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γ-Aminobutyric acid A receptor subunit mutant mice: new perspectives on alcohol actions

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Abstract

γ-Aminobutyric acid A ($GABA_A$) receptors are believed to mediate a number of alcohol's behavioral actions. Because the subunit composition of $GABA_A$ receptors determines receptor pharmacology, behavioral sensitivity to alcohol (ethanol) may depend on which subunits are present (or absent). A number of knock-out and/or transgenic mouse models have been developed (α1, α2, α5, α6, β2, β3, γ2S, γ2L, δ) and tested for behavioral sensitivity to ethanol. Here we review the current $GABA_A$ receptor subunit knock-out and transgenic literature for ethanol sensitivity, and integrate these results into those obtained using quantitative trait loci (QTL) analysis and gene expression assays. Converging evidence from these three approaches support the notion that different behavioral actions of ethanol are mediated by specific subunits, and suggest that new drugs that target specific $GABA_A$ subunits may selectively alter some behavioral actions of ethanol, without altering others. Current data sets provide strongest evidence for a role of α1-subunits in ethanol-induced loss of righting reflex, and α5-subunits in ethanol-stimulated locomotion. However, three-way validation is hampered by the incomplete behavioral characterization of many of the mutant mice, and additional subunits are likely to be linked to alcohol actions as behavioral testing progresses.

Keywords: $GABA_A$; Knock-out; Mouse; Ethanol; Behavior; QTL

γ-Aminobutyric acid A ($GABA_A$) receptors represent the major inhibitory class of neurotransmitter receptors in the mammalian brain. $GABA_A$ receptors have a pentameric structure, with five subunits arranged to form an ion pore. When bound by agonist, $GABA_A$ receptors conduct chloride ions across the cell membrane. The net result is an influx of negatively charged ions, and hyperpolarization of the neuron.

$GABA_A$ receptors mediate a number of drug effects, including sedation/hypnosis, anxiolysis, and anesthesia. Ethanol (alcohol), as well as barbiturates, benzodiazepines, neuroactive steroids, and volatile and intravenous anesthetics enhance $GABA_A$ receptor function in the presence of agonist, resulting in the induction of the above behavioral drug effects. Most native $GABA_A$ receptors are thought to consist of two α, two β, and a γ subunit. However, seven classes of $GABA_A$ receptor subunits have been described to date (α1–6, β1–3, γ1–3, δ, ϵ, θ1–3, π, ρ1–3), allowing for extensive heterogeneity in receptor subunit composition across neuronal cell types and brain regions. Moreover, the subunit composition of $GABA_A$ receptors has profound effects on receptor pharmacology [1] suggesting the possibility that behavioral sensitivity to ethanol (and other drugs that alter $GABA_A$ receptor function) may depend on which subunits are present (or absent) in specific brain circuits. Evidence supporting such pharmacological and behavioral specificity comes from knock-in mouse studies. These mice possess a point mutation that alters one aspect of protein function, leaving all other aspects of protein function in tact. Recent studies with knock-in mice show that whereas α1-receptor subunits

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mediate the sedative, amnestic, and anticonvulsant actions of diazepam [2], α2-receptor subunits mediate the anxiolytic actions of the benzodiazepine [3].

In this paper, we review the actions of alcohol on $GABA_A$ receptor knock-out (null mutant) mice. A number of genetic knock-out models have been developed and tested, including the α1, α2, α5, and α6, the β2 and β3, the γ2L, and the δ knock-out mice. Transgenic mice that overexpress either the γ2S or γ2L splice variants of the $GABA_A$ receptor γ-subunit were also developed and tested. These studies have revealed a complicated picture of subunit specific behavioral pharmacology. Together, they support the notion that specific $GABA_A$ receptor subunits may indeed mediate specific ethanol- or drug-related behavioral phenotypes. Such results have important implications for studies aimed at developing drugs with fewer side effects; compounds could be engineered to target $GABA_A$ receptors possessing specific subunits, thereby altering very specific ethanol-related behaviors. Comparison of the null mutant data with behavioral and gene expression data from the recombinant inbred strains will provide an interesting and powerful new way of examining the link between genes, brain, and behavioral sensitivity to ethanol.

1. α-Subunit knock-out mice

1.1. α1-Subunit

The α1-receptor subunit of the $GABA_A$ receptor is the most widely distributed α-subunit, having expression in the olfactory bulb, cortex, thalamus, hypothalamus, hippocampus, amygdala, midbrain, and cerebellum [4].

Three different groups have developed mutant mice possessing genetically altered α1-receptor subunits. Rudolph et al. [2] were the first to report knock-in mice possessing a point mutation (H101R) that eliminated diazepam-potentiation of GABA currents in vitro, and a second group duplicated the feat with the same point mutation [5]. Both knock-in mouse models were less sensitive to the sedative actions of diazepam. Moreover, Rudolph et al. [2] provided additional evidence that sensitivity to the amnestic and anticonvulsant actions of diazepam were selectively altered in the knock-ins. Although the groups were in disagreement on whether the point mutation attenuated sensitivity to the motor impairing effects of diazepam, the combined data sets provide the first evidence that certain $GABA_A$ receptor subunits may indeed mediate very specific drug-related behaviors. For a more in depth review of the α1-subunit knock-in mouse literature, the reader is referred to Rudolph and Möhler [6].

α1-Subunit knock-out mice were developed by two different groups [7,8]. Whereas the knock-outs developed by Sur et al. [7] displayed only strong body tremor, those developed by Kralic et al. [9] exhibited spontaneous seizures. Nevertheless, both mutant mouse models breed and developed normally, although the number of mutant births observed by both groups was somewhat lower than that predicted by Mendelian genetics.

Binding studies suggest that both populations of α1-subunit knock-out mice lost about 50% of all $GABA_A$ receptors [7], consistent with this subunit's wide distribution throughout the brain. Interestingly, genetic deletion of the α1-subunit also resulted in a relative up-regulation of α2-(37%) and α3-(39%) receptor subunits [7,9] and down-regulation of β2/3-(65%) and γ2-(47%) receptor subunits [9]. There was also a selective down-regulation of α6-receptor subunits in the cerebellum [7]. These alterations may represent some of the compensatory changes that can occur following genetic deletion of specific genes and must be considered when interpreting any resultant behavioral changes in knock-out mice.

In addition to the changes in $GABA_A$ receptor subunit expression, a number of other notable phenotypic changes were observed in α1-null mutant mice. Both α1-subunit knock-out mouse populations had lower body weights that normalized by 3 months of age and displayed handling-induced tremors [7,9]. Moreover, in vitro work demonstrated that the changes in cerebellar inhibitory synaptic currents that are associated with normal development were absent in the null mutants [8], and that hippocampal miniature inhibitory postsynaptic currents (IPSCs) were less frequent, smaller in amplitude, and longer in duration in α1-subunit knock-out mice [10]. Longer IPSC durations were also observed in neurons from the supraoptic nucleus of the hypothalamus [11] and layers II–III of the visual cortex [12], and at least in the visual cortex (layer II–II), these changes were associated with a decreased density of mushroom-shaped dendridic spines [13]. However, despite these observations, the genotypes displayed similar motor abilities and levels of spontaneous locomotion [7].

Table 1 summarizes the results of studies examining the effects of α1-subunit gene deletion on ethanol behavioral sensitivity. Two different studies examined ethanol behavioral sensitivity in α1-subunit knock-out mice, each with a different independently developed population of mutant mice. Both studies indicated that genetic deletion of the α1-subunit gene increased the locomotor stimulant effects of ethanol [14,15]. Blednov et al. [14] showed that the mutation decreased ethanol preference drinking and enhanced the aversive effects of ethanol, without altering ethanol conditioned place preference (ethanol's reinforcing properties) or chronic ethanol withdrawal, using female mutants developed by Sur et al. Deletion of the α1-subunit gene also reduced acute ethanol withdrawal severity in male mice (Blednov et al., unpublished). Using their mice, Kralic et al. [15] showed that α1-subunit knock-out and wild-type mice also did not differ in sensitivity to the anxiolytic, motor incoordinating, or anticonvulsant actions of ethanol, nor did the null mutation alter acute tolerance to ethanol's motor incoordinating effects. Whereas the male mutants tested by Blednov et al. [16]

Table 1
Change in behavioral sensitivity to ethanol in GABA-A subunit knock-out and transgenic mice: a summary of the data

Subunit	Drinking	CTA	CPP	Stimulation	LORR	Hypotherm	Ataxia	Anxiolysis	Anticonv	Acute Tolerance	Chronic Tolerance	Acute Withdrawal	Chronic Withdrawal
α1	↓	↑	=	↑	↓, =		=	=	=	=		↓	=
α2	=				↓			=				=	
α5	↓			↑	=			=		=		↓	
α6					=					=	=		=
β2	=	=	=	=	↓							↑	=
β3					=								
γ2S										↓ (Tg)			
γ2L				=	= (Tg), =		=		↓ (Tg), =		= (Tg)	=	
δ	↓				=	=		=	↓	=	=		↓

Results are from knock-out mice unless otherwise indicated. Results from transgenic (over-expressing) mice are designated by (Tg). *Abbreviations/definitions:* Drinking, preference and/or consumption; CTA, conditioned taste aversion, aversion; CPP, conditioned place preference, reinforcement; stimulation, locomotor stimulation; LORR, loss of righting reflex, hypnotic actions; ataxia, motor incoordination—rotarod; anxiolysis, anxiolytic actions—elevated plus maze; anticonv, anticonvulsant actions; acute tolerance, acute tolerance to motor incoordinating actions—dowel rod; chronic tolerance, chronic tolerance to hypnotic actions—LORR. Details along with references can be found in Appendix A. Up and down arrows indicate increased and decreased sensitivity of mutant mice, respectively. Equal sign indicates no change in sensitivity of mutant mice.

displayed reduced sensitivity to ethanol's hypnotic actions as assessed by the loss of righting reflex assay, the mutants tested by Kralic et al. [15] did not differ. Thus, despite the negative finding in the place conditioning assay, it would appear that the α1-subunit may have some role in modulating ethanol's reinforcing and/or motivational properties. In support of this contention, a recent report suggests that intra-ventral pallidal injections of an α1-selective benzodiazepine antagonist reduce ethanol maintained responding in alcohol-preferring *P* rats [17].

