowed to drink from either a drinking tube filled with tap water or a drinking tube filled with escalating concentrations (3%, 5%, 7%, 9%, and 11% vol/vol) of ethanol. Drinking tubes were set up Monday and made continuously available through Friday. Tubes were weighed Monday, Wednesday, and Friday to determine the weight of each liquid consumed. Fluids in the tubes were refreshed and tube positions oscillated on Wednesdays to control for side biases. The mice remained at each concentration of ethanol for 2 5-day testing sessions, with a single water bottle that was oscillated each weekend (for a total of 2 weeks), after which they would advance to the next highest concentration of ethanol. For each 2-week period, an average number of grams per day per cage was obtained for both the ethanol bottle and the water bottle, and the percent drinking from the ethanol bottle relative to the total amount of liquid consumed was calculated.

Two-bottle choice: sucrose solution versus water

To determine the generality of any effects observed with ethanol, preference for a nonethanol caloric beverage was also assessed. Age-matched, naïve, and paired female mice were assessed in parallel using the same paradigm as above with escalating concentrations (1% and 3% w/v) of sucrose solution versus water (n = 4 cages per genotype).

Loss of righting reflex (LORR)

Duration of LORR is typically thought to measure the anesthetic or sedative actions of ethanol. Here, naïve male and female WT and KO mice (2.5–6 months of age) were injected with ethanol. Three doses of ethanol were tested: (1) 18 (6 male and 3 female WT and KO) mice were assessed following 1.5 g/kg, i.p.; (2) 22 (7 female and 4 male WT, 5 female and 6 male KO) mice were assessed following 2.0 g/kg, i.p.; and (3) 38 (7 female and 12 male WT and KO) mice were assessed following 2.5 g/kg, i.p. Each mouse was placed into a clean holding cage for 1 minute. Mice were then placed in the supine position in a V-shaped trough 2 times consecutively every minute to assess ability to right. LORR was defined as the time interval spanning from the initiation of an inability to right for at least 1 full minute until the mouse could right 2 times consecutively. If no LORR was observed, the test concluded at 20 minutes.

Locomotor activity

Locomotor activity in naïve male and female WT and KO mice (11–13 weeks old) was assessed following i.p. administration of saline, 1.0, 1.5, 2.0, or 2.5 g/kg ethanol (n = 4 per genotype, per dose, and per sex). Following a 20 minute habituation to the test chamber apparatus, mice were assessed for a 1-hour period. Additionally, a group of older WT and KO mice (9 months old, n = 8 per genotype) that had 2 previous exposures to ethanol were also assessed following a

2.0 g/kg, i.p. dose and 15 minutes of habituation, for a 2-hour period (Supplemental Fig. 1).

Determination of BELs

We assessed BEL in male and female WT and KO mice (approximately 50–50 split, as indicated; 2–8 months old) treated with 2.5 g/kg ethanol (i.p.). At selected time points (2, 5, 10, 20, 30, 45, 180 minutes, 19 hours and 24 hours), animals were euthanized and trunk blood was collected for determination of BELs. Blood samples were immediately centrifuged at 3200 rpm for 8 to 12 minutes. The plasma was drawn off, transferred to polypropylene tubes, and then frozen until analysis. Triple determinations of BALs were conducted using a rapid high performance plasma alcohol analysis using alcohol oxidase with an AM1 series analyzer and Analox Kit GMRD-113 (Analox Instruments USA, Lunenberg, MA).[17] This process detects BEL ranges from 0 to 350 mg/dL using an internal standard of 100 mg/dL.

Results

Two-bottle choice

We assessed whether WT and KO mice differed in their preference for drinking ethanol using a two-bottle choice paradigm. WT and KO mice differed in the percent of total drinking that was from the ethanol bottle (Fig. 1A). Overall, KO mice drank a significantly greater volume of ethanol solution versus water as compared with WT mice (2-way analysis of variance [ANOVA], $F_{1,16} = 23.59$, $P < 0.001$). Genotype accounted for 19.85% of the total variance in bottle choice. The magnitude of this difference was dependent on ethanol concentration, which also had overall significance (2-way ANOVA, $F_{4,64} = 19.09$, $P < 0.001$, Bonferroni t tests, $P < 0.001$ at the 5% ethanol concentration; $P < 0.05$ at 3% and 7% ethanol concentrations), which accounted for 34.38% of the variance. This choice behavior resulted in the KO mice consuming more total ethanol per day (on average) than WT mice at every concentration tested (Table 1).

