Glycine receptors containing α2 or α3 subunits regulate specific ethanol-mediated behaviors

Yuri A. Blednov, Jillian M. Benavidez, Mendy Black, Courtney R. Leiter, Elizabeth Osterndorff-Kahanek, R. Adron Harris
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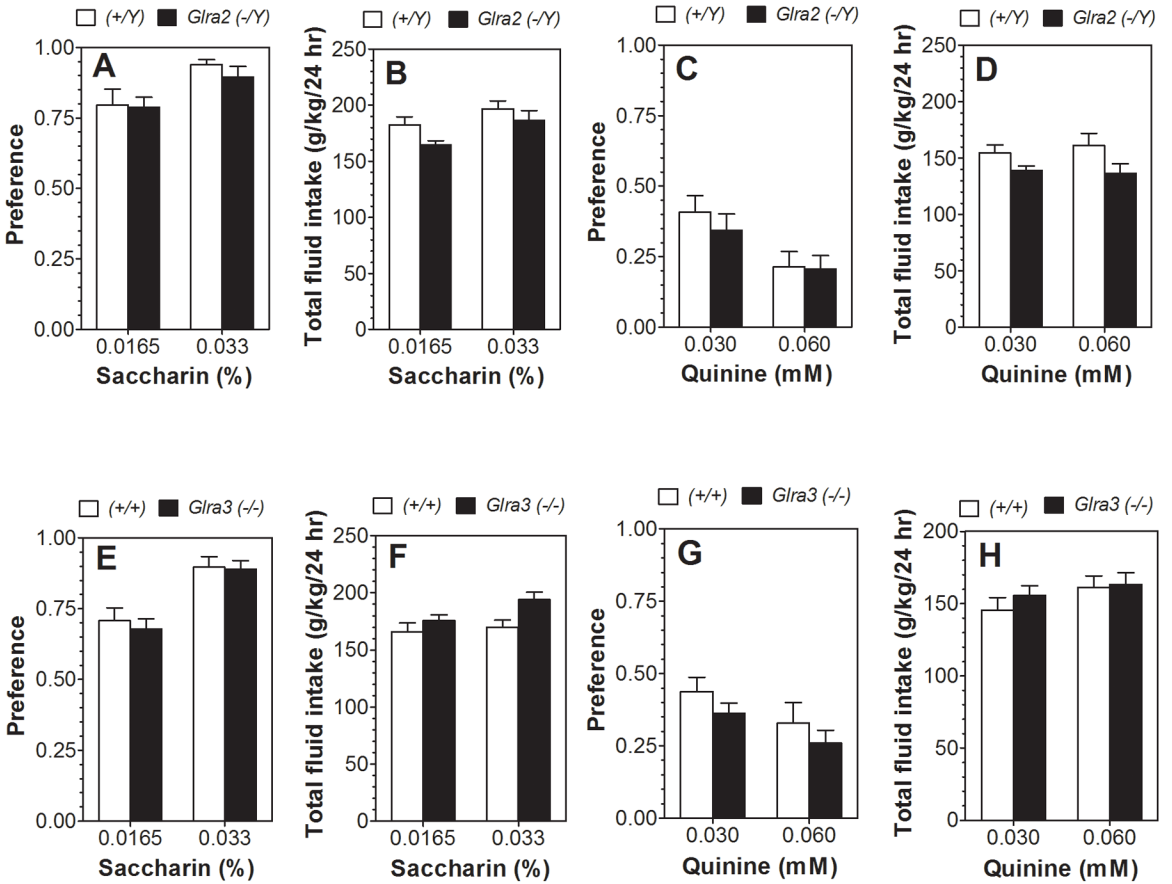
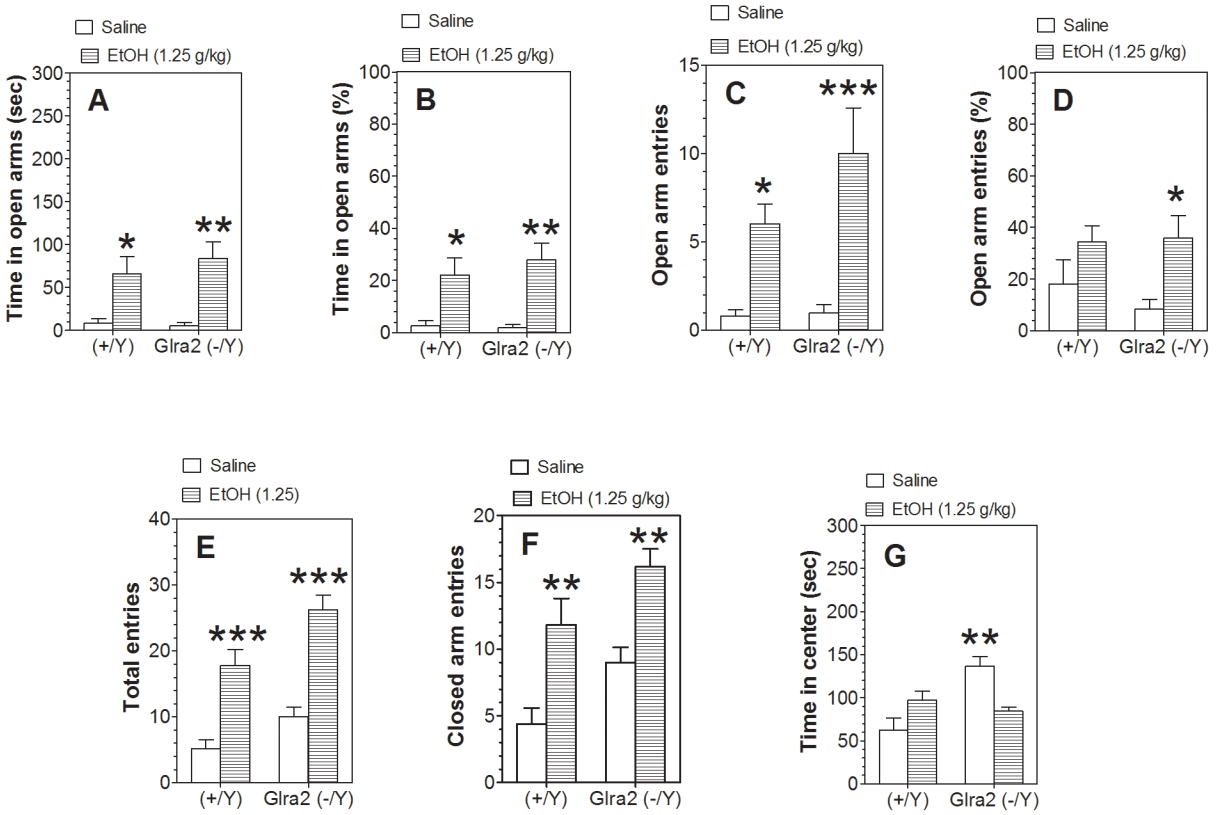
Supplemental Fig. 1