A detailed review on the behavioral sensitivity to all other classes of $GABA_A$ acting drugs is beyond the scope of this review. However, for the interested reader we have included a table that details the actions of other GABAergic compounds on hypnotic (loss of righting reflex) sensitivity in $GABA_A$ receptor-subunit knock-out mice (Table 2). In addition, we have also included a detailed summary of all other behavioral studies that have examined sensitivity to GABAergic drugs in $GABA_A$ receptor-subunit knock-out mice (Appendix A).

1.2. α2-Subunit

The α2-receptor subunit is expressed at low levels in a number of structures throughout the brain, including the cortex, hypothalamus, hippocampus, and amygdala [4]. There are currently no published data on the role of this subunit in the mediation of ethanol sensitivity, likely because compounds that target this subunit do not currently exist. However, it was recently reported that knock-in mice possessing a point mutation (H101R) that selectively blocks diazepam-induced potentiation of GABA currents in vitro eliminates the anxiolytic actions of diazepam in both the light–dark box and elevated plus maze behavioral assays [3], and reduces hypnotic sensitivity to the combined use of diazepam and ethanol [18]. Interestingly, knock-in mice with the same point mutation were also developed by a second group [19,20], and these mice exhibited enhanced sensitivity to the locomotor sedative actions of diazepam. As interpretation of light–dark box and elevated plus maze data depend upon the mobility of the animal, it may be that the reduced anxiolytic actions of diazepam can be explained by the altered locomotor sedative actions of the drug. Nevertheless, we hypothesized that the α2-receptor subunit might also mediate sensitivity to ethanol's anxiolytic and hypnotic actions, in as much as the mechanism of ethanol action is similar to that of diazepam.

α2-Subunit knock-out mice were recently generated by Paul Whiting, Elisabeth Garrett, and Thomas Rosahl at Merck Sharp and Dohme (Harlow, UK). Although early on there was about a 30% decrease in the number of mutants surviving to weaning, α2-subunit knock-outs now appear to breed and develop normally. It is currently not known whether genetic deletion of this subunit altered the expression of other $GABA_A$ receptor subunits.

Table 2
Change in hypnotic sensitivity to various GABAergic compounds in GABA-A subunit knock-out mice: a summary of the data

Subunit	GABA agonist	Barbiturate	Benzodiazepine	Neuroctive steroid	Anesthetic
α1	↓THIP	↓pb; = pb; ↑diaz	↑diaz; ↓flur; ↓mid; ↓zolp	= preg; = alph	↓etom, = etom; = prop
α6		= pb			= enflur; = halo
β2	↓THIP	= pb	↓flur; ↓zolp	= alph	↓etom
β3		= pb	↓mid		↓etom; = enflur; = halo
γ2L		= pb	↑mid; ↑zolp		= etom
δ	↓THIP	= pb	= mid	↓preg; ↓alph	= etom; = enflur; = halo

Hypnotic sensitivity was assessed by loss of righting reflex. *Abbreviations/definitions:* THIP, 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol; pb, pentobarbital;. diaz, diazepam; flur, flurazepam; mid, midazolam; zolp, zolpidem; preg, pregnanolone; alph, alphaxalone; etom, etomidate; prop, propofol; enflur, enflurane; halo, halothane. Details along with references can be found in Appendix A. Up and down arrows indicate increased and decreased sensivitity of mutant mice, respectively. Equal sign indicates no change in senstivity of mutatnt mice.

α2-Subunit knock-out mice are now maintained on a mixed C57BL6J/129SvEv genetic background, and a colony has been established at the University of Texas at Austin for characterization of ethanol sensitivity. No published data exist on the basal behaviors in these mice. However, when tested for locomotor response to novelty, we found that non-habituated α2 knock-out mice were less active compared to their wild-type counterparts (Fig. 1A).

A number of behavioral assays were employed to determine whether behavioral sensitivity to ethanol had been altered in the α2 knock-out mice. We assessed sensitivity to ethanol's hypnotic actions using the loss of righting reflex test, acute ethanol withdrawal by handling-induced convulsion (HIC), ethanol's anxiolytic effects using the elevated plus maze, and ethanol preference drinking using a two-bottle choice drinking procedure. We hypothesized that null mutation of the α2-receptor subunit gene would result in attenuation of ethanol's anxiolytic properties. Although ethanol treatment increased the percentage of time and entries into the open arms of the knock-outs by about 33% (versus nearly 100% in the wild-types), the genotypes did not statistically differ in sensitivity to this action of ethanol (Fig. 2D and E). Furthermore, that α2-subunit knock-out mice tended to display a lower basal level of locomotion (Figs. 2F and 1A) renders interpretation of the present data even more difficult as assessment of ethanol's anxiolytic actions using the elevated plus maze depends upon this parameter. Nevertheless, the knock-outs did display shorter durations of ethanol-induced loss of righting reflex (Fig. 2A), suggesting a role for the α2-subunit in the mediation of ethanol's hypnotic actions. The genotypes also did not significantly differ in sensitivity to acute ethanol withdrawal, although knock-outs tended to experience less severe withdrawal (Fig. 2B and C). Female mutants preferred and consumed significantly less ethanol than did their wild-type counterparts (Fig. 3A and B). However, although not reaching statistical significance, the null mutants also trended toward lower preference for the bitter tasting quinine (data not shown); no difference in taste sensitivity was observed following presentation of the sweet tasting saccharine (data not shown). Thus, some caution must be exercised when interpreting the

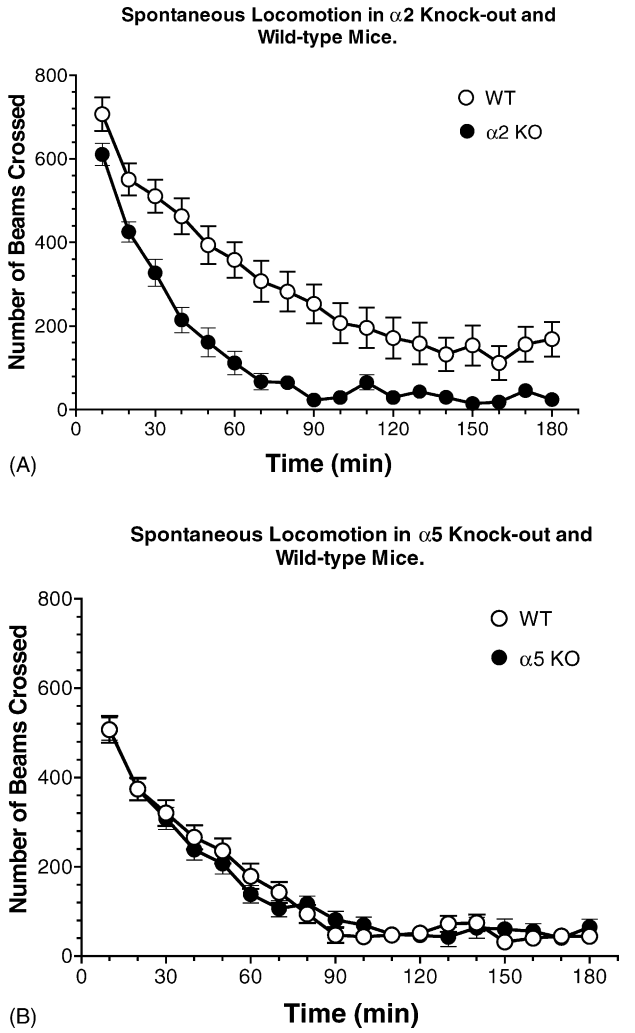

Fig. 1. Locomotor response to novelty in $GABA_A$ α2- and α5-subunit knock-out and wild-type mice. Locomotor activity was assessed using standard mouse cages equipped with infrared sensors. Detailed methods can be found in Blednov et al. [97]. Both male and female mice were tested. However, gender did not interact with any other factor, and data were collapsed over this factor for subsequent analysis. Values represent mean ± S.E.M. (A) α2-Subunit knock-out mice were less active than their wild-type counterparts (repeated measures ANOVA, interaction of time and genotype, $P < 0.001$, $n = 28$ per genoptye). Simple effects analyses showed that α2-subunit knock-out mice were less active at all time points ($P < 0.05$). (B) α5-Subunit knock-out mice displayed a similar locomotor response to novelty.