We also analyzed the total amount of liquid consumed by the WT and KO mice during each 2-week interval as the concentration of ethanol solution available increased. WT and KO mice differed in the amount (grams) of total liquid (ethanol plus water) that they consumed (Fig. 1B). Overall, WT mice consistently drank more total liquid than KO mice (2-way ANOVA, $F_{1,16} = 6.32$, $P = 0.023$), with genotype accounting for 15.8% of the total variance. The concentration of ethanol solution available was also an overall significant factor (2-way ANOVA, $F_{4,64} = 5.12$, $P = 0.0012$, Bonferroni t tests, $P < 0.01$ at the 5% ethanol concentration), accounting for 9.66% of the total variance.

In contrast to ethanol two-bottle choice, WT and KO mice showed no significant difference in the percent of total drinking that was from the sucrose bottle in a sucrose two-bottle choice experiment, at either 1% or 3% concentrations of sucrose (Fig. 1C).

Loss of righting reflex (LORR)

No mice showed LORR following 1.5 g/kg ethanol (n = 9 per genotype, Fig. 2A). At 2.0 g/kg, 5 of 11 KO mice showed LORR that lasted between 25 to

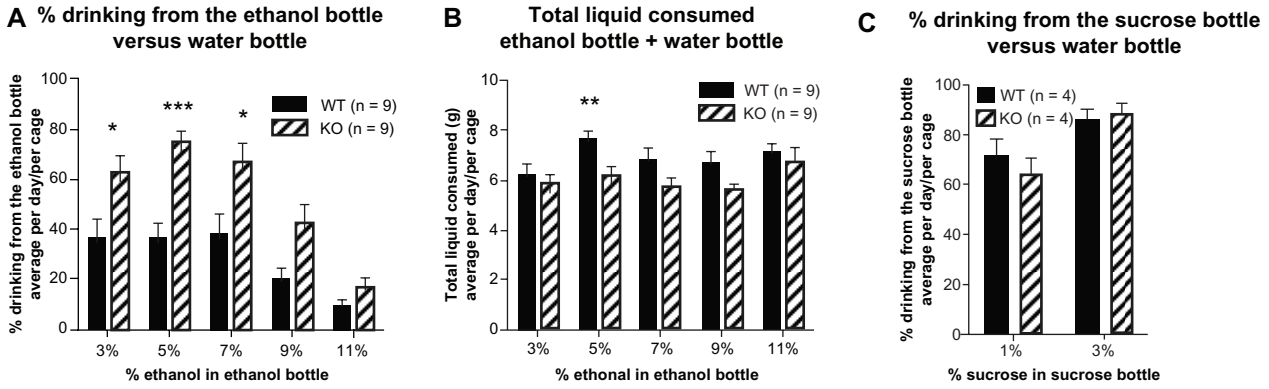


Figure 1. Two-bottle choice. (**A**) KO mice drank a significantly greater volume of ethanol versus water as compared with WT mice ($F_{1,16} = 23.59$, $P = 0.0002$, n = 9 cages per genotype, 2 mice/cage). The magnitude of this difference was dependent on ethanol concentration ($F_{4,64} = 19.09$, $P = 0.0001$, Bonferroni t tests, ***$P < 0.001$; *$P < 0.05$). (**B**) WT mice consistently drank more total liquid than KO mice ($F_{1,16} = 6.32$, $P = 0.023$, n = 9 cages per genotype, 2 mice/cage). The concentration of ethanol available was also an overall significant factor ($F_{4,64} = 5.12$, $P = 0.0012$, Bonferroni t tests, **$P < 0.01$). (**C**) WT and KO mice showed no significant difference in the percent of total drinking that was from the sucrose bottle in a sucrose two-bottle choice experiment, at either 1% or 3% concentrations of sucrose (Fig. 1, right, n = 4 cages per genotype, 2 mice/cage).

