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G-Protein-Gated Inwardly Rectifying Potassium (GIRK) Channel Subunit 3 Knock-Out Mice Show Enhanced Ethanol Reward

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Abstract

Background—G protein-coupled inwardly rectifying potassium (GIRK) channels contribute to the effects of a number of drugs of abuse, including ethanol. However, the roles of individual subunits in the rewarding effects of ethanol are poorly understood.

Methods—We compare conditioned place preference (CPP) in GIRK3 subunit knock-out ($GIRK3^{-/-}$), heterozygote ($GIRK3^{+/-}$), and wild-type (WT) mice. In addition, the development of locomotor tolerance/sensitization and the effects of ethanol intoxication on associative learning (fear conditioning) are also assessed.

Results—Our data show significant ethanol CPP in $GIRK3^{-/-}$ and $GIRK3^{+/-}$ mice, but not in the WT littermates. In addition, we demonstrate that these effects are not due to differences in ethanol metabolism, the development of ethanol tolerance/sensitivity, or associative learning abilities. While there were no consistent genotype differences in the fear conditioning assay, our data do show a selective sensitization of the impairing effects of ethanol intoxication on contextual learning, but no effect on cued learning.

Conclusions—These findings suggest that GIRK3 plays a role in ethanol reward. Further, the selectivity of this effect suggests that GIRK channels could be an effective therapeutic target for the prevention and/or treatment of alcoholism.

Keywords

GIRK channels; reward; conditioned place preference; fear conditioning; ethanol

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Introduction

The neural mechanisms that underlie the rewarding effects of ethanol are highly complex. Although there are clear effects of ethanol on regions thought to underlie drug reward and addiction development, such as the mesolimbic dopamine system (Nestler, 2005; Soderpalm and Ericson, 2013), the number of channels and signaling pathways through which ethanol could be exerting these effects makes it difficult to determine which targets contribute to ethanol reward (Weiss and Porrino, 2002; Luscher and Ungless, 2006).

One such target is the G-protein gated inwardly rectifying potassium (GIRK) channel family. These channels are activated by the Gβγ subunits released from $G_{i/o}$-associated G-protein coupled receptors (GPCRs) and conduct an outward potassium current that hyperpolarizes the cell (Dascal, 1997; Hibino et al., 2010). Ethanol alters the signals induced by a number of GPCRs associated with GIRK channel activation, one or more of which may contribute to ethanol's rewarding properties (Federici et al., 2009; Ding et al., 2014). In addition, GIRK channels can be directly activated by ethanol in a Gβγ-independent manner at concentrations that are relevant to human consumption (Aryal et al., 2009; Bodhinathan and Slesinger, 2013). Given that GIRK channels are expressed in a number of regions associated with reward processing (Saenz del Burgo et al., 2008), we and others (Luscher and Slesinger, 2010) hypothesize that the modulation of GIRK signaling by ethanol could contribute to ethanol reward.

Four neuronal GIRK subunits (GIRK1 - GIRK4) are expressed throughout the brain in distinct but overlapping patterns (Koyrakh et al., 2005; Aguado et al., 2008). Of these, the GIRK2 subunit is the most intensively studied and has been implicated in several alcohol responses (Blednov et al., 2001; Hill et al., 2003); however, much less is known about the role of the other GIRK subunits. Previously, we identified the gene that codes for the GIRK3 subunit (*Kcnj9*) as a quantitative trait gene for ethanol physiological dependence and associated withdrawal in mice (Kozell et al., 2009). Thus, GIRK3 might play a role in a variety of alcohol-related behaviors, including reward, which is significantly genetically correlated with withdrawal in mice (Cunningham, 2014).

Here we use conditioned place preference (CPP), a widely used measure of drug reward (Sanchis-Segura and Spanagel, 2006), to assess the role of GIRK3 in ethanol's rewarding and motivational properties in GIRK3 knock-out ($GIRK3^{-/-}$), heterozygote ($GIRK3^{+/-}$), and wild-type (WT) littermates. In addition, we assess the potential confounding effects of genotype on ethanol metabolism, the development of ethanol tolerance/sensitization, and on the ability of ethanol to alter associative learning processes, all of which could influence CPP expression.

Materials and Methods

Animals

GIRK3 GIRK3+/− mice were originally obtained from Dr. Kevin Wickman (Torrecilla et al., 2002), and have been maintained in our colony for 17 generations using $GIRK3^{+/-}$ × $GIRK3^{+/-}$ breeding with backcrossing to C57BL/6J mice (The Jackson Laboratory, Bar

Harbor, ME) every third generation. Mice were weaned at 21 days of age and housed 2–4 per cage by sex under standard housing conditions (12h:12h light:dark cycle) with access to food and water *ad libitum*. Adult male mice (56 – 100 days old) were used for these studies, and each behavioral assay was performed in a different squad of mice. All studies were approved by the Institutional Animal Care and Use Committee at Oregon Health & Science University or the Portland VA Research Center and were conducted in accordance with the guidelines provided by the National Institutes of Health (NIH Publications No. 8023).

Conditioned Place Preference

Chambers—The apparatus and procedure used for two-compartment CPP training are identical to those described in Hitchcock et al. (2014). Briefly, we used a rectangular apparatus (30 cm wide × 15 cm long × 15 cm high) consisting of clear acrylic walls and two interchangeable floor types: grid (2.3-mm stainless steel rods mounted 6.4 mm apart) and hole (perforated stainless steel with 6.4-mm round holes on 9.5-mm staggered centers). During training, the chamber was bisected with a clear acrylic barrier, which was removed during the preference test. Both the floor type (grid/hole) and side of the chamber (left/right) associated with ethanol were counterbalanced across groups. The CPP chambers were housed in melamine boxes (McCarthy Manufacturing, Gresham, OR). A camera and infrared light were mounted in the center of each box directly above the CPP apparatus.

Training and Testing—On day 1, mice were placed in the chambers and allowed to explore both sides freely for 5 min to test for any initial bias (pre-test). Mice were trained on days 2–9. Training consisted of an i.p. injection of either 2 g/kg ethanol (days 2, 4, 6, and 8) or an equivalent volume of saline (days 3, 5, 7, and 9), followed by a 15-min exposure to the appropriate side of the chamber (CS+ for ethanol, CS− for saline; CS: conditioned stimulus) (Lim et al., 2012). Mice were then tested for preference on day 10 by placing them in the chambers with access to both sides for 15 min.

Scoring—The position and locomotor activity of each animal was recorded and analyzed using Ethovision software (Noldus, Lessburg, VA). Preference was defined as the amount of time (sec/min) each animal spent on the CS+ associated floor during the test session.