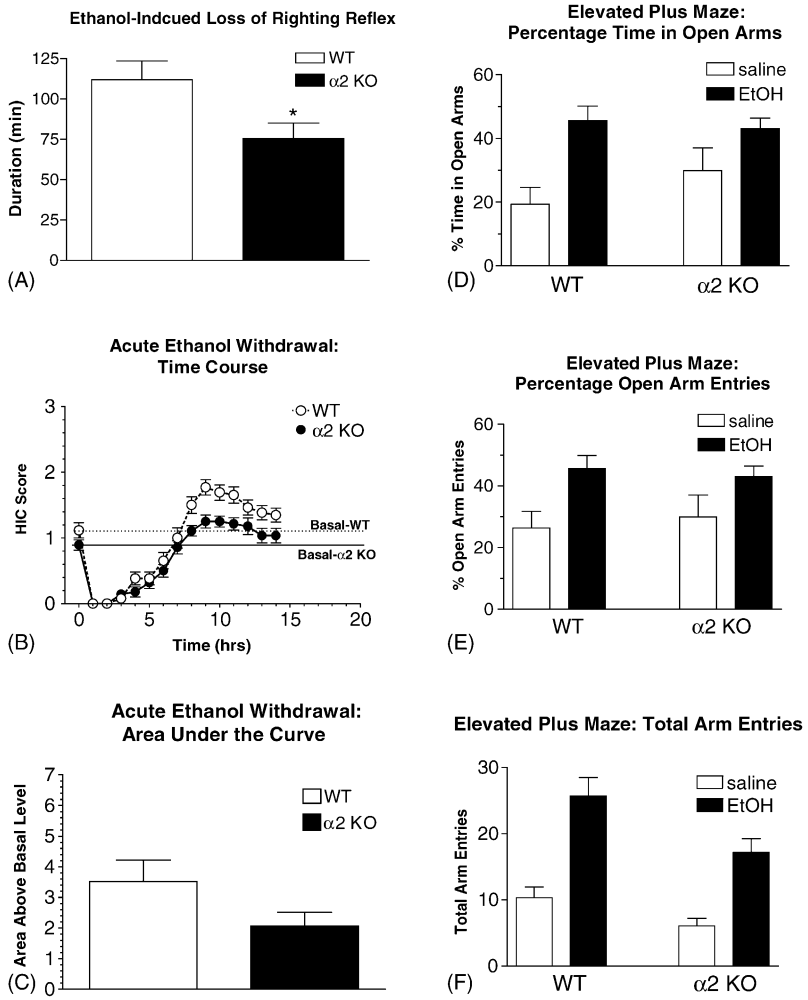

Fig. 2. Sensitivity to the hypnotic and anxiolytic actions of ethanol, and to acute ethanol withdrawal, in $GABA_A$ α2-subunit knock-out and wild-type mice. Although both male and female mice were tested, gender did not interact with any other factor. Thus, data from male and female mice were combined for further analysis. Values represent mean ± S.E.M. (A) Hypnotic sensitivity to ethanol (3.8 g/kg, v/v; i.p.) was assessed by loss of righting reflex. See Blednov et al. [16] for detailed methods. Duration of ethanol-induced loss of righting reflex was reduced in α2-subunit knock-out mice (unpaired *t*-test, t [42] = 2.4, $P < 0.02$, $n = 22$ per genotype). (B and C) Time course and area above baseline of acute ethanol withdrawal in α2-subunit knock-out mice. Mice (n = 26–28 per genotype) received an acute injection of ethanol (4.0 g/kg, v/v; i.p.) and handling-induced convulsions (HICs) were assessed every hour for 14 h. See Blednov et al. [98] for detailed methods. HIC scores differed between the genotypes over the time course of acute withdrawal (repeated measures ANOVA, interaction of time and genotype, F[14, 728] = 2.6, $P = 0.001$); α2-subunit knock-out mice exhibited lower HIC scores over hours 8–11 and 13–14 of the acute ethanol withdrawal time curve (simple effects analysis, $P < 0.03$, panel B). α2-Subunit knock-out mice also tended to experience less severe acute ethanol withdrawal as assessed by positive area under the curve, although this difference did not reach statistical significance (unpaired *t*-test, t [52] = 1.8, $P = 0.08$, panel C). (D–F) Ethanol's anxiolytic actions in α2-subunit knock-out mice. Mice (n = 18–19 per genotype and treatment) were injected with 1.5 g/kg ethanol, v/v (i.p.) and the percentage time and entries into open arms, and total arm entries were assessed. Detailed methods can be found in Boehm II et al. [99]. Saline-treated α2-subunit knock-out and wild-type mice did not differ in the percentage of time or entries into open arms. Ethanol-treatment increased the percentage of time (ANOVA, main effect of treatment, F[1, 67] = 7.8, $P < 0.01$, panel D) and entries (ANOVA, main effect of treatment, F[1, 67] = 32.6, $P < 0.001$, panel E) into the open arms, as well as the total number of arm entries (ANOVA, main effect of treatment, F[1, 67] = 38.6, $P < 0.001$, panel F), but the genotypes did not differ in sensitivity to these behavioral actions of ethanol.

drinking data as female α2-subunit knock-out mice may have preferred and consumed less ethanol because of an enhanced aversion to bitter tastants. Male α2-subunit knock-out mice did not differ in preference or consumption of ethanol (Fig. 3D and E).

1.3. α5-Subunit

The α5-receptor subunit is not widely expressed in brain. Expression is restricted to structures like the hippocampus, olfactory bulb, areas of cortex, and the hypothalamus [4]. Moreover, whereas α1, α2, and α3 receptor subunits are localized to the synapse, studies examining subunit expression in olfactory bulb and hippocampal pyramidal cells suggests that α5-receptor subunits are largely localized to extrasynaptic sites [21,22] where they likely modulate tonic GABAergic inhibition [23]. Interestingly, recent studies suggest that the molecular sites of at least some of ethanol's actions are extrasynaptic, although α5-receptor subunits were not directly studied [24,25].

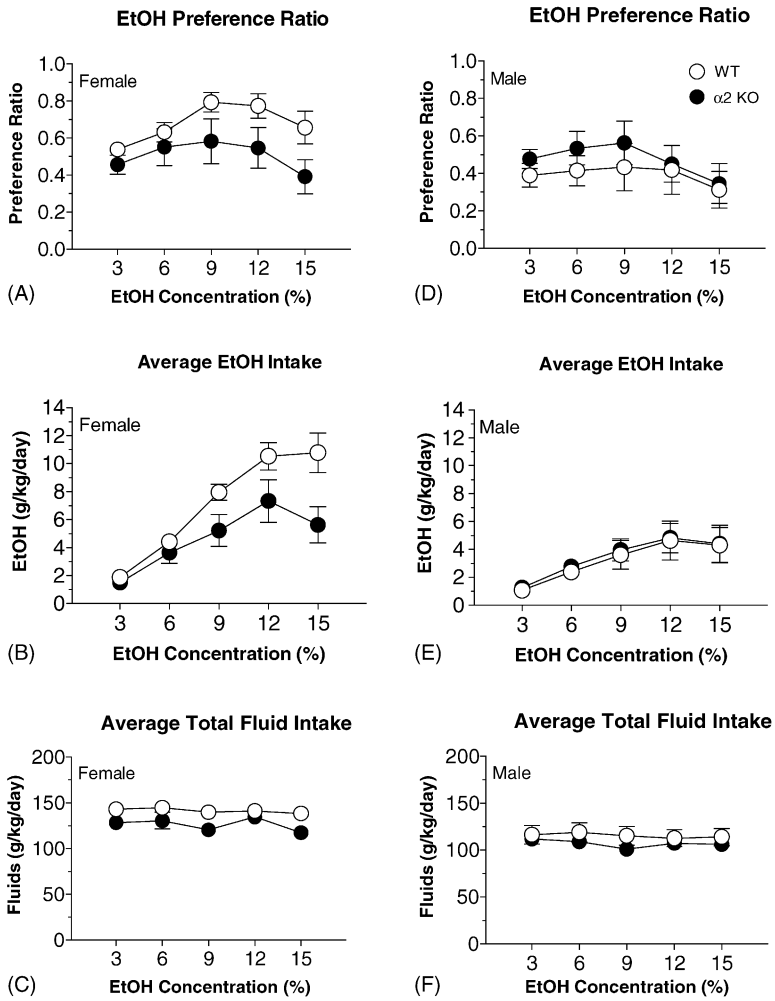

Fig. 3. Ethanol-preference drinking in $GABA_A$ α2-subunit knock-out and wild-type mice. Values represent mean ± S.E.M. Mice (n = 11–19 per sex and genotype) were given a choice between a bottle containing 3, 6, 9, 12, or 15% ethanol, and a second bottle containing water. See Boehm II et al. [99] for detailed methods. Although female α2-subunit knock-out mice exhibited a trend towered reduced ethanol preference (repeated measures ANOVA, main effect of genotype, $F[1, 23] = 3.3$, $P = 0.08$, panel A), ethanol consumption (panel B) did not significantly differ. Male knock-out and wild-type mice did not differ in either of these measures (D and E, respectively). α2-Subunit knock-out and wild-type mice did not differ in total fluid intake, regardless of sex (C and F, respectively).

α5-Receptor subunit knock-in (H105R) mice have been generated [26]. These mice display enhanced trace fear conditioning to threat cues suggesting a role for this subunit in the cognitive processing of spatial memory. Interestingly, these mice also exhibit an unexpected 33% reduction in hippocampal (CA1 and CA3) α5-receptor subunits [26], and in this respect, may be considered a partial knock-out. This was not the case for mutant mice possessing a similar point mutation that were more recently developed [19]. With respect to drug sensitivity, α5-subunit knock-in mice display incomplete myorelaxant sensitivity to diazepam [26], but do not differ in sensitivity to the sedative, anxiolytic, or anticonvulsant actions of the drug [19,26]. As was the case with the other alpha subunit knock-ins, ethanol sensitivity was not investigated.

To date only two published reports have examined the role of α5-receptor subunits in ethanol sensitivity. One study showed that intra-hippocampal (CA1 and CA3) injection of a selective α5-receptor subunit benzodiazepine inverse agonist attenuated ethanol self-administration in rats [27], suggesting that null mutation of the α5-receptor subunit may attenuate alcohol's reinforcing properties. More recently the same group demonstrated that this receptor subunit regulates the reinforcing (ethanol-maintained responding), motor incoordinating (oscillating bar task), and sedative (open field activity) actions of ethanol using a novel benzodiazepine inverse agonist with high affinity for α5-containing $GABA_A$ receptors [28].

α5-Receptor subunit null mutant mice were recently developed, and although hippocampal benzodiazepine binding sites were reduced by 16%, there were no apparent alterations in the expression levels of other $GABA_A$ receptor subunits [29]. Compared to their wild-type counterparts, α5-subunit knock-out mice exhibited improved performance in a water maze of spatial learning, but not in a non-hippocampal learning or anxiety task [29].