Table 1. Average ethanol consumed per day during each 2-week period of ascending concentrations of ethanol availability.

% ethanol	Genotype	g/kg/day/mouse
3%	WT	3.04 ± 0.73
	KO	4.85 ± 0.47
5%	WT	6.35 ± 1.21
	KO	10.13 ± 0.87
7%	WT	7.96 ± 1.65
	KO	12.03 ± 1.49
9%	WT	5.74 ± 1.08
	KO	9.56 ± 1.78
11%	WT	3.2 ± 1.19
	KO	5.32 ± 1.54

110 minutes, whereas none of the 11 WT mice displayed LORR (n = 11 per genotype, Fig. 2B). At 2.5 g/kg, all mice all displayed LORR, and LORR in KO mice continued as much as 40 minutes longer than any WT tested (n = 19 per genotype, Fig. 2C).

Locomotion

Naïve male and female WT and KO mice were administered saline or a 1.0 g/kg, 1.5 g/kg, 2.0 g/kg, or 2.5 g/kg i.p. injection of ethanol (20% vol/vol), and locomotor activity was measured for 60 minutes in an activity chamber at 5-minute intervals. There were no overall significant differences between KO and WT mice of either sex at any of the doses of ethanol tested, but there was a significant difference between measures at different time points (2-way ANOVA performed on each line graph in Fig. 3: genotype, ns; time points, $F_{12,72} = 2.77$ to 12.60, $P < 0.01$ in each analysis, Bonferroni *t* tests as indicated in Fig. 3 line graphs). Cumulative counts over the 60-minute test interval were also analyzed (Fig. 3, bar graphs). Female KO mice showed significantly lower levels of total locomotion over the 60-minute test period following exposure to 2.5 g/kg ethanol (*t* test, $P < 0.01$, Fig. 3E), as did male KO mice following exposure to 1.0 g/kg ethanol (*t* test, $P < 0.01$, Fig. 3B). Also, when male and female data were combined and analyzed, KO mice showed significantly lower levels of total locomotion over the 60-minute test period following exposure to 2.5 g/kg ethanol (*t* test, $P < 0.01$, Fig. 3E). Lastly, an additional group of WT and KO mice (n = 6 male and 2 female for each genotype) which had been exposed for the first time to ethanol on each of the 2 previous days was tested on the third day with a 2.0 g/kg dose of ethanol (i.p.) and locomotor activity was measured for 120 minutes in an activity chamber at 5-minute intervals (Supplemental Fig. 1). Overall, a similar pattern was observed, with KO mice showing significantly lower locomotor activity as compared with WT mice (2-way ANOVA, genotype and time: genotype, $F_{1,14} = 5.85$, $P < 0.03$; time, $F_{24,336} = 11.73$, $P < 0.001$; Bonferroni *t* tests, as indicated, $P < 0.01$).

Blood ethanol level (BEL)

To investigate whether WT and KO mice differ in their ability to metabolize ethanol, BELs were measured following injection of 2.5 g/kg ethanol (i.p.) in separate groups of WT and KO mice and then euthanized at different time points (0.03–24 hours postinjection; number and sex of mice are matched between genotypes and are indicated in Fig. 4, top). Data were analyzed using noncompartmental pharmacokinetic analysis (PKSolver, see Zhang et al).[18] All pharmacokinetic parameters between genotypes were almost