Blood Ethanol Concentrations

Mice were assessed for blood ethanol concentrations (BECs) at 45 min, 75 min, 3 h, 4 h, and 6 h post-ethanol (4 g/kg, i.p.) administration. Beginning at 45 min post-injection, the tail of the mouse was snipped and a blood sample (20 μl) was taken using a capillary tube. The samples were processed and ethanol levels were determined using gas chromatography, as described in (Finn et al., 2007).

Locomotor Activity

Testing for changes in locomotor responses was performed in rectangular conditioning chambers (24 cm wide × 29 cm long × 20 cm high; Med-Associates, St. Albans, VT). Each chamber was housed in an individual sound-attenuating box. On day 1, mice were injected (i.p.) with saline to determine baseline locomotion. On days 2–5, mice were injected with 2 g/kg ethanol. After each injection, mice were immediately placed into the chambers for 15

min. Locomotor behavior was recorded with Ethovision software (Noduls), and the total distance traveled (cm) was used as a measure of locomotor activity.

Fear Conditioning

Apparatus—Training and contextual testing were conducted in 14.5 cm circular Plexiglas chambers mounted on a rod floor. Each chamber was housed in an individual sound-attenuating box (Med-Associates). An 85 dB white noise CS was administered through a sound generator (Coulbourn Instruments, Whitehall, PA), and a 0.35 mA footshock (unconditioned stimulus: US) was administered through the rod floor via a shock scrambler/generator (Coulbourn). The apparatus was cleaned with 1% acetic acid prior to conditioning and testing. The training and context testing sessions were controlled by an IBM-PC running Graphic State software (Coulbourn). Testing for cued fear conditioning was conducted in rectangular conditioning chambers with Plexiglas floors (Med-Associates). Each chamber was housed in an individual sound-attenuating box and located in a novel procedural room. In these chambers, the CS was generated with ANL-926 (Med-Associates) and administered through speakers mounted on the left wall of the chambers. The CS presentations were controlled by an IBM-PC running MED-PC IV (Med-Associates). The cue testing chambers were cleaned with 70% ethanol.

Drug Administration—All mice received a total of four injections (one per day for four days) and were trained immediately after receiving their fourth injection. The saline group received four saline injections, the first ethanol exposure group received three saline injections and an ethanol injection (2 g/kg) on training day, and the fourth ethanol exposure group received four ethanol injections (2 g/kg).

Training—Prior to training, animals were injected with either saline or 2 g/kg ethanol (see above) and immediately placed into the training chambers. Each training session began with the activation of a house light. After 2 min, a 30-sec CS was activated and co-terminated with a 2-sec footshock (US). All animals received two CS-US pairings in a 6.5-min session.

Testing—To evaluate contextual learning, mice were returned to the training chambers 24 h after training. The houselight was activated, and behavior was assessed for 12 min. To test cued learning, mice were placed in the novel cue testing apparatus 48 h after training. After a 3-min pre-CS interval, the animals were exposed to two 3-min CS presentations separated by a 3-min inter-CS interval and followed by a 3-min post-CS period, for a total test time of 15 min.

Scoring—The context and CS tests were handscored by an experimenter who was blind to the genotype and treatment condition of each subject. Animals were scored at 10-s intervals for freezing, which was defined as the absence of all movement except respiration (Blanchard and Blanchard, 1969). The data are presented as the percentage of observations (six observations per minute) in which the animal was determined to be freezing. For contextual learning, we report the average total freezing for the full 12-min context test. Data for cued learning includes the average freezing during the non-CS periods (pre-CS, inter-CS, and post-CS) and during the two CS presentations.

Data Analysis

For all experiments, the data are presented as the mean ± standard error (SEM). The data were analyzed using one- or two-way ANOVAs followed by Tukey's Honestly-Significant-Difference Test to compare individual groups. $p<0.05$ was considered significant.

Results

Conditioned Place Preference

There was no evidence of a baseline floor bias in any of the groups during the pre-test and no effect of genotype for time spent on either floor (Figure 1A; $p>0.6$ for all measures). Following CPP training (4 CS+ and 4 CS− trials), we found a significant effect of genotype ($F_{2,46}=4.3$, $p=0.019$) for the time spent on the CS+ side of the chamber (Figure 1B). Both the GIRK3$^{+/-}$ ($p=0.026$) and GIRK3$^{-/-}$ ($p=0.043$) groups spent significantly more time on the ethanol-paired floor than the WT mice, indicating enhanced preference in these groups. To assess preference within each genotype, a simple planned comparison of time on the grid floor (grid paired with ethanol vs. grid paired with saline) for each group was conducted (Raybuck et al., 2013). While both the GIRK3$^{-/-}$ ($p<0.01$) and GIRK3$^{+/-}$ ($p<0.001$) groups showed robust preference, we did not detect preference in the WT mice ($p=0.18$). This is unsurprising, as ethanol preference in C57BL/6 mice typically does not appear after only four pairings (Nocjar et al., 1999; Gabriel and Cunningham 2008). There were no differences in total distance moved during the preference test ($p>0.2$ for all comparisons), suggesting that these results are not due to differences in general locomotor behavior during testing.

These data suggest that reduced GIRK3 expression results in enhanced ethanol preference, but a number of other factors could also drive this effect. Consistent with results from GIRK2$^{-/-}$ mice (Blednov et al., 2001) and recent work from another group assessing BEC after a 2 g/kg injection in GIRK3$^{-/-}$ mice (Herman et al., 2015), we found that the loss of GIRK3 did not alter initial BEC values ($p=0.81$) or clearance rates ($p>0.1$ for all timepoints) following an injection of 4 g/kg ethanol (Figure 2). In addition, other groups have shown that GIRK3$^{-/-}$ and WT mice do not differ in general appetitive learning, such as operant responding for food rewards (Morgan et al., 2003; Pavetoni and Wickman, 2008). However, potential differences in the development of tolerance or sensitization to ethanol's effects and differences in associative learning have not been investigated. Thus, we designed the following experiments to investigate the effect of GIRK3 expression on these potential confounding effects.

Locomotor Effects of Ethanol

Repeated exposures to ethanol can result in the development of sensitization or tolerance to individual ethanol effects (Linsenbardt et al., 2009, Matson et al., 2014), and differences in tolerance/sensitivity, especially to the locomotor effects of ethanol, could bias the measure of ethanol CPP. While our CPP test data did not reveal differences in locomotor behavior (see above), this test was given under non-ethanol conditions, and is thus not informative of ethanol-induced locomotor activity or potential changes in this behavior across the four ethanol exposures used during training. To determine if the GIRK3 genotypes differed in the

development of tolerance or sensitization to ethanol's locomotor effects, we measured locomotor behavior in an open field following the administration of saline and four subsequent injections of 2 g/kg ethanol.