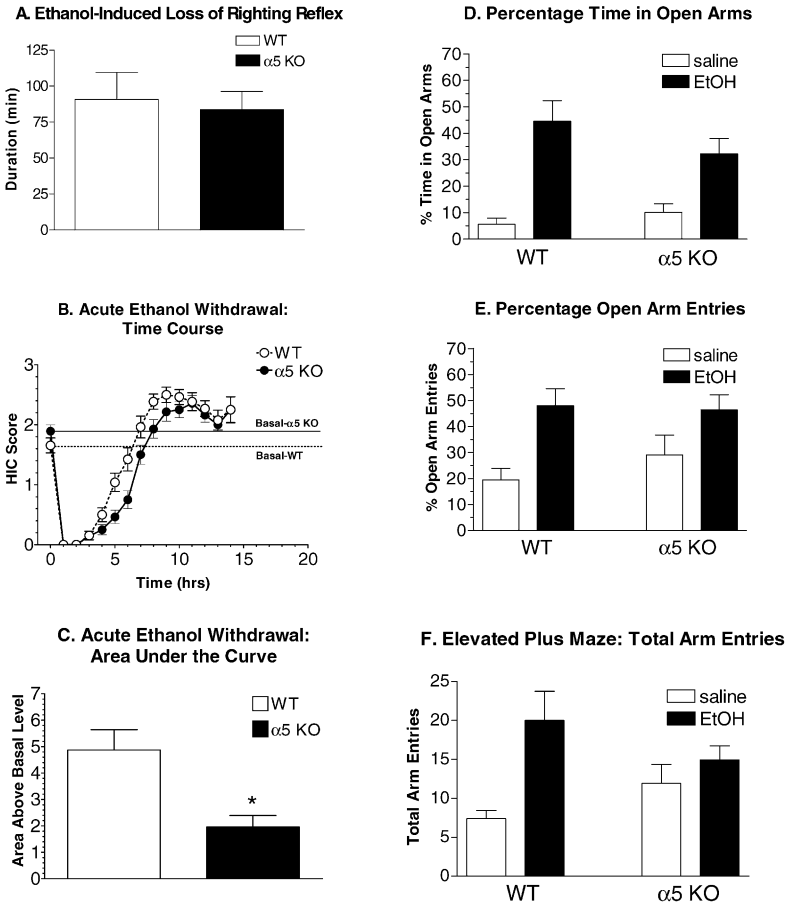

Fig. 4. Sensitivity to the hypnotic and anxiolytic actions of ethanol, and to acute ethanol withdrawal, in $GABA_A$ α5-subunit knock-out and wild-type mice. Although both male and female mice were tested, gender did not interact with any other factor. Thus, data from male and female mice were combined for statistical analysis. Values represent mean ± S.E.M. (A) Hypnotic sensitivity to ethanol (3.8 g/kg, v/v; i.p.) was assessed by loss of righting reflex. See Blednov et al. [16] for detailed methods. α5-Subunit knock-out and wild-type mice ($n = 12$ per genotype) did not differ in duration of ethanol-induced loss of righting reflex. (B and C) Time course and area above baseline of acute ethanol withdrawal in α5-subunit knock-out mice. Mice ($n = 26$–28 per genotype) received an acute injection of ethanol (4.0 g/kg, v/v; i.p.) and handling-induced convulsions (HICs) were assessed every hour for 15 h. Detailed methods can be found in Blednov et al. [98]. The time course of acute ethanol withdrawal did not differ between the genotypes (panel B). However, α5-subunit knock-out mice experienced less severe acute ethanol withdrawal (reduced positive area under the curve, t [52] = 3.4, $P < 0.01$, panel C). (D–F) Ethanol's anxiolytic effects in α5-subunit knock-out mice. Mice ($n = 12$–13 per genotype and treatment) were injected with 1.5 g/kg ethanol, v/v (i.p.) and the percentage time and entries into open arms, and total arm entries were assessed. See Boehm II et al. [99] for detailed methods. Saline-treated α5-subunit knock-out and wild-type mice did not differ in the percentage of time or number of open arm entries. Although ethanol-treatment increased the number of percentage of time (ANOVA, main effect of treatment, $F[1, 47] = 33.4$, $P < 0.001$, panel D) and entries (ANOVA, main effect of treatment, $F[1, 47] = 13.9$, $P = 0.001$, panel E) into the open arms, the genotypes did not differ in sensitivity to this ethanol effect. However, ethanol-treated α5-subunit knock-out mice tended to exhibit a blunted locomotor stimulant response, as indexed by the total number of arm entries (ANOVA, interaction of genotype and treatment, $F[1, 47] = 3.8$, $P = 0.06$, panel F).

However, the genotypes did not differ in locomotor response to novelty (Fig. 1B). α5-Subunit knock-out mice also displayed lower IPSC amplitudes, and greater facilitation of paired-pulse facilitation of field excitatory post-synaptic potential amplitudes in hippocampal brain slices [29]. Taken together, these results suggest that α5-receptor subunits have an important role in mediating hippocampal-dependent learning processes.

A colony of α5-receptor subunit knock-out mice (of a C57BL6J/129SvEv mixed genetic background) was recently established at the University of Texas at Austin for ethanol studies. Sensitivity to ethanol's hypnotic (loss of righting reflex), anxiolytic (elevated plus maze), and reinforcing (ethanol preference drinking) properties were examined, and acute ethanol withdrawal severity (HICs) was assessed. Based on the work of June et al. [27,28], we predicted that genetic deletion of the α5-subunit gene would reduce ethanol preference drinking. α5-Subunit knock-out mice did not differ in sensitivity to ethanol-induced loss of righting reflex (Fig. 4A) or ethanol's anxiolytic effects (Figs. 4D and E). However, the knock-outs displayed lower HIC scores, suggesting a blunted acute withdrawal syndrome (Fig. 4B and C). Moreover, male null mutants preferred and consumed less ethanol than did their male wild-type counterparts (Fig. 5D and E) in the absence of any differences in preference for the

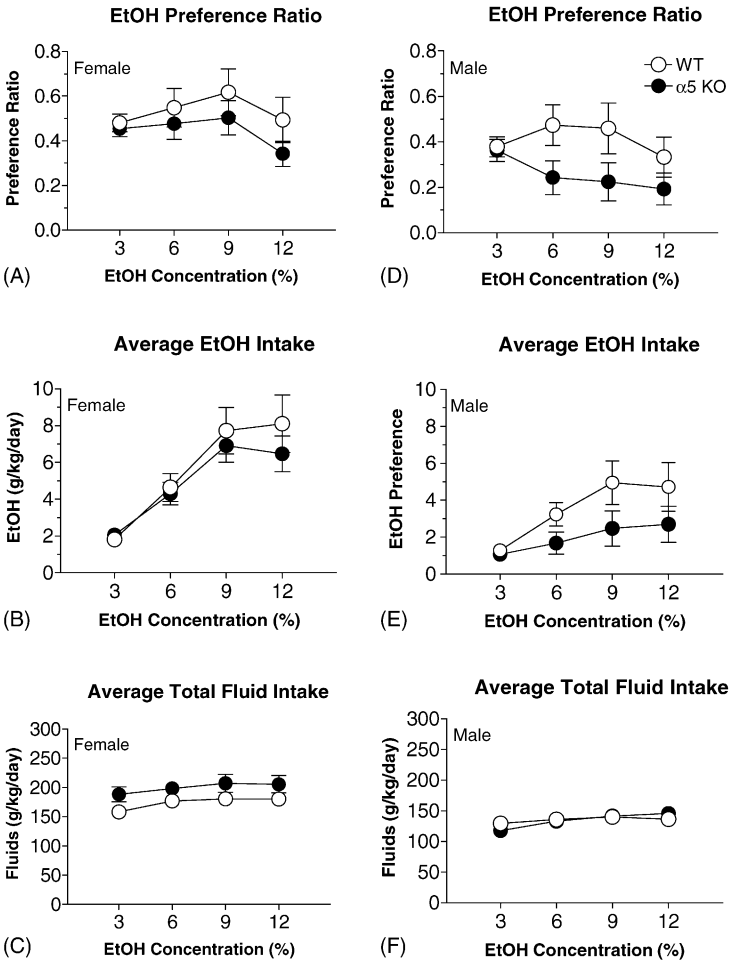

Fig. 5. Ethanol-preference drinking in $GABA_A$ α5-subunit knock-out and wild-type mice. Values represent mean ± S.E.M. Mice (n = 15–17 per sex and genotype) were given a choice between a bottle containing 3, 6, 9, or 12% ethanol, and a second bottle containing water. Detailed methods can be found in Boehm II et al. [99]. Male α5-subunit knock-out mice preferred (repeated measures ANOVA, interaction of genotype and ethanol concentration, $F[3, 84] = 3.4$, $P < 0.05$) and consumed (repeated measures ANOVA, interaction of genotype and ethanol concentration, $F[3, 84] = 2.6$, $P = 0.05$) less ethanol. Follow-up simple effects analyses showed that compared to male wild-types, male α5-subunit knock-outs tended to prefer ($P = 0.06$, panel D) and consume ($P = 0.09$, panel E) less of the 6% ethanol solution. Female knock-out and wild-type mice did not differ in preference (panel A) or consumption (panel B) of ethanol. α5-Subunit knock-out and wild-type mice did not differ in total fluid intake, regardless of sex (C and F, respectively).

sweet tasting saccharine or the bitter tasting quinine (data not shown), suggesting that deletion of the α5-subunit gene may have also altered ethanol's reinforcing properties. Interestingly, the null mutation also appeared to blunt sensitivity to ethanol's locomotor stimulant effects, as assessed by counting the total number of arm entries in the elevated plus maze (Fig. 4F). Deletion of the α5-subunit gene did not alter acute tolerance to ethanol's motor incoordinating effects (data not shown). Thus, α5-subunit knock-out may have also altered the motivational effects of ethanol. However, this result should be interpreted with some caution as sensitivity to ethanol's locomotor stimulant effects have not been directly assessed. Taken together, these data suggest a role for the α5-subunit in the mediation of acute ethanol withdrawal. Moreover, they support the work of June and colleagues in suggesting a role for this receptor subunit in the mediation of ethanol's reinforcing and/or motivational effects.

1.4. α6-Subunit

Expression of $GABA_A$ α6-receptor subunits are restricted to the post-migratory granule cells of the cerebellum and cochlear nuclei [30–32]. Indeed, some 40–60% of cerebellar $GABA_A$ receptors contain the α6-subunit [33,34]. Given the role of the cerebellum in motor control [35] the above pattern of expression makes α6-receptor subunits attractive candidates for the mediation of ethanol's motor impairing actions. In support of this notion, a recent investigation reported that $GABA_A$ receptors composed of α6β3δ or α4β3δ are 10 times more sensitive to ethanol concentrations known to have physiological actions in humans [25].

Mutant mice lacking the α6-receptor subunit were developed by two different research groups [36,37]. Deletion of the α6-subunit resulted in a reduction in cerebellar β2, β3, and γ2 subunits [38]. δ-Subunit expression was also reduced, despite the presence of a normal mRNA level

[37]. These results suggest that δ may associate with α6 in vivo [37]. However, caution should be exercised when interpreting phenotypic data from these animals as α1 and β2 subunit expression levels were also reduced in the forebrain, a structure that does not posses α6-receptor subunits [39]. These results indicate that insertion of the neomycin resistance cassette into the α6-subunit gene likely had the unexpected effect of down-regulating expression of other subunits clustered very near the α6-subunit on mouse chromosome 11 [39]. Finally, binding affinity for muscimol was reduced in the cerebellum of the mutant mice [36], as was tonic conductance in cerebellar granule cells [40].