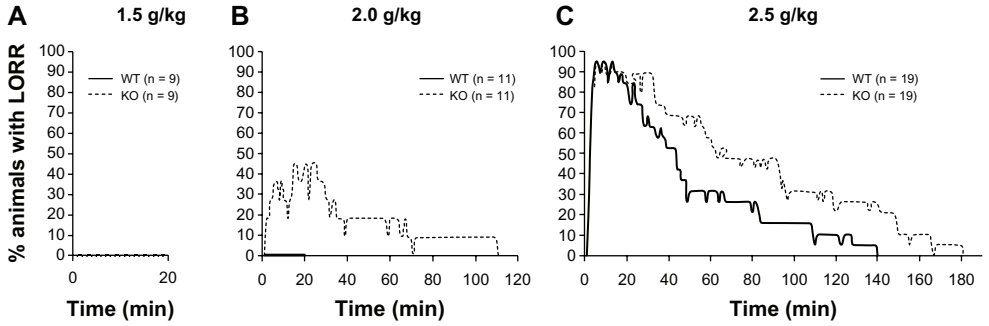


Figure 2. Loss of righting reflex (LORR). Naïve WT (solid lines) and KO (dotted lines) mice were treated with either a 1.5 g/kg, 2.0 g/kg, or 2.5 g/kg dose of ethanol (i.p.) and tested for LORR. (**A**) At 1.5 g/kg ethanol, no mice showed LORR (n = 9 per genotype). (**B**) At 2.0 g/kg, 5 of 11 KO showed LORR that lasted between 25 and 110 minutes, whereas none of 11 WT displayed LORR. (**C**) At 2.5 g/kg, 19 KO and 19 WT mice all displayed LORR, and LORR in KO mice continued as much as 40 minutes longer than any WT tested.

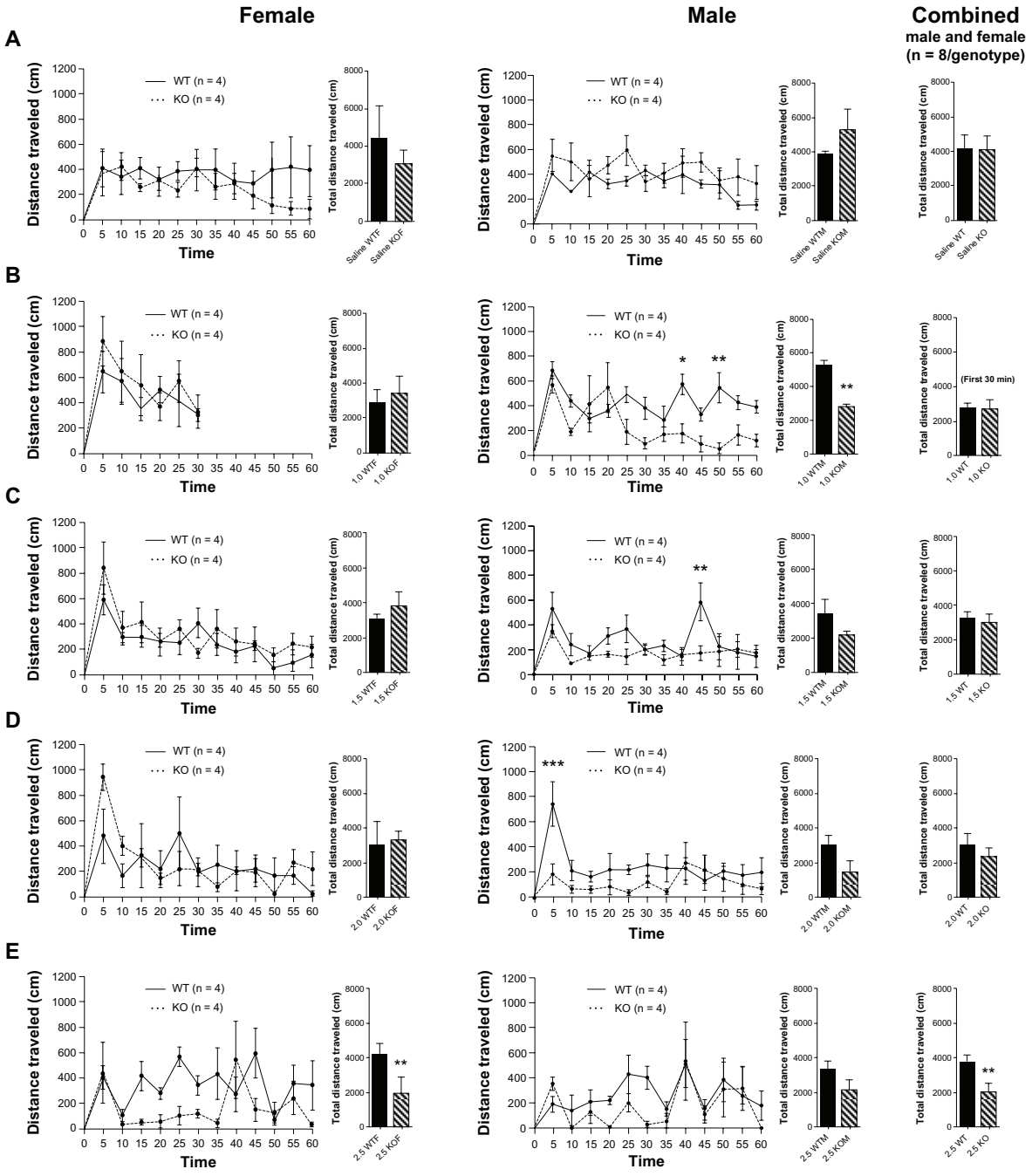
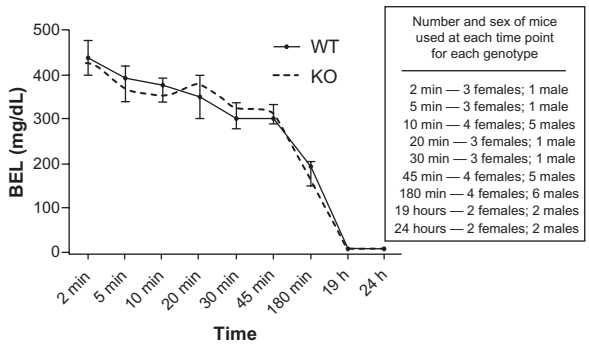