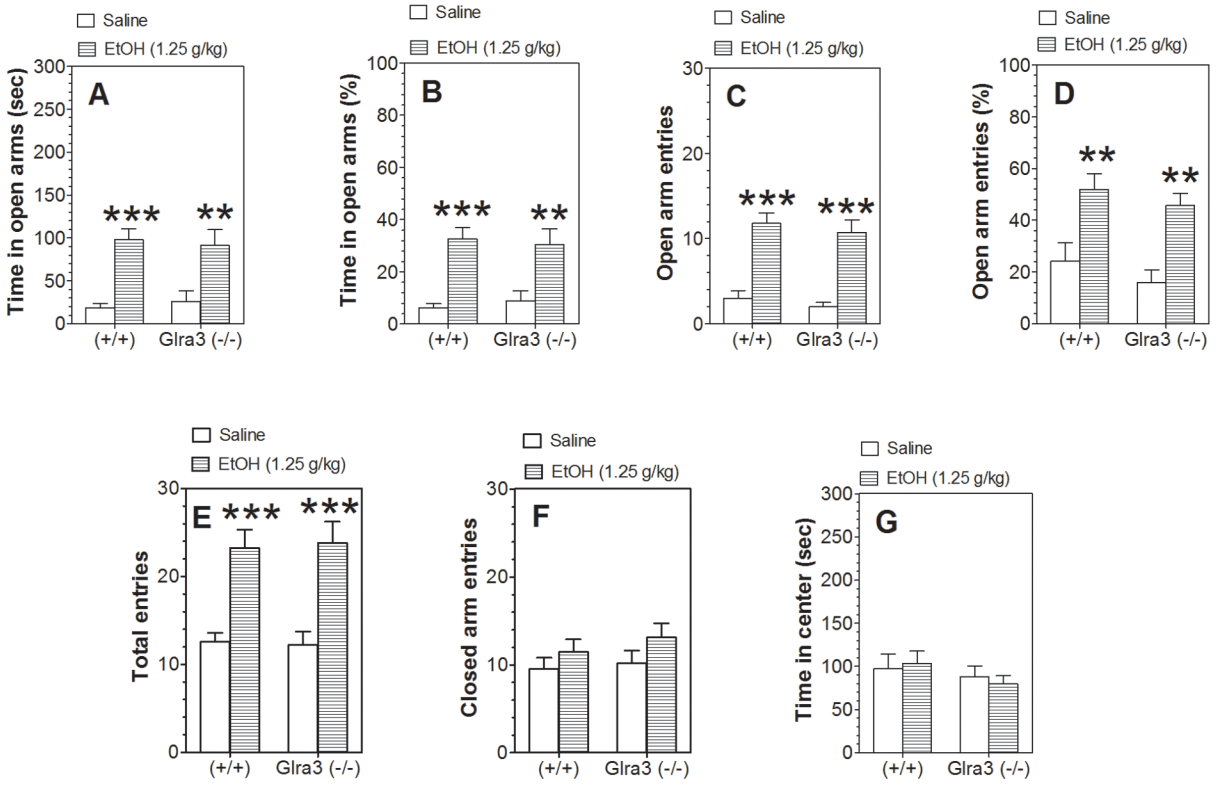
Supplemental Figure 1. Voluntary saccharin and quinine consumption did not differ in *Glra2 (-/Y)* or *Glra3 (-/-)* and wild type mice in the two-bottle choice test. A-D. Male *Glra2 (-/Y)* and wild type mice (n=10 per genotype). E-H. Male *Glra3 (-/-)* and wild type mice (n=8-12 per genotype). A,E. Preference for saccharin. B,F. Total fluid intake for saccharin. C,G. Preference for quinine. D,H. Total fluid intake for quinine. Values represent mean ± SEM. Data were analyzed by two-way repeated measures ANOVA followed by Bonferroni *post hoc* test.