Despite the above changes in subunit expression, the α6-deficient and wild-type mice developed by Jones et al. [37] appeared to breed and develop normally. Moreover, when tested for the mutation's effects on naïve behavior, the genotypes displayed similar levels of open field activity, and did not differ in performance on the horizontal wire task [37]. The α6-subunit knock-out mice developed by Homanics et al. [36] were also viable and fertile, and had grossly normal cerebellar cytoarchitecture. However, no basal behavioral data were published on these animals.

Given the fairly strong expression of the α6-subunit in the cerebellum, one would predict that mice lacking this subunit would display altered behavioral responses to ethanol. Several ethanol-related behaviors were examined in α6-subunit knock-out and wild-type mice. However, the knock-outs did not differ in sensitivity to ethanol's hypnotic effects as measured using the loss of righting reflex test [36], nor did they develop different acute or chronic tolerance to ethanol, or chronic ethanol withdrawal [41]. These results are summarized in Table 1. Thus, despite the strong cerebellar expression, deletion of the α6-subunit gene did not alter behavioral sensitivity to ethanol. However, a recent report suggests that neuronal adaptations may have countered the loss of this subunit, allowing for normal sensitivity to ethanol's motor impairing actions. Brickley et al. [40] showed that normal cerebellar granule cell excitability was maintained by an adaptive enhancement in voltage-independent K^+ leak conductance in α6-subunit knock-out mice.

One of the benefits of summarizing all the $GABA_A$ receptor knock-out data in one place is that it allows for identification of gaps in our knowledge. Several studies have demonstrated that a naturally occurring point mutation in the α6-subunit gene is responsible for ethanol's impairment of postural reflexes in rat lines genetically selected to differ in ethanol sensitivity [42]. Consequently, it may be that α6-subunit gene deletion altered sensitivity to ethanol's motor incoordinating effects, but that the appropriate behavioral tasks were not employed. Finally, as the available evidence suggests that this same mutation may also alter ethanol preference [43,44], α6-subunit knock-out and wild-type mice should also be tested in a two-bottle choice-drinking paradigm, or in other paradigms that assess ethanol's reinforcing and/or motivational properties.

2. β-Subunit knock-out mice

2.1. β2-Subunit

β2-Subunits are the most abundant of the beta subunits, and $GABA_A$ receptors containing them are found in virtually all brain structures, including olfactory bulb, cortex, hippocampus, thalamus, hypothalamus, amygdala, cerebellum, and midbrain [4]. Given this wide distribution, one would predict that β2-subunits are important for normal GABAergic functioning, and that they may also be important mediators of ethanol's behavioral effects.

β2-Subunit knock-in mice possessing a point mutation rendering the subunit insensitive to etomidate (β2/3-selective intravenous anesthetic) in vitro have been developed [45]. These mice displayed reduced etomidate-induced sedation, but normal etomidate-induced anaesthesia indicating that efficacy at the β3 subunit of the $GABA_A$ receptor alone is sufficient to induce general anaesthesia. These data were further corroborated by the same group using mice lacking the β2 subunit [46]. Consistent with its widespread distribution, deletion of the β2-subunit gene reduced the total number of brain $GABA_A$ receptors. In addition, the expression of all six alpha subunits was reduced (40–70% reduction). β3-Subunit expression was not up-regulated. These results suggest that β2-subunits can co-assemble with any alpha subunit, and that other β-subunits do not substitute for β2.

Despite the wide-spread distribution of β2-subunits, these mutants bred and developed normally, and did not display any spontaneous seizures [7]. The knock-outs also did not show any major deficits in motor function as measured by the rotarod, balance beam, or swimming ability tasks. However, they displayed greater spontaneous locomotor activity [7]. When ethanol sensitivity was assessed, knock-outs were less sensitive to ethanol's hypnotic actions as assessed by the loss of righting reflex test [16]. This differential action of ethanol was only seen among male mice. Although it is not immediately clear why females knock-outs did not display a similar reduction in hypnotic sensitivity, it is notable that the dose response curves for the male knock-outs overlapped the curves for the female knock-outs and wild-types. Thus, it is possible that a floor effect masked any further reduction in hypnotic sensitivity in the female β2-subunit knock-outs. Gender specific effects were also seen when β2-subunit knock-out mice were assessed for acute ethanol withdrawal severity; male knock-outs experienced more severe acute ethanol withdrawal (Blednov et al., unpublished). The genotypes did not differ in sensitivity to ethanol's locomotor stimulant effects, nor did they differ in ethanol preference drinking, ethanol conditioned taste aversion, or ethanol

conditioned place preference [14]. β2-Subunit knock-out mice exhibited more robust chronic ethanol withdrawal. However, they also consumed more of the chronic ethanol liquid diet [14], making interpretation of the data difficult. These results are summarized in Table 1.

2.2. β3-Subunit

β3-Receptor subunits are strongly expressed in the olfactory bulb, cortex, hypothalamus, and amygdala, but can be found in many different cell types throughout the brain [4] and spinal cord [47]. Its strong expression in cerebellar granule cells [4] makes it an attractive candidate for mediating behavioral sensitivity to ethanol. Indeed, a recent study suggests that $GABA_A$ receptors composed of α6, β3, and δ receptors are particularly sensitive to ethanol [25].

Knock-in mice possessing a point mutation in the β3-subunit gene (N265M) that abolishes the actions of some general anesthetics have been developed [48]. The point mutation attenuated etomidate- and enflurane-induced loss of righting reflex, as well as etomidate- and enfurane-induced suppression of hindlimb withdrawal reflex. However, ethanol sensitivity has not yet been examined.

β3-Receptor knock-out mice were first developed by Homanics et al. [49]. Knock-out of the β3-subunit gene reduced the total number of brain $GABA_A$ receptors containing α2- and α3-subunits [50], and severely impaired $GABA_A$ receptor function as assessed by recordings from dorsal root ganglion [49] and cortical neurons [50]. However, brain morphology was grossly normal. Although most β3 null mutants died shortly after birth, not all the deaths were associated with cleft palate. This result is significant in light of earlier work showing that cleft palate and neonatal death result from irradiation of the segment of mouse chromosome 7 containing the β3-subunit, and that these phenotypes can be rescued by introduction of the β3-subunit transgene [51]. β3-Subunit knock-out mice that survived were runted, but achieved normal body weight after weaning. The null mutants also had a shortened life span, but bred normally.

Consistent with the wide-spread distribution of the subunit, β3-subunit knock-out mice displayed a number of abnormal behaviors [49]. β3-subunit knock-out mice were hyperactive and hyperresponsive to sensory stimuli. When lifted by the tail, the mutants showed signs of neurological impairment, and experienced difficulty walking on grids, platforms, and rotarods. β3-Deficient mice also displayed occasional epileptic seizures, confirmed by electroencephalographic recordings. Interestingly, the above phenotypes are consistent with Angelman syndrome, a severe human neurodevelopmental disorder resulting form deletion and/or mutation of maternal chrmosome 15q11-13 [52]. Importantly, the β3-subunit gene is contained within this chromosomal region.

β3-Receptor subunit knock-out mice were found to differ in sensitivity to a number of $GABA_A$ acting compounds (see Table 2 and Appendix A). However, only one ethanol-related behavior has been assessed. This may be, in part, due to the severe behavioral consequences of β3-subunit knock-out that are detailed above. Compared to their wild-type counterparts, β3-subunit knock-out mice did not differ in sensitivity to ethanol's hypnotic effects as measured by the loss of righting reflex task [53].

3. γ2-Subunit knock-out and transgenic mice

The γ2-subunit can be alternatively spliced, creating two different splice variants, the γ2S (short) and γ2L (long). The γ2L splice variant differs from its shorter relative because it posses eight additional amino acids in the third intracellular loop. Importantly, these additional amino acids contain a protein kinase C phosphorylation site, and work by Wafford et al. [54] suggested that these eight amino acids are required for ethanol-enhancement of $GABA_A$ receptors possessing the γ2-subunit. However, another study did not support this finding [55].

The distribution of γ2S and γ2L are fairly similar throughout the brain. However, the relative expression intensity varies depending on brain region and neuronal cell type. For example, whereas γ2S-subunits were more abundant in the olfactory bulb, cortex, and hippocampus, γ2L-subunits exhibited stronger expression in cerebellar Purkinje cells [56].

3.1. γ2S-Subunit

Mice lacking the γ2S-subunit have not been generated. However, transgenic mice that over-express the γ2S-subunit were developed by Wick et al. [57]. There are no available data on basal behaviors in these mutant mice. However, when ethanol sensitivity was studied, it was determined that γ2S-subunit transgenic mice developed less acute tolerance to ethanol's motor incoordinating effects. Thus, it appears that the γ2S-subunit may also have a role in mediating behavioral sensitivity to ethanol. Nevertheless, more work will be necessary to elucidate the role of γ2S in the mediation of ethanol's behavioral actions.

3.2. γ2L-Subunit

Knock-out mice lacking the γ2L-subunit were developed by Homanics et al. [58]. These mice were viable, and indistinguishable from wild-type mice. However, despite some in vitro evidence suggesting that this $GABA_A$ receptor subunit is important for ethanol's actions [54], γ2L-subunit deficient mice displayed similar sensitivities to ethanol's hypnotic (loss of righting reflex), anxiolytic (elevated plus maze), and locomotor stimulant actions [58]. Moreover, the genotypes developed similar chronic ethanol withdrawal and acute tolerance to ethanol's motor

incoordinating actions. Electrophysiological recordings in dorsal root ganglion neurons also did not reveal any differences between γ2L-subunit knock-out and wild-type mice. However, a caveat was that total γ2-subunit protein levels were unchanged in the knock-outs, suggesting that the γ2S-subunit may have substituted for the γ2L-subunit in these mutant mice.