Figure 3. Locomotion. Male and female WT (solid lines and bars) and KO (dotted lines and striped bars) were administered saline (**A**), 1.0 g/kg (**B**), 1.5 g/kg (**C**), 2.0 g/kg (**D**) or 2.5 g/kg (**E**) ethanol (20% vol/vol) by i.p. injection, and locomotor activity was measured in 5-minute intervals for 60 minutes (n = 4 per genotype per dose). There were no overall significant differences between KO and WT mice of either sex at any of the doses of ethanol tested, but there was an overall significant difference between measures at different time points (2-way ANOVA, $F_{12,72} = 2.77$ to 12.60, $P < 0.01$ in each analysis, Bonferroni *t* tests $^{*}P < 0.05$, $^{**}P < 0.01$). Female KO mice showed significantly lower levels of total locomotion over the 60-minute test period following exposure to 2.5 g/kg ethanol (*t* test, $^{**}P < 0.01$, row E), as did male KO mice following exposure to 1.0 g/kg ethanol (*t* test, $^{**}P < 0.01$, row B). With both sexes combined, KO mice showed significantly lower levels of total locomotion over the 60-minute test period following exposure to 2.5 g/kg ethanol (*t* test, $^{**}P < 0.01$, row E).

identical (see Fig. 4, bottom), including elimination half-life ($t_{1/2}$), maximal concentration (C_{max}), area under the curve from 0 to last time (AUC_{0-t}), clearance rate (Cl), volume of distribution based on terminal slope (V_z) and mean residence time (MRT).

Discussion

The present study demonstrates that deletion of TAAR1 in mixed-strain (background 75% C57BL/6J and 25% 129S1/Sv) mice results in adult KO mice that show a greater preference for ethanol and



Pharmacokinetic parameters

Genotype	$t_{1/2}$ (h)	C_{max} (mg/dL)	AUC_{t-0} (mg/dL*h)	Cl (g/(mg/dL)/h)	V_z (g/mg/dL)	MRT (h)
TAAR1 KO	4.04	438.6	2234	0.00109	0.0064	3.74
Wild-type	4.10	437.4	2497	0.00098	0.0058	3.78