Supplemental Fig. 2



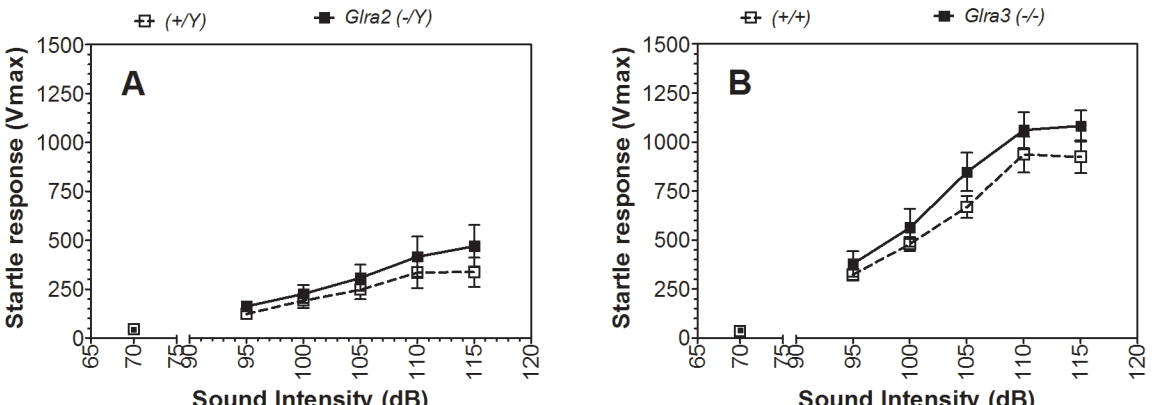
Supplemental Figure 2. Ethanol reduced anxiety to a similar extent in *Glra2 (-/Y)* and wild type mice in the elevated plus-maze test. A. Time in the open arms expressed in seconds. B. Time in the open arms expressed as percent of total time. C. Number of entries into the open arms. D. Number of entries into the open arms expressed as percent of total number of entries. E. Total number of entries. F. Number of entries into the closed arms. G. Time in the center expressed in seconds. Values represent mean ± SEM. Data were analyzed by two-way ANOVA followed by Bonferroni *post hoc* test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. saline group of corresponding genotype (n=5 per group and genotype). EtOH=1.25 g/kg ethanol.