There was a significant effect of genotype ($F_{2,100}$=9.0, p<0.001) on the distance traveled (Figure 3), but this effect was only apparent for the initial saline day ($F_{2,20}$=7.4, p=0.004), in which WT mice moved significantly more than GIRK3$^{+/-}$ or GIRK3$^{-/-}$ mice (p<0.02 for all comparisons). There were no differences across the different treatment days for any group (repeated measures post-hoc: p>0.4 for all comparisons) and no genotype differences on any of the ethanol days (p>0.1 for all comparisons).

Associative Learning

In addition to reward sensitivity, CPP relies on the ability of the animal to form an association between the subjective effects of the drug and the specific location in which the drug is given (Hitchcock et al., 2014). Theories of addiction development emphasize the importance of learned cue associations that are predictive of drug availability (Robbins and Everitt, 2002; Everitt and Robbins, 2005), and the modulation of learning processes are thought to underlie the development of addictive behavior (Hyman et al., 2006). Thus, differences in CPP could be due to potential genotype effects on either baseline associative learning or the sensitivity of these learning processes to modulation by ethanol. To test this, we compared the GIRK3 genotypes in another form of Pavlovian learning, delay fear conditioning, following the administration of saline, a single ethanol injection, or their fourth ethanol injection.

In the context test (Figure 4A), we found a significant effect of treatment ($F_{2,78}$=33.8, p<0.001), but no effect of genotype. For WT and GIRK3$^{-/-}$ mice, there was a significant difference between freezing in saline-treated mice and both ethanol groups (p<0.001 for all comparisons) and for freezing between the single and fourth ethanol exposure groups (p<0.001). Although we did not detect an effect of genotype on these responses, we did observe an interesting effect of the number of ethanol exposures on contextual learning. In the WT and GIRK3$^{-/-}$ mice, the single ethanol exposure group froze significantly less than the saline-treated group, and the fourth exposure group froze significantly less than the first exposure group. A similar trend was also observed for the GIRK3$^{+/-}$ mice. These data suggest that the impairing effects of ethanol on contextual learning actually sensitize across repeated pairings.

For the CS test, there were no significant effects of genotype or treatment on freezing prior to the first CS presentation (SAL: GIRK3$^{+/+}$ 3.7±2.2, GIRK3$^{+/-}$ 5.6±2.5, GIRK3$^{-/-}$ 8.6±2.8; 1 ETOH: GIRK3$^{+/+}$ 4.3±2.4, GIRK3$^{+/-}$ 8.6±3.0, GIRK3$^{-/-}$ 5.6±1.7; 4 ETOH: GIRK3$^{+/+}$ 3.3±1.8, GIRK3$^{+/-}$ 5.6±2.5, GIRK3$^{-/-}$ 4.3±2.1) or for any of the other non-CS periods (data not shown; p>0.1 for all comparisons). We did find a significant effect of genotype ($F_{2,78}$=4.5, p=0.014) and treatment ($F_{2,78}$=8.3, p=0.001) for freezing during the CS presentations (Figure 4B); however, analysis of the individual groups revealed that these effects were both driven exclusively by the GIRK3$^{+/-}$ mice. GIRK3$^{+/-}$ mice froze more than WT mice for the saline-treated groups (p=0.008), but there were no differences between the GIRK3$^{+/-}$ and GIRK3$^{-/-}$ or the GIRK3$^{-/-}$ and WT groups (p>0.2 for all comparisons).

Similarly, the treatment effects were only apparent in the GIRK3$^{+/-}$ group, which showed a significant difference between the fourth ethanol exposure group and the saline group ($p=0.005$) and initial ethanol exposure ($p=0.006$) groups; however, these differences are due primarily to high baseline responding and do not reflect differences across the genotypes in the ethanol treated groups. The WT and GIRK3$^{-/-}$ groups did not show a treatment effect, and there were no differences across genotypes for either of the ethanol-treated groups ($p>0.1$ for all comparisons). Thus, we found no consistent strain or treatment effects on freezing to the CS, suggesting that the effect of repeated ethanol exposure on associative processes is not responsible for differences in ethanol CPP expression.

Discussion

Our data show that GIRK3$^{-/-}$ and GIRK3$^{+/-}$ mice have enhanced ethanol CPP compared to WT littermates. Further, we show that this effect is not due to general differences in BECs, the development of ethanol tolerance/sensitization, or the ability of ethanol to alter learning and memory. Our findings contribute to a growing body of literature implicating GIRK3-containing channels as having an important role in alcohol/drug abuse and dependence/withdrawal (Cruz et al., 2008; Kozell et al., 2009). Additionally, our data demonstrate a novel, selective sensitization of contextual learning to ethanol exposure that raises an interesting point about the relationship between CPP and associative processes. While the traditional conception of CPP involves the association of the context with a drug state, our data demonstrate that contextual association processes are significantly impaired by ethanol intoxication at this dose and that this impairment may show sensitization across multiple ethanol exposures. Although there are potential differences in associative processes involving reward and those involving fear, our data would suggest that it is unlikely that ethanol CPP development relies primarily on contextual processing. Instead, our findings support the hypothesis that mice may use more discrete cue associations, which are not as robustly impaired by repeated ethanol exposure (Cunningham and Noble, 1992; Cunningham et al., 2006). Given that the effects of ethanol on associative learning were apparent in all three genotypes, it is unlikely that ethanol modulation of associative processes is driving the observed differences in ethanol CPP; however, the robust, sensitizing effect of ethanol on contextual learning compared to cued learning in the fear conditioning task highlights the ability of ethanol to differentially modulate specific processes and brain regions involved in this task. Specifically, our data are in agreement with previous work suggesting that associative processes involving the hippocampus are more sensitive to disruption by ethanol than non-hippocampal tasks (White et al., 2000; Weitemeir and Rayabinin, 2003; Gulick and Gould, 2007).

Potential Mechanisms

The enhanced ethanol preference observed in our study leads to the question of how a reduction in GIRK3 expression might result in increased ethanol reward. While GIRK3 is expressed in a number of brain regions, GIRK3$^{-/-}$ mice show little or no change in baseline electrophysiological properties, including baclofen-induced currents and post-synaptic potentials, compared to WT mice (Labouebe et al., 2007; Arora et al., 2010; Hearing et al., 2013), suggesting that our results are not due to significant alterations in GIRK-mediated

currents or neuronal excitability. However, recent work has shown that the ability of high concentrations of ethanol to increase the firing rate of dopamine neurons in the ventral tegmental area (VTA), and the subsequent increase in dopamine release to the nucleus accumbens, is reduced in GIRK3$^{-/-}$ mice (Herman et al., 2015), suggesting that this subunit may play a more modulatory role in GIRK channel function. In agreement with this, the existing literature on GIRK3 points to two alternative mechanisms that could contribute to these effects: differences in ethanol's modulation of GIRK signaling and/or differences in GIRK trafficking.