The same group that developed the γ2S-subunit transgenic mice also developed transgenic mice that over-express the γ2L-subunit [57]. These mice were also impaired in their ability to develop acute tolerance to ethanol's motor incoordinating actions. Thus, whereas the γ2L-subunit appears to alter acute tolerance in transgenic mice, it has no such effect in knock-out mice. These results are contrary to the prediction that the effects of gene knock-out would be phenotypically opposite those of transgenic over-expression, and provide a mixed support, at best, for a role of the γ2L-subunit in mediating ethanol sensitivity. Furthermore, it should also be noted that both γ2S- and γ2L-subunit transgenic mice displayed similar ethanol phenotypes (i.e., impaired acute tolerance), arguing against the importance of the eight amino acid segment in mitigating ethanol sensitivity in γ2-containing $GABA_A$ receptors. Nevertheless, given the normal γ2-subunit protein levels and lack of ethanol phenotype in the γ2L-subunit knock-outs, it might be prudent to assess ethanol sensitivity in the heterozygous, global γ2-subunit knock-out mice developed by Günther et al. [59].

A summary of the above ethanol studies can be found in Table 1. A summary of data addressing behavioral sensitivity to other GABAergic compounds in the γ2S- and γ2L-subunit mutants discussed above can be found in Fig. 2 and Appendix A.

4. δ-Subunit knock-out mice

4.1. δ-Subunit

δ-Receptor subunits are found in most brain structures, albeit with limited abundance [4]. The δ-subunit is more prevalant in cerebellar granule cells where its localization is almost exclusively extrasynaptic [60]. Interestingly, a recent study suggests that extrasynaptic δ-containing $GABA_A$ receptors are particularly sensitive to ethanol [25,61]. Furthermore, recent work indicates that δ-containing $GABA_A$ receptors, especially those also composed of α4 and β, may be up-regulated during hormonal states (i.e., premenstrual syndrome) associated with enhanced sensitivity to ethanol [61]. Thus, δ-subunit knock-out mice would be expected to display altered behavioral sensitivity to ethanol.

δ-Subunit knock-out mice were developed by Mihalek et al. [62]. Immunoaffinity chromatography of cerebellar extracts indicated an increased co-assembly of α6 and γ2 subunits in the δ-subunit knock-outs [63]. Because 97% of all δ-subunits were co-assembled with α6-subunits in the cerebellum of wild-type mice, the above results suggest that α6βγ2 and αβ replaced δ-containing $GABA_A$ receptors in the cerebellum of δ-subunit deficient mice. Thus, it appears that the availability of the δ-subunit influences the assembly of γ2 with other subunits, even though δ- and γ2-subunits do not co-assemble in the same receptor. Indeed, similar results were seen in forebrain where δ-subunits normally co-assemble with α4 subunits; γ2-subunit expression and co-assembly with α4 was increased in δ-subunit knock-out mice [64,65]. δ-Subunit gene deletion also reduced α4-subunit expression in forebrain [65] and hippocampus [66].

Hippocampal slices from δ-subunit knock-out mice displayed a faster decay of mIPSCs, but no change in mIPSC amplitude or frequency [62]. However, the mutants bred and developed normally [62]. Moreover, basal behaviors were not altered by the δ-subunit null mutation. Mutants and wild-types displayed similar anxiety levels, and normal fear conditioning, exploratory activity, and pain sensitivity [62]. Interestingly, δ-subunit knock-outs were insensitive to the hypnotic actions of several neuroactive steroids [62], and another neuroactive steroid failed to prolong IPSCs in the cerebellar granule cells of the knock-outs [67]. Similar results were seen in hippocampal slices from δ-subunit knock-out mice [66,68]. These results suggest a role for δ-subunits in mediating neuroactive steroid sensitivity.

δ-Subunit knock-out mice preferred and consumed less ethanol, exhibited reduced chronic ethanol withdrawal severity, and displayed reduced sensitivity to the anticonvulsant actions of ethanol [69]. The hypnotic (loss of righting reflex), anxiolytic (elevated plus maze), and hypothermic actions of ethanol were not changed, nor was chronic tolerance to the hypnotic actions, or acute tolerance to the motor incoordinating actions, of ethanol. These data are summarized in Table 1. Consequently, the behavioral data appears to support in vitro studies suggesting a role for this $GABA_A$ receptor subunit in the mediation of ethanol sensitivity. Based on work suggesting a role neurosteroids in the modulation of ethanol's actions at δ-containing receptors [61], future studies should assess the combined actions of ethanol and various neuroactive steroids in δ-subunit knock-out mice.

Although the behavioral actions of other GABAergic drugs have also been tested in δ-subunit knock-out mice, a detailed discussion of these studies is beyond the scope of this review. Nevertheless, the results of these studies are summarized in Table 2 and Appendix A.

5. $GABA_A$ receptor subunit-associated quantitative trait loci (QTL)

Another way to determine whether different $GABA_A$ receptor subunits alter specific ethanol-related behavioral

phenotypes is to examine the available quantitative trait loci literature. Ethanol-related behaviors are complex traits. This means that many different genes influence genetic vulnerability to ethanol's behavioral actions. Over the past decade QTL analysis has emerged as a strategy for mapping the many chromosomal regions that contain genes influencing sensitivity to a number of ethanol-related behaviors.

The basic premise of QTL analysis is simple [70]. First, one must measure a specific phenotype within a population. Next, the population must be genotyped at a hundred or more marker loci distributed across the genome, and the genotype (at each marker locus) of each individual animal compared with its phenotypic score. The idea is to identify animals of one genotype that score differently on the phenotypic trait than animals of another genotype. If such animals are found, a QTL is detected and mapped to the chromosomal region containing the associated marker.

To date a number of significant QTLs for different alcohol-related traits in mice have been identified. Most of these have recently been reviewed by Crabbe et al. [71] and Crabbe [72], and include QTLs for acute alcohol withdrawal severity, alcohol reinforcement (alcohol preference drinking, conditioned taste aversion, conditioned place preference, locomotor stimulation, and locomotor sensitzation), hypnosis (loss of righting reflex), hypothermia, and motor incoordination. Moreover, a number of candidate genes are known to reside within the regions spanned by these QTLs. However, confirmation of these is difficult as the initial mapping of a QTL usually occurs with a resolution of 10–30 cM [71], a region containing hundreds of possible candidate genes. Thus, it is essential to proceed from QTL to gene by both narrowing the region spanned by the QTL and by testing any suggested candidate genes lying within the region spanned by the QTL.

A number of QTLs span chromosomal regions known to contain $GABA_A$ receptor subunit genes. These genes are clustered on five different chromosomes, including 4, 5, 7, 11, and the X chromosome [73] (Table 3). For example, a gene cluster containing the α1-, α6-, β2-, and γ2-subunits can be found on chromosome 11, at about 23 cM. Interestingly, a QTL for acute ethanol withdrawal spans this region [74]. Other notable gene clusters include the α2-, α4-, β1-, and γ1-subunit cluster on chromosome 5 (40 cM), the α5-, β3-, and γ3-subunit cluster on chromosome 7 (28 cM), the ρ1- and ρ2-subunit cluster on chromosome 4 (11 cM), and the α3-, θ-, and ϵ-subunit cluster on the X chromosome (29 cM). The δ-subunit is located by itself on chromosome 4 (79 cM).

Table 3
Chromosomal localization of known GABA-A receptor gene clusters in mice

Chromosome	Location (cM)	GABA-A gene
4	10.5	ρ1, ρ2
4	79	δ
5	40	α2, α4, β1, γ1
7	28	α5, β3, γ3
11	23	α1, α6, β2, γ2
X	29	α3, θ, ϵ

As mentioned above, the region spanned by a QTL can harbor hundreds of genes, any of which may influence the trait of interest. Besides narrowing the region spanned by the QTL, another strategy has been to turn to mutant mice for confirmation or exclusion of candidate genes. QTLs overlapping the locations of known $GABA_A$ receptor subunit genes are listed in Table 4. Not all of the listed QTLs are among the most highly significant in the studies in which they were detected. However, all span regions that overlap or are in close proximity to the locations of known $GABA_A$ receptor genes. For example, inspection of Table 4 reveals that a number of significant QTLs for such phenotypes as acute ethanol withdrawal, chronic ethanol withdrawal, loss of righting reflex, ethanol-induced motor incoordination, ethanol conditioned taste aversion, ethanol-induced hypothermia, and chronic tolerance to ethanol were detected that span a region of mouse chromosome 11 [74–80]. Importantly, this particular region contains a cluster of $GABA_A$ receptor genes that includes the α1-, α6-, β2-, and γ2-subunit genes. Because it is known that ethanol modulates $GABA_A$ receptor function, it is reasonable to include these subunits among a list of potential candidate genes responsible for the significant QTLs.

Comparison of the QTL results in Table 4 with the data obtained from $GABA_A$ subunit knock-out and transgenic mouse models summarized in Table 1 reveals some interesting trends. For example, a significant QTL for ethanol's hypnotic actions as assessed by the loss of righting reflex test was detected on chromosome 11 in a region containing the α1-, α6-, β2-, and γ2-subunit genes [78]. Interestingly, genetic deletion of the α1- and β2-subunits also reduced sensitivity to ethanol's hypnotic actions as assessed by the same behavioral assay [16]. Thus, the knock-out data provide strong evidence that these two receptor subunits likely account for the QTL. Moreover, that genetic deletion of the α6-[36] and γ2L- [58] subunits, or overexpression of the γ2L-subunit [56], had no such effect allows for the elimination of these subunits as possible candidate genes for this QTL. Similar convergence was seen regarding a significant QTL for ethanol conditioned taste aversion also detected on chromosome 11 [77]. The QTL spanned the same $GABA_A$ receptor gene cluster, and α1-subunit knock-out mice developed greater ethanol conditioned taste aversion [14].