Supplemental Fig. 3



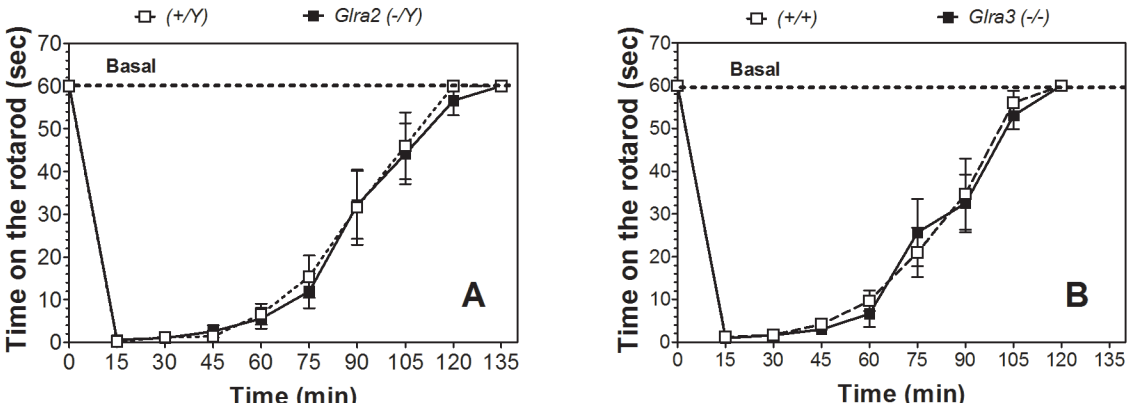
Supplemental Figure 3. Ethanol reduced anxiety to a similar extent in *Glra3 (-/-)* and wild type mice in the elevated plus-maze test. A. Time in the open arms expressed in seconds. B. Time in the open arms expressed as percent of total time. C. Number of entries into the open arms. D. Number of entries into the open arms expressed as percent of total number of entries. E. Total number of entries. F. Number of entries into the closed arms. G. Time in the center expressed in seconds. Values represent mean ± SEM. Data were analyzed by two-way ANOVA followed by Bonferroni *post hoc* test. ***P* < 0.01, ****P* < 0.001 vs. saline group of corresponding genotype (n=10-11 per group and genotype). EtOH=1.25 g/kg ethanol.

Supplemental Fig. 4



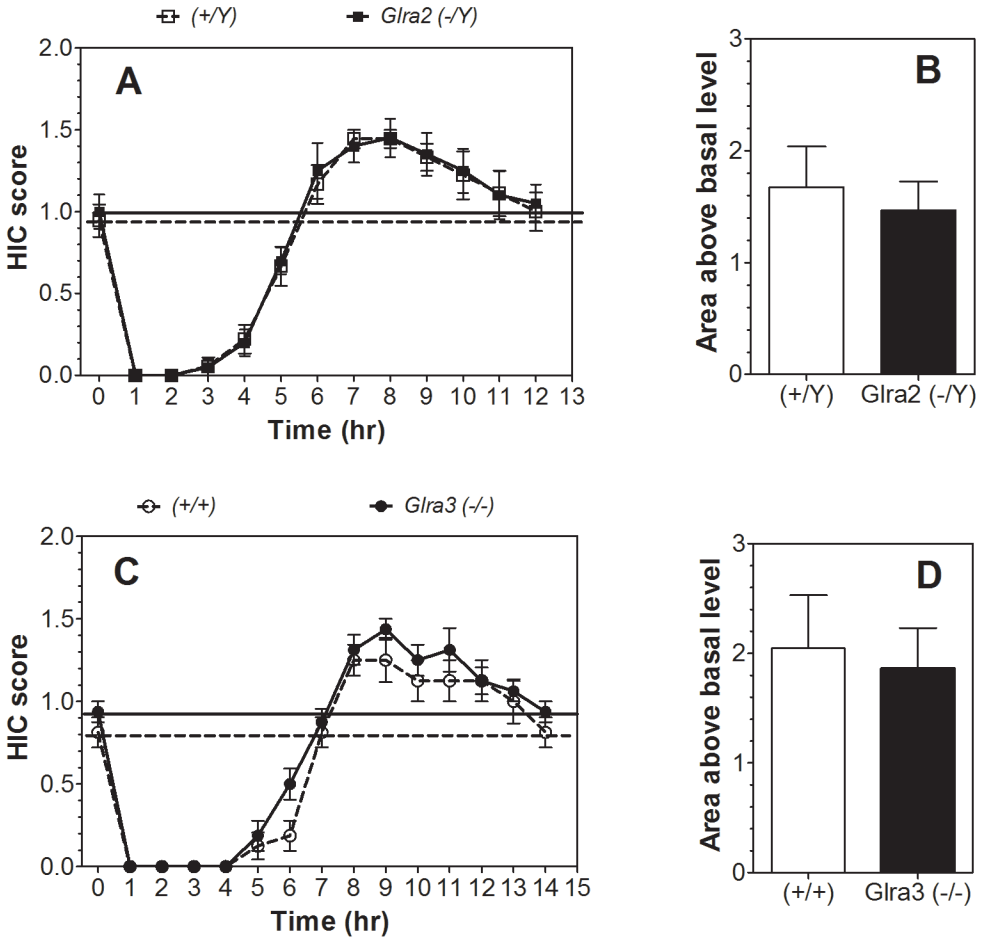
Supplemental Figure 4. Acoustic startle response is not altered in mutant mice. Data represent the maximum startle amplitude (Vmax) as a function of sound intensity in decibels (dB). A. Male *Glra2 (-/Y)* and wild type mice (n=7 per genotype). B. Male *Glra3 (-/-)* and wild type mice (n=9 per genotype). Values represent mean ± SEM. Data were analyzed by two-way repeated measures ANOVA followed by Bonferroni *post hoc* test.