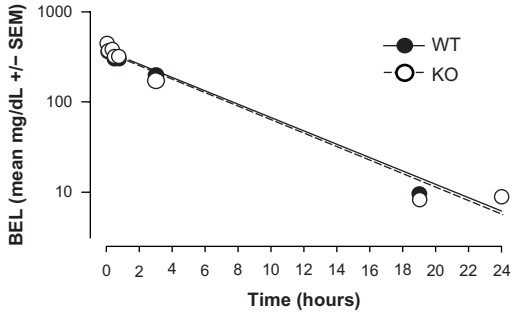


Figure 4. Blood ethanol level (BEL). We assessed BELs in KO and WT mice (n = 4–11 mice per time point, as indicated) that were administered a 2.5 g/kg dose of ethanol (i.p.). Pharmacokinetic analysis showed no differences between genotype for any relevant parameter associated with ethanol metabolism.

consume more ethanol than their WT counterparts. The TAAR1 KO mice in this study also had a greater sensitivity to the sedative effects of ethanol and showed a longer duration of motor impairment following ethanol exposure, yet KO and WT mice showed similar ethanol pharmacokinetics. Consistent with this observation, a greater reduction in locomotion in response to ethanol was also observed in the KO mice as compared with WT mice. These findings are the first to implicate a role for TAAR1 in the behavioral and reinforcement-related effects of ethanol and raise the question of whether specific drugs that target TAAR1 could potentially reduce alcohol consumption in humans with AUDs.

In the absence of any overt behavioral abnormalities under baseline conditions, KO mice show a pharmacogenic phenotype of an enhanced locomotive response to amphetamine and methamphetamine, coincident with an increase in the release of biogenic amines.[2,16,19] Consistent with the augmented locomotor response, KO mice acquire methamphetamine-induced conditioned place preference (CPP) earlier than WT mice and retain CPP longer during extinction training.[19] Notably though, both genotypes display similar levels of morphine-induced CPP. These data suggest that TAAR1 plays a modulatory role in the behavioral sensitization to amphetamine-based psychostimulants and a selective role in the conditioned reinforcing effects of methamphetamine versus morphine. This differential effect may involve dopamine in that dopamine released by amphetamine (or methamphetamine) interacts with dopamine D1 (and D2) receptors to establish CPP,[20] whereas D1 receptors are reportedly not required for morphine-induced CPP.[21] In this regard, other drugs of abuse also involve dopamine receptor-dependent reward mechanisms, including ethanol. Both dopamine D1 and D2 receptor mechanisms have been implicated in ethanol-seeking behavior in mice.[22] Dopamine D3 receptors are also implicated in ethanol seeking and relapse behaviors in mice and rats[23–25] as well as in ethanol preference and intake in alcohol-preferring in rats.[26] Collectively, and in the context of the present study, the pharmacogenic phenotypes observed in KO mice along with the ability of TAAR1 activation to regulate dopamine suggest that TAAR1 is a modulator of dopamine-mediated rewarding effects of drugs of abuse. Notably, this includes amphetamine-like psychostimulants such as methamphetamine, which directly binds to the receptor, as well as ethanol, which may indirectly alter TAAR1 signaling via its ability to affect levels of endogenous TAAR1 agonists (eg, dopamine, but also trace amines). Additionally, TAAR1-mediated effects on NET and SERT function suggest a pharmacology for the TAAR1 receptor that is unique and distinct from other monoamine receptors. In this regard, the present study warrants further investigation of TAAR1 as a potential therapeutic target for addictive disorders generally and for AUDs specifically that may function in a distinctly different manner than current pharmacotherapies.[9,10,13,27]