Of the remaining QTLs, several others overlapping $GABA_A$ receptor gene clusters were also supported by results from knock-out mice. QTLs for ethanol-induced loss of righting reflex were detected on mouse chromosome 5 that spanned a region containing a cluster of genes coding for the α2-, α4-, β1-, and γ1-subunits [81,82]. Whereas knock-outs for the α4-, β1-, and γ1-subunits are not currently available, we showed that α2-subunit knock-out mice exhibit reduced sensitivity to ethanol's hypnotic actions as assessed by the loss of righting reflex test (Fig. 2A), supporting the α2-subunit as a viable candidate gene underlying

Table 4
GABA-A receptor subunit gene expression patterns are associated with elhanol behavioral QTLs that map to chromosomal regions harboring these subunits

Chromosome (cM)	Ethanol phonotvpe	Mouse population	Reference	Maps near (chromosome, cM)	[a]Correlated with subunit expression value (*P* value)
11:16–19	Acute withdrawal	BXD	Buck et al. [74]	α1, α6, β2, γ2 (11:23)	
11:46	Chronic tolerance-hypothermia	BXD	Crabbe et al. [75J	α1, α6, β2, γ2 (11:23)	[b]α1, CB (0.002); [b]β2, CB (0.001); [b]γ2, CB (0.052)
11:15–36	Chronic withdrawal	IP2 X IR1 F2	Bergeson et al. [80]	α1, α6, β2, γ2 (11:23)	
11:40–45	CTA	BXD	Risinger et al. [77]	α1, α6, β2, γ2 (11:23)	
11:12	Hypothermia	BXD	Crabbe et al. [75]	a1, a6, β2, γ2 (11:23)	[b]α1, FB (0.014); [b]β2, CB (0.028); [b]γ2, FB (0.004)
11:26	LORR	BXD	Browman and Crabbe [78]	α1, α6, β2, γ2 (11:23)	[c]α1, FB (0.059)
11:29–43	Motor incoodination-dowel	BXD	Kirstein et al. [79]	α1, α6, β2, γ2 (11:23)	[b]α1, CB (0.051); [b]γ2, CB (0.028)
11:35–40	Motor incoodination-grid	BXD	Phillips et al. [76]	α1, α6, β2, γ2 (11:23)	[b]β2, FB (0.011)
11:26–32	Motor incoordination-screen	BXD	Browman and Crabbe [78]	α1, α6, (β2, y2 (11:23)	[b]α1, CB (0.041)
5:58	Chronic tolerance-grid	BXD	Phillips et al. [76]	α2, α4, β1, γ1 (5:40)	
5:25–63	Chronic tolerance-hypothermia	BXD	Crabbe et al. [75]	α2, α4, β1, γ1 (5:40)	
5:47	Hypothermia	BXD	Crabbe et al. [75]	α2, α4, β1, γ1 (5:40)	[b]α2, FB (0.057)
5:20	LORR	B6 X D2 F2	Radcliffe et al. [81]	α2, α4, β1, γ1 (5:40)	
5:17	LORR	BXD	Rodriguez et al. [82]	α2, α4, β1, γ1 (5:40)	
5:26	LORR-female	B6 X D2 F2	Radcliffe et al. [81]	α2, α4, β1, γ1 (5:40)	
7:7	Acute tolerance-dowel	BXD	Gallaher et al. [87]	α5, β3, γ3 (7:28)	
7:50	Chronic tolerance-grid	BXD	Phillips et al. [76]	α5, β3, γ3 (7:28)	
7:10–15	Chronic tolerance-hypothermia	BXD	Crabbe et al. [75]	α5, β3, γ3 (7:28)	[b]β3, FB (0.044); [b]β3, CB (0.026)
7:36	Hypothermia	BXD	Crabbe et al. [75]	α5, β3, γ3 (7:28)	[b]β3, CB (0.026)
7:6–20	Locomotor stimulation	BXD	Phillips et al. [76]	α5, β3, γ3 (7:28)	[c]α5, CB (0.053)
7:10–16	Locomotor stimulation	BXD	Cunningham [85]	α5, β3, γ3 (7:28)	[b]β3, FB (0.035)
7:13	LORR	BXD	Rodriguez et al. [82]	α5, β3, γ3 (7:28)	
7:27	Preference	BXD	Phillips et al. [86]	α5, β3, γ3 (7:28)	
7:13	Preference	B6 X D2 F2	Tarantino et al. [83]	α5, β3, γ3 (7:28)	[b]β3, CB (0.013)
7:11	Preference	BXD	Phillips et al. [84]	α5, β3, γ3 (7:28)	
7:22	Preference	BXD	Rodriguez el al. [82]	α5, β3, γ3 (7:28)	[b]β3, CB (0.038)
4:81	Acute tolerance-dowel	BXD	Gallaher et al. [87]	δ (4:79)	[b]δ, FB (0.016)
4:70	Chronic tolerance-grid	BXD	Phillips et al. [76]	δ (4:79)	[b]δ, FB (0.056)
4:46–79	CPP	BXD	Cunningham [85]	δ (4:79)	
4:75–94	CTA	BXD	Risinger et al. [77]	δ (4:79)	
4:60–69	Hypothermia	BXD	Crabbe et al. [75]	δ (4:79)	[b]δ, FB (0.014)
4:66–78	Locomotor stimulation	BXD	Demarest et al. [100]	δ (4:79)	[b]δ, FB (0.054)
4:81	Preference	BXD	Phillips et al. [86]	δ (4:79)	
4:65	Preference	B6 X D2 F2	Tarantino et al. [83]	δ (4:79)	
4:57–61	Preference	BXD	Phillips et al. [84]	δ (4:79)	
4:0–22	Acute tolerance-dowel	BXD	Gallaher et al. [87]	ρ1, ρ2 (4:11)	[b]ρ2, FB (0.005); [b]ρ2, CB (0.008)
4:14	Acute withdrawal	BXD	Buck et al. [74]	ρ1, ρ2 (4:11)	[b]ρ1, FB (0.009)
4:0–16	Chronic tolerance-grid	BXD	Phillips et al. [76]	ρ1, ρ2 (4:11)	
4:25	Chronic tolerance-hypothermia	BXD	Crabbe et al. [75]	ρ1, ρ2 (4:11)	[b]ρ2, CB (0.042)
4:2	LORR	BXD	Rodriguez et al. [82]	ρ1, ρ2 (4:11)	
4:0–26	Motor incoordination-dowel	BXD	Gallaher et al. [87]	ρ1, ρ2 (4:11)	ρ2, CB (0.037)
4:35–36	Motor incoordination-screen	BXD	Browman and Crabbe 178]	ρ1, ρ2 (4:11)	

Abbreviations: CB, cerebellum; FB, forebrain.

[a] WebQTL (www.webqtl.org) provides access to behavioral and gene expression data from the BXD recombinant inbred strains. These data can then be accessed for data mining purposes. We used WebQTL to perform correlational analyses between the ethanol behavioral parameters above and GABA-A receptor subunit gene expression in the BXD recombinant inbred strains.

[b] Change in subunit expression is consistent with a significant QTL.

[c] Change in subunit expression is consistent with a significant QTL and data from knock-out mice.

the QTL. QTLs for ethanol preference drinking [82–84] and ethanol-induced locomotor stimulation [75,85] were detected on chromosome 7 that span the same region as the α5-, β3-, and γ3-subunit genes. Although β3-subunit knock-out mice have not been tested and γ3-subunit knock-outs have yet to be developed, Fig. 5 shows that α5-subunit knock-out mice prefer and consume less ethanol, and Fig. 4F shows that these same mutants may also be more sensitive to

ethanol's locomotor stimulant properties. Thus, these results provide additional support for the α5-subunit as a possible candidate gene for both ethanol-related QTLs. Finally, three QTLs for ethanol preference drinking were detected on chromosome 4 in a region harboring the δ-subunit gene [84,86] and δ-subunit knock-out mice exhibited reduced ethanol preference drinking [69]. Thus, the mutant mouse literature can be used to confirm the possibility that suspected candidate genes contained within a region spanned by a QTL indeed underlie the QTL.

As can also be seen by comparison of Tables 1 and 4, there are also a number of QTLs for which $GABA_A$ receptor subunits are viable candidate genes, but the corresponding knock-out and/or transgenic mouse models were not assessed using the appropriate behavioral assays. For example, whereas three different groups identified QTLs for sensitivity to ethanol's motor incoordinating actions in a region of mouse chromosome 11 that contains the α1-, α6-, β2-, and γ2-subunit genes [76,78,79], only α1-subunit knock-out mice were tested for sensitivity to this ethanol-related trait [15], and these mice did not differ. Moreover, whereas a number of QTLs were detected that span the α5-, β3-, and γ3-subunit gene cluster on mouse chromosome 7 [75,82–84,87], only the α5-subunit knock-outs were tested in more than one behavioral assay. Indeed, β3-subunit knock-out mice were only tested for sensitivity to ethanol's hypnotic actions using the loss of righting reflex test [53], and γ3-subunit knock-out mice have yet to even be developed. Finally, a number of QTLs were detected that overlap a cluster of genes coding for the ρ1- and ρ2-subunits on mouse chromosome 4 [74,75,77, 82,87]. Although ρ2-subunit knock-out mice do not currently exist, two different groups have recently developed the ρ1-subunit knock-out and wild-type mice [88,89]. Given that behaviors such as acute tolerance to ethanol, acute ethanol withdrawal, chronic tolerance to ethanol, ethanol-induced loss of righting reflex, and ethanol-induced motor incoordination have each been mapped to this region, it will be interesting to assess ethanol sensitivity in these new mutant mice.

6. $GABA_A$ receptor subunit-associated gene expression patterns

A genetic difference in DNA sequence within the coding region of an underlying gene is one mechanism by which significant QTLs might influence their associated traits. Such a mechanism might alter the amino acid sequence of the subunit, thereby altering $GABA_A$ receptor function and changing behavioral sensitivity to ethanol. For example, a recent report identified a polymorphism in the γ2-subunit gene that predicts a difference in amino acid sequence between the C57BL6/J (B6) and DBA/2J (D2) inbred strains, the progenitors of the BXD recombinant inbred strains [90]. This polymorphism was shown to associate with acute ethanol withdrawal severity, likely explaining the earlier detection of a QTL that spanned a region of chromosome 11 harboring the γ2-subunit gene.