Supplemental Fig. 5



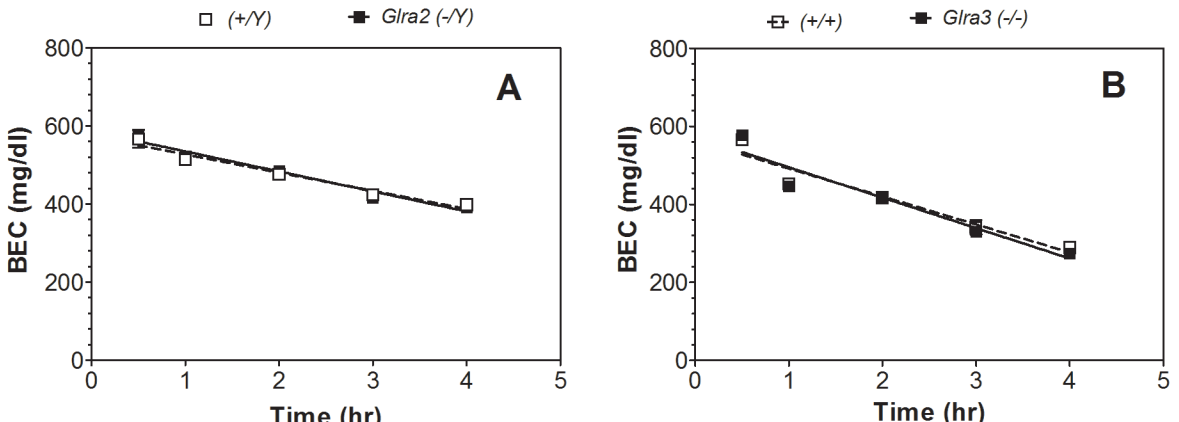
Supplemental Figure 5. Recovery from ethanol-induced motor incoordination in *Glra2 (-/Y)* or *Glra3 (-/-)* mice. Data represent time in seconds (sec) on the rotarod after injection of ethanol (2 g/kg). A. Male *Glra2 (-/Y)* vs. wild type mice (n=5 per genotype). B. Male *Glra3 (-/-)* vs. wild type mice (n=6 per genotype). Values represent mean ± SEM. Data were analyzed by two-way repeated measures ANOVA followed by Bonferroni *post hoc* test.