It is currently unclear whether GIRK3-containing channels show altered sensitivity to ethanol compared to other GIRK channel subtypes; however, it has been shown that GIRK2/3 channels have reduced sensitivity to Gβγ activation (Jelacic et al., 2000). If GIRK2/3 channels are likewise less sensitive to activation by ethanol, then reducing GIRK3 expression could result in a general increase in ethanol's ability to modulate GIRK signaling. Thus, GIRK signaling in GIRK3$^{-/-}$ mice may be more sensitive to ethanol modulation, potentially enhancing ethanol reward processing. Alternatively, reduced GIRK3 expression could alter channel trafficking and thus the adaptation of these cells to repeated ethanol exposure. GIRK3 subunits associate with sorting nexin 27 (SNX27), which regulates GIRK channel expression levels by targeting GIRK3-containing channels to early endosomes, thereby reducing surface expression and resulting in smaller GIRK currents (Lunn et al., 2007; Balana et al., 2013). SNX27 itself has also been implicated in the rewarding effects of drugs of abuse (Munoz and Slesinger, 2014), suggesting that the regulation of GIRK signaling via this mechanism is an important adaptation to drug exposure. While the effects of ethanol on channel trafficking via SNX27 are unknown, it is possible that this mechanism could play a role in adapting to repeated ethanol exposure and might contribute to the altered dopamine signaling observed following repeated *in vivo* ethanol exposure (Perra et al., 2011). Future investigations into these possibilities may help address the question of how the loss of GIRK3 can alter ethanol responses without dramatically affecting GIRK-mediated currents or neuronal excitability.

While our data in combination with the existing literature discussed here suggests a number of molecular mechanisms through which GIRK3 could alter ethanol reward processing, more work is needed to determine how the loss of GIRK3 alters the interaction between ethanol and the GIRK system. Future work using *Kcnj9*/GIRK3 RNA interference and/or other targeted approaches will be important to rule out the possibility of compensation events that might contribute to the current results, and directly assess the role of GIRK3 in ethanol reward, as well as the potential brain regions and mechanisms involved. Interestingly, the same group that reported a decrease in ethanol stimulation of VTA dopamine neurons in GIRK3$^{-/-}$ mice also found that the loss of GIRK3 increased ethanol consumption, but only under binge-like conditions (Herman et al., 2015). They hypothesize that the loss of GIRK3 may selectively reduce sensitivity to the reinforcing effects of ethanol; however, our CPP results would suggest that GIRK3$^{-/-}$ mice are actually more sensitive to ethanol reward, which could also result in increased consumption. A major limitation of both the work reported here and that by Herman and colleagues with regard to assessing reward sensitivity is that only a single, commonly rewarding dose of ethanol was assessed in each paradigm. To determine if altered GIRK3 expression increases or decreases

behavioral sensitivity to ethanol reward, the assessment of responses to a range of ethanol doses will be required.

Differential Effects of GIRK2$^{-/-}$ and GIRK3$^{-/-}$

A number of previous studies have looked at the effects of the GIRK2 subunit on ethanol-related behaviors. GIRK2 subunits are typically considered to be the dominant GIRK subunit and are capable of forming homomeric channels and heteromeric channels with either GIRK1 or GIRK3 (Hibino et al., 2010). Interestingly, GIRK2$^{-/-}$ mice consume more ethanol that WT mice, but fail to develop ethanol CPP (Blednov et al., 2001; Hill et al., 2003), suggesting that ethanol reward sensitivity may actually be reduced in these mice. In addition, while the GIRK currents from several neuron populations, including those associated with reward signaling, are largely unaltered in GIRK3$^{-/-}$ mice, the loss of GIRK2 significantly decreases GIRK currents in these cell types (Cruz et al., 2004; Koyrakh et al., 2005; Arora et al., 2010, Hearing et al., 2013). Thus, while both previous work with GIRK2 and the current results with GIRK3 support the hypothesis that GIRK signaling plays an important role in ethanol's behavioral effects, they suggest that channels composed of various subunits may have different roles (Table 1).

Conclusions

Our data demonstrate that GIRK3$^{+/-}$ and GIRK3$^{-/-}$ mice show enhanced ethanol CPP that is independent of locomotor sensitization/tolerance and associative learning effects. These findings support the hypothesis that GIRK channels play an important role in the rewarding effects of ethanol. In addition, our results, along with previous findings, strongly suggest that GIRK-based therapeutics, particularly those targeted to specific GIRK subunits, could be effective treatments for alcohol addiction and relapse (Sugaya et al., 2012; Bodhinathan and Slesinger, 2014; Herman et al., 2015).

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References

Aguado C, Colon J, Ciruela F, Schlaudraff F, Cabanero MJ, Perry C, Watanabe M, Liss B, Wickman K, Lujan R. Cell type-specific subunit composition of G protein-gated potassium channels in the cerebellum. J Neurochem. 2008; 105:497–511. [PubMed: 18088366]

Arora D, Haluk DM, Kourrich S, Pravetoni M, Fernandez-Alacid L, Nicolau JC, Lujan R, Wickman K. Altered neurotransmission in the mesolimbic reward system of Girk mice. J Neurochem. 2010; 114:1487–1497. [PubMed: 20557431]

Aryal P, Dvir H, Choe S, Slesinger PA. A discrete alcohol pocket involved in GIRK channel activation. Nat Neurosci. 2009; 12:988–995. [PubMed: 19561601]

Balana B, Bahima L, Bodhinathan K, Taura JJ, Taylor NM, Nettleton MY, Ciruela F, Slesinger PA. Ras-association domain of sorting Nexin 27 is critical for regulating expression of GIRK potassium channels. PLoS One. 2013; 8:e59800. [PubMed: 23536889]

Bjorklund A, Dunnett SB. Dopamine neuron systems in the brain: an update. Trends Neurosci. 2007; 30:194–202. [PubMed: 17408759]

Blanchard RJ, Blanchard DC. Crouching as an index of fear. J Comp Physiol Psychol. 1969; 67:370–375. [PubMed: 5787388]