TAAR1 was discovered in 2001,[1,4] but specific drugs that target the receptor did not come along until recently.[11,28–32] The discovery of compounds that specifically target TAAR1 is now enabling deeper investigations into the role of TAAR1 in the rewarding effects of drugs of abuse. For example, Revel et al[29] showed that the TAAR1 agonist RO5166017 inhibited the firing frequency of dopamine and serotonin

neurons in regions where TAAR1 is expressed (eg, VTA and dorsal raphe nucleus) and blocked dopamine-dependent hyperlocomotion in cocaine-treated mice and DAT knockout mice as well as hyperactivity induced by an NMDA antagonist. Mice injected with cocaine displayed elevated locomotor activity, and RO5166017 given orally prevented this effect in a dose-dependent manner. RO5166017 alone had little or no effect on locomotion. In KO mice, cocaine elevated locomotor activity to a similar extent as in WT mice, but only in WT mice did RO5166017 prevent the cocaine-induced hyperlocomotion. Interestingly, RO5166017 also inhibited stereotypies induced by cocaine in WT mice, and this effect was lost in the KO mice. Lastly, the first potent and selective TAAR1 partial agonist, RO5203648, specifically blocked cocaine self administration in rats.[30] The drug did not significantly alter operant responding for 10% sucrose or increases in the reinforcing effectiveness of sucrose under a progressive ratio schedule of reinforcement, suggesting that a TAAR1 partial agonist does not impair motor or motivational processes. Accordingly, compounds that target TAAR1 are capable of modifying self administration of cocaine, and accordingly, in the context of the present data implicating a role for TAAR1 in modulating ethanol-induced behaviors, it is reasonable to speculate that these compounds may be efficacious in altering self administration of ethanol as well.

It is notable that TAAR1 mRNA and protein have been detected in a variety of brain areas including many of the areas that are critical to the "alcohol" circuitry, such as the nucleus accumbens, amygdala, hippocampus, substantia nigra, ventral tegmental area, and raphe nuclei.[1–3,10] While this receptor has been studied extensively in the monoamine system, it is largely unstudied in other brain regions. TAAR1 agonist treatment of striatal or thalamic synaptosomes promotes changes in DAT/SERT and NET kinetic activity, respectively, by promoting PKA and PKC phosphorylation cascades,[9,10] so it is likely that TAAR1 expressed in other brain regions can also promote PKA and PKC signaling cascades upon stimulation.

In summary, this is the first study to investigate the involvement of TAAR1 in the biological and behavioral effects of ethanol. The enhanced preference, consumption, and sedative effects of ethanol observed in KO mice demonstrate a role for TAAR1 in modulating the behavioral and reinforcement-related effects of ethanol. These findings suggest that TAAR1 may be part of a novel mechanism contributing to the effects of ethanol in the brain and that TAAR1-targeted compounds may have potential as candidate medications for AUDs. Defining a role for TAAR1 in modulating the effects of ethanol can have a large potential impact. If efficacious, TAAR1-targeted drugs would function through a mechanism distinct from other clinically available drugs used for treating alcoholism/AUDs or those that are in preclinical or clinical trials. Further, they may have fewer side effects, in that they may regulate brain monoamines without binding directly to monoamine transporters and/or classic monoamine receptors.[8,13,27]

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Author Contributions

Conceived and designed the experiments: GMM, LJL, KAS, EJV, DMP. Analyzed the data: GMM, LJL, EJV, JKR, DMP. Wrote the first draft of the manuscript: GMM, LJL. Contributed to the writing of the manuscript: GMM, LJL, DMP, JKR. Agree with manuscript results and conclusions: GMM, LJL, KAS, EJV, JKR, DMP. Jointly developed the structure and arguments for the paper: GMM, LJL, DMP. Made critical revisions and approved final version: GMM, DMP. All authors reviewed and approved of the final manuscript.

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Competing Interests

Author(s) disclose no potential conflicts of interest.

Disclosures and Ethics

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published

elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

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Supplemental Figure

Figure S1. Locomotion. An additional group of WT and KO mice (n = 6 male and 2 female for each genotype) which had been exposed for the first time to ethanol on each of 2 days prior days was tested on the third day with a 2.0 g/kg dose of ethanol and locomotor activity was measured for 120 minutes in an activity chamber at 5-minute intervals. KO mice showed significantly lower locomotor activity as compared with WT mice (2-way ANOVA, genotype and time: genotype, $F_{1,14} = 5.85$, $P < 0.03$; time, $F_{24,336} = 11.73$, $P < 0.001$; Bonferroni *t* tests $^{**}P < 0.01$).