Supplemental Fig. 6



Supplemental Figure 6. Severity of acute ethanol-induced withdrawal did not differ in *Glra2 (-/Y)* or *Glra3 (-/-)* and wild type mice. A. HIC score in *Glra2 (-/Y)* vs. wild type mice. B. Area under the HIC score and above the basal level in *Glra2 (-/Y)* vs. wild type mice. C. HIC score in *Glra3 (-/-)* vs. wild type mice. D. Area under the HIC score and above the basal level in *Glra3 (-/-)* vs. wild type mice. The dotted and solid lines represent the average basal HIC scores for wild type and mutant mice, respectively, before administration of ethanol. No differences between the two genotypes were found (Student's t-test). Values represent mean ± SEM. (n=12-13 for *Glra2 (-/Y)* and corresponding wild type and n=8 for *Glra3 (-/-)* and corresponding wild type mice). HIC=handling induced convulsions.

Supplemental Fig. 7



Supplemental Figure 7. Clearance of ethanol (4 g/kg) in *Glra2 (-/Y)* or *Glra3 (-/-)* mice. A. BEC (mg/dl) in *Glra2 (-/Y)* vs. wild type mice (n=4 per genotype). B. BEC (mg/dl) in *Glra3 (-/-)* vs. wild type mice (n=4 per genotype). Values represent mean ± SEM. Data were analyzed by two-way repeated measures ANOVA followed by Bonferroni *post hoc* test. BEC=blood ethanol concentration.

Supplemental Table 1. Statistical analyses of ethanol intake in the two-bottle choice test in *Glra2 (-/Y)* and *Glra3 (-/-)* mice.

Strain	Factors	Two-bottle choice		
		Amount of ethanol consumed (g/kg/24 hr)	Preference	Total fluid intake (g/kg/24 hr)
Glra2 (-/Y) (n = 9-10)	Genotype	**F(1,17)=10.3; p<0.01**	**F(1,17)=5.8; p<0.05**	F(1,17)=3.3; p>0.05
	Concentration	**F(4,68)=49.6; p<0.001**	**F(4,68)=33.5; p<0.001**	**F(4,68)=22.7; p<0.001**
	Interaction	**F(4,68)=3.4; p<0.05**	F(4,68)=2.3; p>0.05	F(4,68)=1.5; p>0.05
Glra3 (-/-) (n = 8-12)	Genotype	F(1,18)=0.7; p>0.05	F(1,18)=0.1; p>0.05	F(1,18)=0,4; p>0.05
	Concentration	**F(4,72)=48.3; p<0.001**	**F(4,72)=20.7; p<0.001**	**F(4,72)=16.4; p<0.001**
	Interaction	F(4,72)=0.7; p>0.05	F(4,72)=0.8; p>0.05	F(4,72)=1.1; p>0.05

Statistically significant results are shown in bold font (two-way ANOVA).

Supplemental Table 2. Statistical analyses of saccharin and quinine intake in the two-bottle choice test in *Glra2 (-/Y)* and *Glra3 (-/-)* mice.

Tastant	Strain	Factors		
			Preference	Total fluid intake (g/kg/24 hr)
Saccharin	*Glra2 (-/Y)* (n = 10)	Genotype	F(1,18)=0.3; p>0.05	F(1,18)=2.6; p>0.05
		Concentration	**F(1,18)=13.6; p<0.01**	**F(1,18)=17.3; p<0.001**
		Interaction	F(1,18)=2.3; p>0.05	F(1,18)=0.8; p>0.05
	Glra3 (-/-) (n = 8-12)	Genotype	F(1,18)=0.1; p>0.05	**F(1,18)=4.7; p<0.05**
		Concentration	**F(1,18)=124.6; p<0.001**	**F(1,18)=4.9; p<0.05**
		Interaction	F(1,18)=0.4; p>0.05	F(1,18)=2.0; p>0.05
Quinine	*Glra2 (-/Y)* (n = 10)	Genotype	F(1,18)=0.3; p>0.05	F(1,18)=3.9; p>0.05
		Concentration	**F(1,18)=17.6; p<0.001**	F(1,18)=0.2; p>0.05
		Interaction	F(1,18)=0.5; p>0.05	F(1,18)=0.7; p>0.05
	Glra3 (-/-) (n = 8-12)	Genotype	F(1,18)=1.3; p>0.05	F(1,18)=0.3; p>0.05
		Concentration	**F(1,18)=10.9; p<0.01**	**F(1,18)=5.6; p<0.05**
		Interaction	F(1,18)=0.1; p>0.05	F(1,18)=0.7; p>0.05

Statistically significant results are shown in bold font (two-way ANOVA).

Supplemental Table 3. Statistical analyses of ethanol intake in the two-bottle choice test with intermittent access to ethanol in *Glra2 (-/Y)* and *Glra3 (-/-)* mice.