Blednov YA, Stoffel M, Chang SR, Harris RA. Potassium channels as targets for ethanol: studies of G-protein-coupled inwardly rectifying potassium channel 2 (GIRK2) null mutant mice. J Pharmacol Exp Ther. 2001; 298:521–530. [PubMed: 11454913]

Bodhinathan K, Slesinger PA. Molecular mechanism underlying ethanol activation of G-protein-gated inwardly rectifying potassium channels. Proc Natl Acad Sci U S A. 2013; 110:18309–18314. [PubMed: 24145411]

Bodhinathan K, Slesinger PA. Alcohol modulation of G-protein-gated inwardly rectifying potassium channels: from binding to therapeutics. Front Physiol. 2014; 5:76. [PubMed: 24611054]

Bunney EB, Appel SB, Brodie MS. Electrophysiological effects of cocaethylene, cocaine, and ethanol on dopaminergic neurons of the ventral tegmental area. J Pharmacol Exp Ther. 2001; 297:696–703. [PubMed: 11303060]

Cruz HG, Ivanova T, Lunn ML, Stoffel M, Slesinger PA, Luscher C. Bi-directional effects of GABA(B) receptor agonists on the mesolimbic dopamine system. Nat Neurosci. 2004; 7:153–159. [PubMed: 14745451]

Cunningham CL, Noble D. Conditioned activation induced by ethanol: role in sensitization and conditioned place preference. Pharmacol Biochem Behav. 1992; 43:307–313. [PubMed: 1409816]

Cunningham CL, Patel P, Milner L. Spatial location is critical for conditioning place preference with visual but not tactile stimuli. Behav Neurosci. 2006; 120:1115–1132. [PubMed: 17014262]

Cunningham CL. Genetic Relationship Between Ethanol-Induced Conditioned Place Preference and Other Ethanol Phenotypes in 15 Inbred Mouse Strains. Behav Neurosci. 2014; 128:430–445. [PubMed: 24841742]

Dascal N. Signalling via the G protein-activated K+ channels. Cell Signal. 1997; 9:551–573. [PubMed: 9429760]

Ding ZM, Ingraham CM, Rodd ZA, McBride WJ. The reinforcing effects of ethanol within the posterior ventral tegmental area depend on dopamine neurotransmission to forebrain cortico-limbic systems. Addict Biol. 2014; 20:458–468. [PubMed: 24674134]

Everitt BJ, Robbins TW. Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat Neurosci. 2005; 8:1481–1489. [PubMed: 16251991]

Federici M, Nistico R, Giustizieri M, Bernardi G, Mercuri NB. Ethanol enhances GABAB-mediated inhibitory postsynaptic transmission on rat midbrain dopaminergic neurons by facilitating GIRK currents. Eur J Neurosci. 2009; 29:1369–1377. [PubMed: 19309316]

Finn DA, Snelling C, Fretwell AM, Tanchuck MA, Underwood L, Cole M, Crabbe JC, Roberts AJ. Increased drinking during withdrawal from intermittent ethanol exposure is blocked by the CRF receptor antagnoist D-Phe-CRF(12–41). Alcohol Clin Exp Res. 2007; 31:939–949. [PubMed: 17403068]

Gabriel KI, Cunningham CL. Effects of maternal strain on ethanol responses in reciprocal F1 C57BL/6J and DBA/2J hybrid mice. Genes Brain Behav. 2008; 7:276–287. [PubMed: 17711451]

Hearing M, Kotecki L, Marron Fernandez de Velasco E, Fajardo-Serrano A, Chung HJ, Lujan R, Wickman K. Repeated cocaine weakens GABA(B)-Girk signaling in layer 5/6 pyramidal neurons in the prelimbic cortex. Neuron. 2013; 80:159–170. [PubMed: 24094109]

Herman MA, Sidhu H, Stouffer DG, Kreifeld M, Le D, Gates-Gatto C, Munoz MB, Roberts AJ, Parsons LH, Roberto M, Wickman K, Slesinger PA. GIRK3 gates activation of the mesolimbic dopaminergic pathway by ethanol. Proc Natl Acad Sci. 2015; 112:7091–7096. [PubMed: 25964320]

Hibino H, Inanobe A, Furutani K, Murakami S, Findlay I, Kurachi Y. Inwardly rectifying potassium channels: their structure, function, and physiological roles. Physiol Rev. 2010; 90:291–366. [PubMed: 20086079]

Hill KG, Alva H, Blednov YA, Cunningham CL. Reduced ethanol-induced conditioned taste aversion and conditioned place preference in GIRK2 null mutant mice. Psychopharmacology (Berl). 2003; 169:108–114. [PubMed: 12721779]

Hitchcock LN, Cunningham CL, Lattal KM. Cue configuration effects in acquisition and extinction of a cocaine-induced place preference. Behav Neurosci. 2014; 128:217–227. [PubMed: 24773441]

Hyman SE, Malenka RC, Nestler EJ. Neural mechanisms of addiction: the role of reward-related learning and memory. Annu Rev Neurosci. 2006; 29:565–598. [PubMed: 16776597]

Jelacic TM, Kennedy ME, Wickman K, Clapham DE. Functional and biochemical evidence for G-protein-gated inwardly rectifying K+ (GIRK) channels composed of GIRK2 and GIRK3. J Biol Chem. 2000; 275:36211–36216. [PubMed: 10956667]

Koyrakh L, Lujan R, Colon J, Karschin C, Kurachi Y, Karschin A, Wickman K. Molecular and cellular diversity of neuronal G-protein-gated potassium channels. J Neurosci. 2005; 25:11468–11478. [PubMed: 16339040]

Kozell LB, Walter NA, Milner LC, Wickman K, Buck KJ. Mapping a barbiturate withdrawal locus to a 0.44 Mb interval and analysis of a novel null mutant identify a role for Kcnj9 (GIRK3) in withdrawal from pentobarbital, zolpidem, and ethanol. J Neurosci. 2009; 29:11662–11673. [PubMed: 19759313]

Labouebe G, Lomazzi M, Cruz HG, Creton C, Lujan R, Li M, Yanagawa Y, Obata K, Watanabe M, Wickman K, Boyer SB, Slesinger PA, Luscher C. RGS2 modulates coupling between GABAB receptors and GIRK channels in dopamine neurons of the ventral tegmental area. Nat Neurosci. 2007; 10:1559–1568. [PubMed: 17965710]

Lim JP, Zou ME, Janak PH, Messing RO. Responses to ethanol in C57Bl/6 versus C57BL/6×129 hybrid mice. Brain Behav. 2012; 2:22–31. [PubMed: 22574271]

Linsenbardt DN, Moore EM, Griffin KD, Gigante ED, Boehm SL 2nd. Tolerance to ethanol's ataxic effects and alterations in ethanol-induced locomotion following repeated binge-like ethanol intake using the DID model. Alcohol Clin Exp Res. 2011; 35:1246–1255. [PubMed: 21410484]

Lister RG. The effects of ethanol on exploration in DBA/2 and C57Bl/6 mice. Alcohol. 1987; 4:17–19. [PubMed: 3828059]

Lunn ML, Nassirpour R, Arrabit C, Tan J, McLeod I, Arias CM, Sawchenko PE, Yates JR 3rd, Slesinger PA. A unique sorting nexin regulates trafficking of potassium channels via a PDZ domain interaction. Nat Neurosci. 2007; 10:1249–1259. [PubMed: 17828261]

Luscher C, Ungless MA. The mechanistic classification of addictive drugs. PLoS Med. 2006; 3:e437. [PubMed: 17105338]

Luscher C, Slesinger PA. Emerging roles for G protein-gated inwardly rectifying potassium (GIRK) channels in health and disease. Nat Rev Neurosci. 2010; 11:301–315. [PubMed: 20389305]

Matson LM, Kasten CR, Boehm SL, Grahame NJ. Selectivly bred crossed hihg-alcohol-preferring mice drink to intoxication and develop functional tolerance, but not locomotor sensitization during free-choice ethanol access. Alcohol Clin Exp Res. 2014; 38:267–274. [PubMed: 23909817]

Michaeli A, Yaka R. Dopamine inhibits GABA(A) currents in ventral tegmental area dopamine neurons via activation of presynaptic G-protein coupled inwardly-rectifying potassium channels. Neuroscience. 2010; 165:1159–1169. [PubMed: 19944748]

Munoz MB, Slesinger PA. Sorting nexin 27 regulation of G protein-gated inwardly rectifying K(+) channels attenuates in vivo cocaine response. Neuron. 2014; 82:659–669. [PubMed: 24811384]

Nestler EJ. Is there a common molecular pathway for addiction? Nat Neurosci. 2005; 8:1445–1449. [PubMed: 16251986]

Nocjar C, Middaugh LD, Tavernetti M. Ethanol consumption and place-preference conditioning in the alcohol-preferring C57BL/6 mouse: relationship with motor activity patterns. Alcohol Clin Exp Res. 1999; 23:683–692. [PubMed: 10235304]

Perra S, Clements MA, Bernier BE, Morikawa H. In vivo ethanol experience increases D(2) autoinhibition in the ventral tegmental area. Neuropsychopharmacology. 2011; 36:993–1002. [PubMed: 21248720]

Quadros IM, Souza-Formigoni ML, Fornari RV, Nobrega JN, Oliveira MG. Is behavioral sensitization to ethanol associated with contextual conditioning in mice? Behav Pharmacol. 2003; 14:129–136. [PubMed: 12658073]

Raybuck JD, McCleery EJ, Cunningham CL, Wood MA, Lattal KM. The histone deacetylase inhibitory sodium butyrate modulates acquisition and extinction of cocaine-induced conditioned place preference. Pharmacol Biochem Behav. 2013; 106:109–116. [PubMed: 23454534]

Robbins TW, Everitt BJ. Limbic-striatal memory systems and drug addiction. Neurobiol Learn Mem. 2002; 78:625–636. [PubMed: 12559840]

Rodd ZA, Melendez RI, Bell RL, Kuc KA, Zhang Y, Murphy JM, McBride WJ. Intracranial self-administration of ethanol within the ventral tegmental area of male Wistar rats: evidence for involvement of dopamine neurons. J Neurosci. 2004; 24:1050–1057. [PubMed: 14762123]

Saenz del Burgo L, Cortes R, Mengod G, Zarate J, Echevarria E, Salles J. Distribution and neurochemical characterization of neurons expressing GIRK channels in the rat brain. J Comp Neurol. 2008; 510:581–606. [PubMed: 18698588]

Sanchis-Segura C, Spanagel R. Behavioural assessment of drug reinforcement and addictive features in rodents: an overview. Addict Biol. 2006; 11:2–38. [PubMed: 16759333]

Soderpalm B, Ericson M. Neurocircuitry involved in the development of alcohol addiction: the dopamine system and its access points. Curr Top Behav Neurosci. 2013; 13:127–161. [PubMed: 22094880]

Sugaya N, Ogai Y, Kakibuchi Y, Senoo E, Ikeda K. Influence of GIRK channel inhibition on relapse risk in Japanese alcohol-dependent inpatients. Nihon Shinkei Seishin Yakurigaku Zasshi. 2012; 32:165–167. [PubMed: 22834107]

Torrecilla M, Marker CL, Cintora SC, Stoffel M, Williams JT, Wickman K. G-protein-gated potassium channels containing Kir3.2 and Kir3.3 subunits mediate the acute inhibitory effects of opioids on locus ceruleus neurons. J Neurosci. 2002; 22:4328–4334. [PubMed: 12040038]

Weiss F, Porrino LJ. Behavioral neurobiology of alcohol addiction: recent advances and challenges. J Neurosci. 2002; 22:3332–3337. [PubMed: 11978808]

Weitemier AZ, Ryabinin AE. Alcohol-induced memory impairment in trace fear conditioning: a hippocampus-specific effect. Hippocampus. 2003; 13:305–315. [PubMed: 12722971]

White AM, Best PJ. Effects of ethanol on hippocampal place-cell and interneuron activity. Brain Res. 2000; 876:154–165. [PubMed: 10973604]