			Two-bottle choice (intermittent access)		
Strain	Concentration of ethanol	Factors	Amount of ethanol consumed (g/kg/24 hr)	Preference	Total fluid intake (g/kg/24 hr)
Glra2 (-/Y) (n = 9-10)	15%	Genotype	F(1,28)=0.8 p>0.05	F(1,28)=063 p>0.05	F(1,28)=013 p>0.05
		Time	**F(5,140)=2.8 p<0.05**	**F(5,140)=2.4 p<0.05**	F(5,140)=1.5 p>0.05
		Interaction	F(5,140)=0.7 p>0.05	F(5,140)=1.1 p>0.05	F(5,140)=1.6 p>0.05
	20%	Genotype	F(1,28)=0.9 p>0.05	F(1,28)=063 p>0.05	F(1,28)=0.1 p>0.05
		Time	**F(5,140)=2.8 p<0.05**	**F(5,140)=2.6 p<0.05**	**F(5,140)=1.7 p<0.05**
		Interaction	F(5,140)=0.1 p>0.05	F(5,140)=0.2 p>0.05	F(5,140)=0.7 p>0.05
Glra3 (-/-) (n = 8-12)	15%	Genotype	**F(1,27)=4.3 p<0.05**	**F(1,27)=7.7 p<0.05**	**F(1,27)=8.1 p<0.01**
		Time	**F(5,135)=13.9 p<0.001**	**F(5,135)=13.7 p<0.001**	F(5,135)=1.3 p>0.05
		Interaction	**F(5,135)=4.2 p<0.01**	**F(5,135)=4.6 p<0.001**	F(5,135)=1.3 p>0.05
	20%	Genotype	F(1,27)=1.3 p>0.05	**F(1,27)=4.2 p<0.05**	**F(1,27)=9.8 p<0.01**
		Time	**F(5,135)=3.1 p<0.05**	**F(5,135)=2.3 p<0.05**	**F(5,135)=4.4 p<0.001**
		Interaction	F(5,135)=9.2 p>0.05	F(5,135)=1.7 p>0.05	F(5,135)=1.0 p>0.05

Statistically significant results are shown in bold font (two-way ANOVA).

Supplemental Table 4. Statistical analyses of ethanol-induced conditioned taste aversion in *Glra2 (-/Y)* mice.

			Wild type		*Glra2 (-/Y)*	
		Factors	Saline (n = 20)	Ethanol 2.5 g/kg (n = 22)	Saline (n = 20)	Ethanol 2.5 g/kg (n = 23)
Glra2 (-/Y)	Saline	Genotype	F(1,38)=1.7 p>0.05			
		Trial	**F(4,152)=7.1 p<0.001**			
		Interaction	F(4,152)=0.5 p>0.05			
	Ethanol 2.5 g/kg	Treatment			**F(1,41)=41.8 p<0.001**	
		Trial			**F(4,164)=17.5 p<0.001**	
		Interaction			**F(4,164)=7.4 p<0.001**	
Wild type	Saline	Treatment		**F(1,40)=17.8 p<0.001**		
		Trial		**F(4,160)=28.1 p<0.001**		
		Interaction		**F(4,160)=12.1 p<0.001**		
	Ethanol 2.5 g/kg	Genotype				F(1,43)=1.0 p>0.05
		Trial				**F(4,172)=58.2 p<0.001**
		Interaction				F(4,172)=1.1 p>0.05

Statistically significant results are shown in bold font (two-way ANOVA).

Supplemental Table 5. Statistical analyses of ethanol-induced conditioned taste aversion in *Glra3 (-/-)* mice.

			Wild type		*Glra3 (-/-)*	
		Factors	Saline (n = 15)	Ethanol 2.5 g/kg (n = 14)	Saline (n = 15)	Ethanol 2.5 g/kg (n = 15)
Glra3 (-/-)	Saline	Genotype	F(1,28)=0.1 p>0.05			
		Trial	F(4,112)=0.9 p>0.05			
		Interaction	F(4,112)=2.1 p>0.05			
	Ethanol 2.5 g/kg	Treatment			**F(1,28)=19.7 p<0.001**	
		Trial			**F(4,112)=10.4 p<0.001**	
		Interaction			**F(4,112)=11.7 p<0.001**	
Wild type	Saline	Treatment		**F(1,27)=4.3 p<0.05**		
		Trial		**F(4,108)=8.2 p<0.001**		
		Interaction		**F(4,108)=3.0 p<0.05**		
	Ethanol 2.5 g/kg	Genotype				**F(1,27)=9.4 p<0.01**
		Trial				**F(4,108)=20.2 p<0.001**
		Interaction				F(4,108)=1.4 p>0.05

Statistically significant results are shown in bold font (two-way ANOVA).