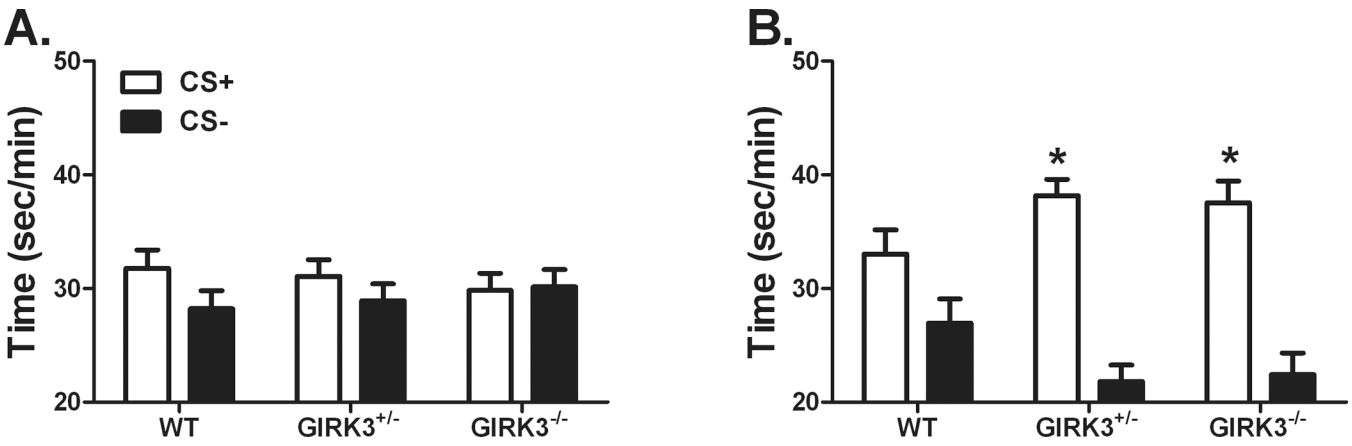

Figure 1. Ethanol Conditioned Place Preference

Average time (sec/min) spent by GIRK3 WT, GIRK3$^{+/-}$, and GIRK3$^{-/-}$ mice on the CS+ and CS− sides of the chamber (A) during the pre-test and (B) following training. (A) There was no evidence of a floor bias or genotype differences during the pre-test. (B) On test day, GIRK3$^{+/-}$ and GIRK3$^{-/-}$ mice spent significantly more time on the CS+ side compared to WT littermates. WT n= 15, GIRK3$^{+/-}$ n= 14, GIRK3$^{-/-}$ n= 21; * $p<0.05$ vs. WT.

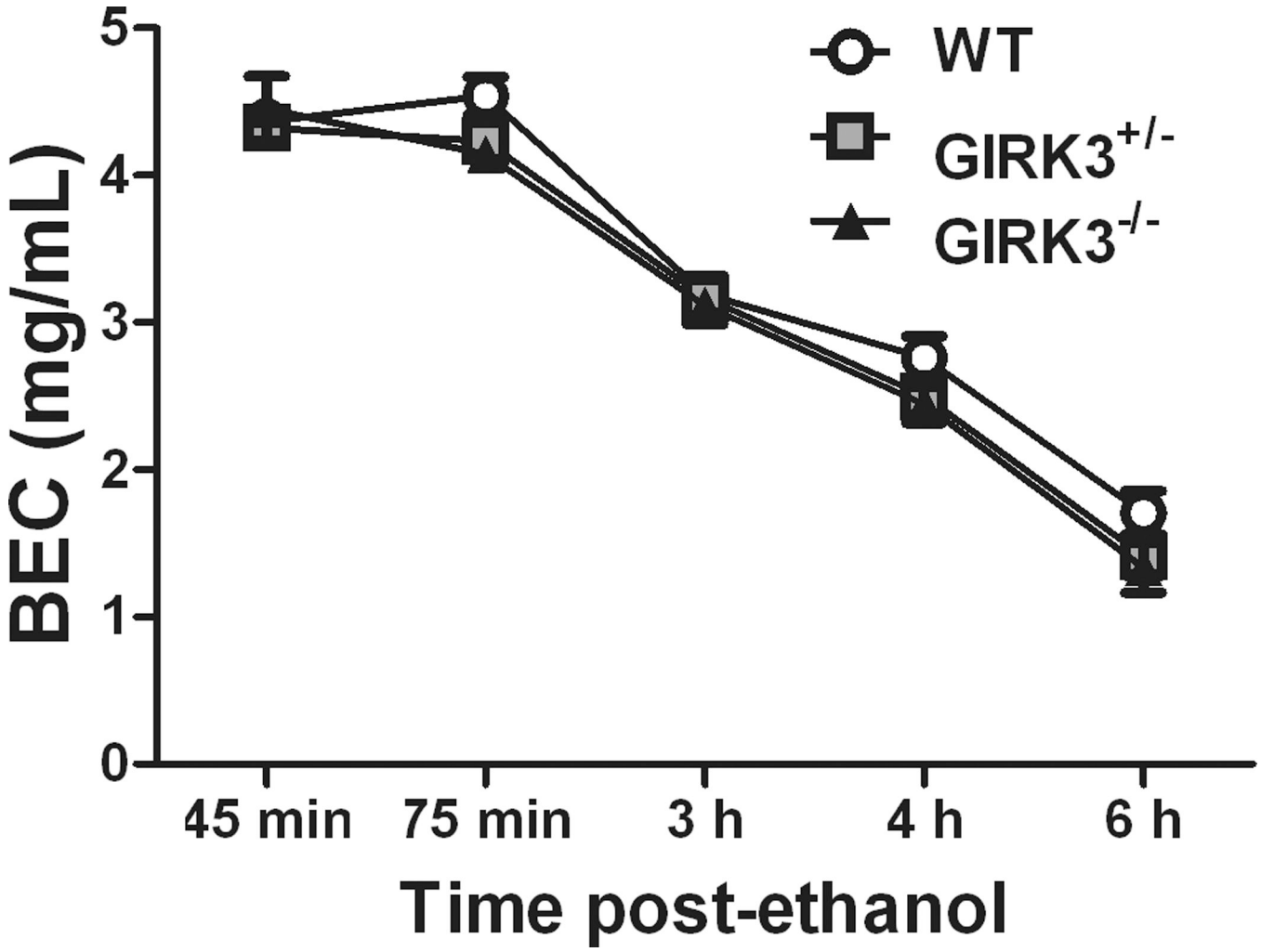

Figure 2. Blood Ethanol Concentrations

Blood ethanol concentrations (BEC) in WT, GIRK3$^{+/-}$ and GIRK3$^{-/-}$ mice following a 4 g/kg dose of ethanol. No genotype differences were detected ($p>0.1$ for all time points). WT n= 10, GIRK3$^{+/-}$ n= 14, GIRK3$^{-/-}$ n= 7.