Supplemental Table 6. Statistical analyses of lithium chloride-induced conditioned taste aversion in *Glra2 (-/Y)* mice.

			Wild type		*Glra2 (-/Y)*	
		Factors	Saline (n = 9)	LiCl (6 mEq/kg) (n = 9)	Saline (n = 9)	LiCl (6 mEq/kg) (n = 9)
Glra2 (-/Y)	Saline	Genotype	F(1,16)=2.8 p>0.05			
		Trial	**F(4,64)=7.4 p<0.001**			
		Interaction	F(4,64)=1.6 p>0.05			
	LiCl 6 mEq/kg	Treatment			**F(1,16)=28.8 p<0.001**	
		Trial			**F(4,64)=12.6 p<0.001**	
		Interaction			**F(4,64)=10.3 p<0.001**	
Wild type	Saline	Treatment		**F(1,16)=24.5 p<0.001**		
		Trial		**F(4,64)=27.7 p<0.001**		
		Interaction		**F(4,64)=20.9 p<0.001**		
	LiCl 6 mEq/kg	Genotype				F(1,16)=0.1 p>0.05
		Trial				**F(4,64)=45.3 p<0.001**
		Interaction				F(4,64)=1.5 p>0.05

Statistically significant results are shown in bold font (two-way ANOVA).

Supplemental Table 7. Statistical analyses of lithium chloride (6 mEq/kg)-induced conditioned taste aversion in *Glra3 (-/-)* mice.

			Wild type		*Glra3 (-/-)*	
		Factors	Saline (n = 7)	LiCl (6 mEq/kg) (n = 10)	Saline (n = 5)	LiCl (6 mEq/kg) (n = 7)
Glra3 (-/-)	Saline	Genotype	F(1,26)=0.3 p>0.05			
		Trial	F(4,104)=0.5 p>0.05			
		Interaction	F(4,104)=0.3 p>0.05			
	LiCl 6 mEq/kg	Treatment			**F(1,16)=4.5 p<0.05**	
		Trial			**F(4,64)=9.1 p<0.001**	
		Interaction			**F(4,64)=7.8 p<0.001**	
Wild type	Saline	Treatment		**F(1,25)=4.7 p<0.05**		
		Trial		**F(4,100)=7.4 p<0.001**		
		Interaction		**F(4,100)=6.9 p<0.001**		
	LiCl 6 mEq/kg	Genotype				F(1,15)=0.8 p>0.05
		Trial				**F(4,60)=18.0 p<0.001**
		Interaction				F(4,60)=1.0 p>0.05

Statistically significant results are shown in bold font (two-way ANOVA).

Supplemental Table 8. Statistical analyses of lithium chloride (10 mE/kg)-induced conditioned taste aversion in *Glra3 (-/-)* mice.

			Wild type		*Glra3 (-/-)*	
		Factors	Saline (n = 7)	LiCl (10 mEq/kg) (n = 10)	Saline (n = 5)	LiCl (10 mEq/kg) (n = 7)
Glra3 (-/-)	Saline	Genotype	F(1,26)=0.3 p>0.05			
		Trial	F(4,104)=0.5 p>0.05			
		Interaction	F(4,104)=0.3 p>0.05			
	LiCl 10 mEq/kg	Treatment			**F(1,17)=7.8 p<0.05**	
		Trial			F(4,68)=0.6 p>0.05	
		Interaction			F(4,68)=0.3 p>0.05	
Wild type	Saline	Treatment		**F(1,23)=34.3 p<0.001**		
		Trial		**F(4,92)=8.2 p<0.001**		
		Interaction		**F(4,92)=9.2 p<0.001**		
	LiCl 10 mEq/kg	Genotype				**F(1,14)=10.0 p<0.01**
		Trial				**F(4,56)=5.3 p<0.01**
		Interaction				**F(4,56)=3.6 p<0.05**

Statistically significant results are shown in bold font (two-way ANOVA).