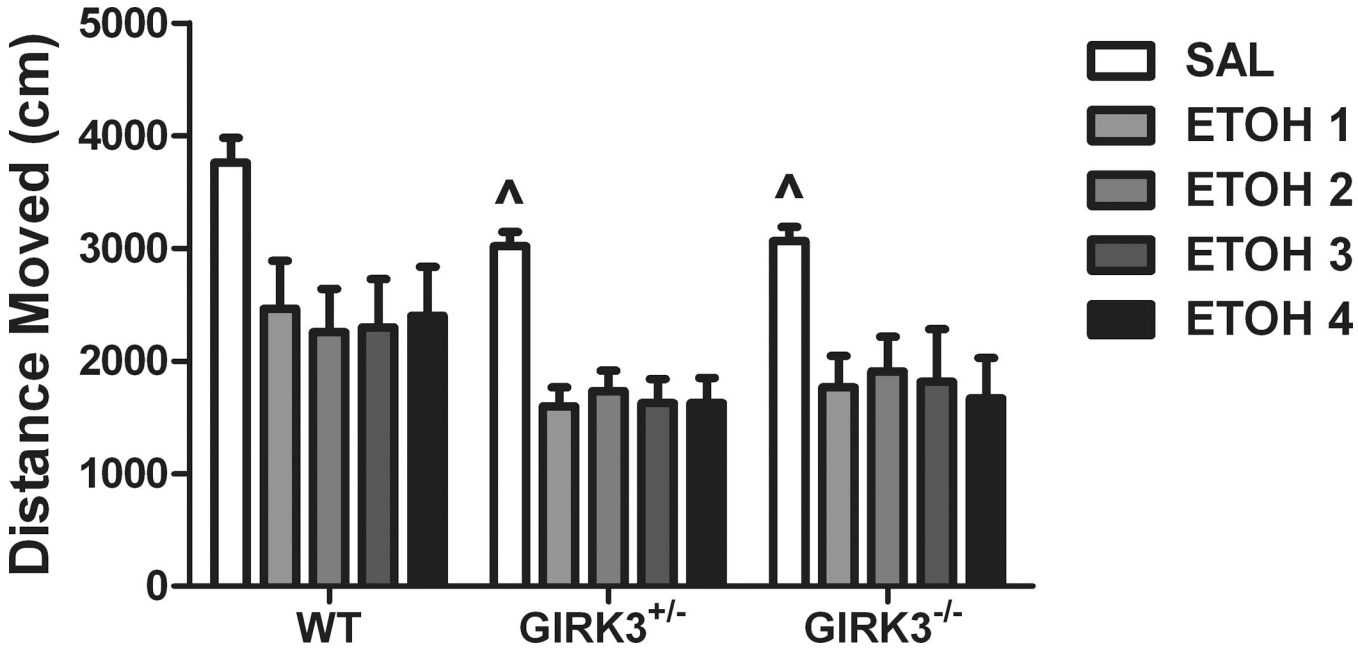

Figure 3. Locomotor Behavior

Average distance traveled (cm) during a 15-min locomotor assay. Animals were injected (i.p.) with saline (SAL) on day 1, followed by four consecutive days of ethanol (ETOH 1 – ETOH 4) injections (2 g/kg). GIRK3$^{+/-}$ and GIRK3$^{-/-}$ mice showed less locomotor behavior than WT mice on the saline day; however, there were no genotype differences on any of the ethanol exposure days and no differences across ethanol days for any genotype. n=8 per genotype; ^ $p<0.05$ vs. WT.

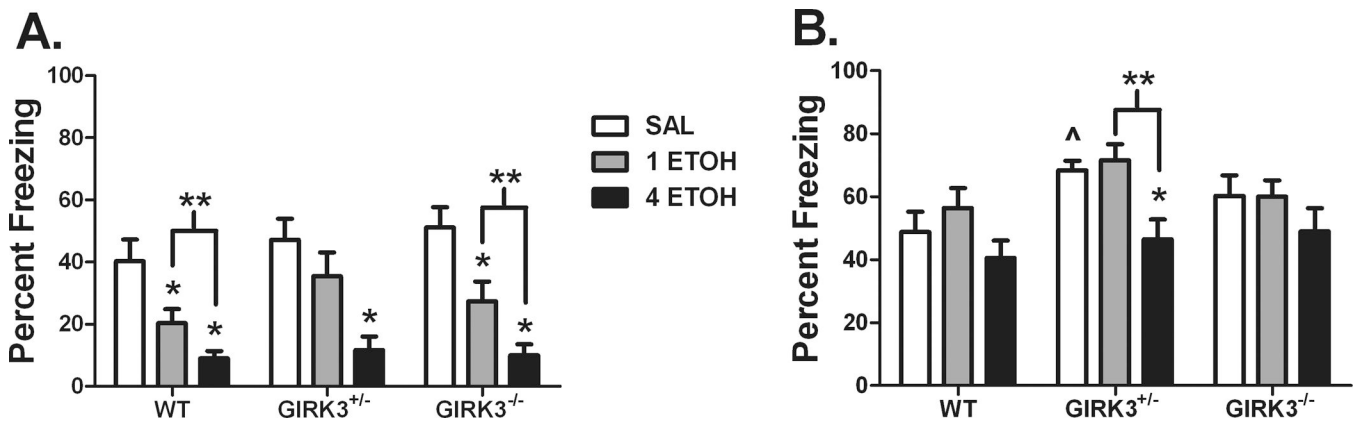

Figure 4. Delay Fear Conditioning

Percent freezing to the (A) context and (B) CS for mice trained with delay fear conditioning immediately after injection with saline (SAL), their first ethanol exposure (1 ETOH), or their fourth ethanol exposure (4 ETOH). (A) Ethanol (2 g/kg) exposure during training impaired contextual learning in all genotypes. This effect was more robust in mice trained after their fourth exposure than mice trained after their first exposure. (B) During the CS test, saline-treated GIRK3$^{+/-}$ mice froze more than WT mice in response to the CS, but no other genotype effects were observed. Similarly, the fourth exposure group of GIRK3$^{+/-}$ mice froze less than the saline-treated and first exposure groups, but no other treatment effects were observed. SAL: WT n=9, GIRK3$^{+/-}$ n=10, GIRK3$^{-/-}$ n=10; 1 ETOH: WT n=9, GIRK3$^{+/-}$ n=9, GIRK3$^{-/-}$ n=10; 4 ETOH: WT n=10, GIRK3$^{+/-}$ n=11, GIRK3$^{-/-}$ n=9; ^ $p<0.05$ vs. WT; * $p<0.05$ vs. SAL; **$p<0.05$ vs 1 ETOH.

Table 1

Comparison of GIRK2$^{-/-}$ and GIRK3$^{-/-}$

Comparison of reported differences in GIRK-mediated signaling and ethanol-related behaviors for mice lacking GIRK2 or GIRK3.

	GIRK2$^{-/-}$	GIRK3$^{-/-}$	References
GIRK-mediated currents	< WT	= WT	Aurora et al., 2010; Hearing et al., 2013
Ethanol CPP	< WT	> WT	Blednov et al., 2001; Figure 1
BEC	= WT	= WT	Blednov et al., 2001; Figure 2
Ethanol withdrawal induced convulsions	<WT	<WT	Blednov et al., 2001; Kozell et al., 2009