Despite the story for γ2-subunit gene and acute ethanol withdrawal, all to often detection of significant QTLs are not always followed by the identification of sequence variants. Indeed, the B6 and D2 strains exhibited identical polypeptide sequences for the α1 [91] and β2 [92] subunit genes, also contained within the spanned region of the acute ethanol withdrawal QTL on chromosome 11 [90]. Although it is possible that these two $GABA_A$ receptor subunits do not contribute to acute ethanol withdrawal severity, it may be that they actually do contribute by virtue of differing genotypic expression patterns of the α1- and β2-subunit genes. In other words, differences in subunit expression patterns may also regulate $GABA_A$ receptor function, altering behavioral sensitivity to ethanol.

WebQTL (www.webqtl.org) is a web-based resource for the analysis of complex traits [93,94] containing more than 600 published phenotypes tested in a panel of mouse strains. These strains include the B6 and D2 inbred strains, their F1 hybrids, and their 32 derived BXD recombinant inbred strains [95,96]. Importantly, WebQTL also contains estimates of gene expression patterns across these 35 strains. Messenger ribonucleic acid (mRNA) expression patterns in forebrain and cerebellum were determined in naïve mice using Affymetrix (forebrain, U74Av2; cerebellum, M430) micorarrays [95] (see www.webqtl.org for animal, sample, and preparation details). In sum, WebQTL allows for the comparisons of gene expression patterns with various ethanol-related phenotypes. More specifically, WebQTL allows for calculating genetic correlations between mRNA expression and behavioral variables, which provide yet another way to verify potential candidate genes that might underlie significant QTLs.

Using WebQTL, we calculated Pearson's correlations between various ethanol-related behavioral traits and $GABA_A$ receptor subunit mRNA abundance values in the BXD recombinant inbred strains. Our goal was to relate these findings to ethanol behavior QTLs that overlap the known locations of subunit gene clusters, and ultimately to data from $GABA_A$ receptor subunit knock-out mice. Thus, with a few exceptions, we restricted the number of correlations to only those that involved subunits for which knock-out mice are currently available. This approach yielded about 125 significant correlations.

Of the ethanol related-traits that correlated with $GABA_A$ receptor subunit gene expression, only a handful were consistent with previously mapped ethanol behavior QTLs (Table 4). Correlated differences in gene expression were observed for traits like ethanol preference drinking, ethanol-induced locomotor stimulation, ethanol-induced loss of righting reflex, ethanol-induced hypothermia and motor incoordination, as well as toler-

ance to these effects, and acute ethanol withdrawal. For example, QTL analysis suggests that each of the $GABA_A$ receptor gene clusters (except for the one on mouse chromosome 4 that codes for the ρ-subunits) may contain viable candidate genes influencing sensitivity to ethanol's hypothermic actions [75]. Whereas any number of the genes contained within these clusters may actually underlie the QTLs, correlational analysis reveals strong associations between this ethanol behavioral phenotype and expression of α1-, α2-, β2-, β3-, γ2-, and δ-subunits (Table 4). These results support a role for these $GABA_A$ receptor subunits in the mediation of ethanol's hypothermic actions, and effectively eliminate α5- and α6-subnits (at least in the brain regions for which mRNA expression data are available) as viable candidate genes.

Perhaps the most intriguing of the significant correlations are those that are consistent with both a previously mapped ethanol behavior QTL and the $GABA_A$ receptor subunit knock-out mouse literature. This was not the case for the above ethanol hypothermia correlations. In fact, except for δ, none of the subunits whose expression correlated with the behavior were even tested using mutant mouse models, and the δ-subunit knock-out and wild-type mice did not differ in sensitivity to this action of ethanol (Table 1). Inspection of Table 4 reveals just two cases where a significant correlation consistent with both QTL and knock-out data was found. Sensitivity to loss of righting reflex correlated with α1-subunit mRNA expression in forebrain, confirming a significant QTL for this behavior detected on mouse chromosome 11 [78], and coinciding with data showing the α1-subunit knock-out mice are less sensitive this action of ethanol [16]. Moreover, three-way convergence was also found for a correlation between ethanol's locomotor stimulant actions and α5-subunit expression in cerebellum. This result is consistent with a significant QTL on mouse chromosome 7 [76], as well as our data suggesting that α5-subunit knock-out mice are more sensitive to this ethanol effect (Fig. 4F). Thus, comparison of phenotypic data from these very different behavioral genetic approaches can yield powerful evidence for or against suspected candidate genes, in this case $GABA_A$ receptor subunits.

Finally, we earlier discussed a very interesting series of ethanol behavioral QTLs that mapped to the proximal portion of mouse chromosome 4. These QTLs spanned or were very close to a cluster of genes coding for the ρ1- and ρ2-subunit genes [74–77,87]. We pointed out that although ρ2-subunit knock-out mice are not currently available, ρ1-subunit knock-outs were recently developed [88,89], and have yet to be tested for sensitivity to ethanol's behavioral actions. Interestingly, several highly significant correlations were detected between ρ1- and ρ2-subunit expression and several different ethanol-related traits. Of particular interest is highly significant correlation between ρ1-subunit expression in forebrain and acute ethanol withdrawal severity. Buck et al. [74] detected an acute ethanol withdrawal QTL very near the ρ-gene cluster. These results may suggest a role for ρ1-subunits in mediating acute ethanol withdrawal severity, and that future work should examine this ethanol-related behavior in the new knock-out mice.

7. Conclusion

$GABA_A$ receptors are known to have a role in the modulation of a number of ethanol's behavioral actions. Evidence suggests that the subunit composition of individual $GABA_A$ receptors may determine behavioral sensitivity to ethanol [1]. Indeed, studies of subunit knock-out mice have yielded considerable insights into the subunit specificity of ethanol's behavioral actions. These studies complement experiments aimed at mapping chromosomal regions (QTLs) underlying behavioral sensitivity to ethanol. A number of ethanol behavioral QTLs span regions harboring $GABA_A$ receptor subunit gene clusters, and examination of the knock-out literature provides a means by which to investigate the potential that these subunit genes might underlie these QTLs.

Subunit gene expression data has also been generated for many of the inbred strains used in the above QTL analyses. These data sets have been uploaded to an online database (WebQTL) allowing for the correlation of $GABA_A$ receptor subunit gene expression with various ethanol-related traits. The results of these correlational analyses may also be compared to the results of QTL studies to more precisely determine the role of certain $GABA_A$ receptor subunits in behavioral sensitivity to ethanol.

Convergence of results between knock-out, QTL, and gene expression studies offer the strongest evidence supporting the role of any particular $GABA_A$ receptor subunit in mediating sensitivity to a specific ethanol-related behavior. Although future studies will undoubtedly refine the above techniques, the converging results of these studies will very likely aid in the development of subunit specific drugs for the treatment of alcohol abuse and dependence. These studies indicate that it may be possible to engineer drugs that selectively alter certain behavioral actions of ethanol. As just one example, convergent evidence from knock-out, QTL, and gene expression data suggest that drugs that target α5-containing $GABA_A$ receptors may be particularly useful in altering ethanol's reinforcing/motivational properties. Interestingly, such drugs are currently being developed and tested in animal models [27].

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Appendix A. Change in behavioral sensitivity to GABAergic drugs in GABA-A subunit knock-out and transgenic mice

Subunit	Drug phenotype	References
α1	GABA-A Agonist/antagonist	
	↑ Convulsive action of bicuculline	Kralic et al. [9]
	↓ Hypnotic action of THIP	Blednov et al. [16]
	Barbiturate	
	↓ Hypnotic action of pentobarbital	Kralic et al. [15]
	= Hypnotic action of pentobarbital	Blednov et al. [16]
	Benzodiazepine	
	↑ Anxiolytic action of diazepam	Kralic et al. [101]
	↑ Sedative action of diazepam	Kralic et al. [101], Reynolds et al. [102]
	↓ Anticonvulsant action of diazepam	Kralic et al. [101]
	↑ Hypnotic action of diazepam	Kralic et al. [101]
	↓ Hypnotic action of flurazepam	Blednov et al. [16]
	↓ Hypnotic action of midazolam	Kralic et al. [15]
	↓ Hypnotic action of zolpidem	Blednov et al.[16], Kralic et al. [101]
	Neuroactive steroid	
	= Anticonvulsant action of allopregnanolone	Kralic et al. [101]
	= Hypnotic action of pregnanolone	Kralic et al. [101]
	= Hypnotic action of alphaxalone	Boehm II et al., unpublished
	Intravenous and volatile anesthetic	
	↓ Hypnotic action of etomidate—females only	Kralic et al. [15]
	= Hypnotic action of etomidate	Blednov et al. [16]
	= Hypnotic action of propofol	Kralic et al. [15]
	Ethanol	
	↓ Ethanol consumption—females only	Blednov et al. [14]
	↑ Ethanol aversion	Blednov et al. [14]
	= Ethanol conditioned place preference	Blednov et al. [14]
	↑ Locomotor stimulant action of ethanol	Blednov et al. [14], Kralic et al. [15]
	= Chronic ethanol withdrawal	Blednov et al. [14]
	= Anxiolytic action of ethanol	Kralic et al. [15]
	= Motor incoordinating action of ethanol	Kralic et al. [15]
	= Anticonvulsant action of ethanol	Kralic et al. [15]
	= Acute tolerance to motor incoordinatining	Kralic et al. [15]
	action of ethanol	Kralic et al. [15]
	↓ Acute ethanol withdrawal—males only	Blednov et al., unpublished
	= Hypnotic action of ethanol	Kralic et al. [15]
	↓ hypnotic action of ethanol—males only	Blednov et al. [16]
α2	Ethanol	
	= Ethanol preference drinking	Current report
	= Anxiolytic action of ethanol	Current report
	= Acute ethanol withdrawal	Current report
	↓ Hypnotic action of ethanol	Current report
α3	Currently unavailable	
α4	Currently unavailable	
α5	Ethanol	
	↓ Ethanol preference drinking—males only	Current report
	↑ Locomotor stimulant action—elevated plus maze	Current report
	= Anxiolytic action of ethanol	Current report
	= Acute tolerance to motor incoordinating	Boehm II et al., unpublished
	action of ethanol	
	↓ Acute ethanol withdrawal	Current report
	= Hypnotic action of ethanol	Current report

Appendix A (*Continued*)

Subunit	Drug phenotype	References
α6	Barbiturate	
	= Hypnotic action of pentobarbital	Homanics et al. [36]
	Benzodiazepine	
	↑ Motor incoordinating action of diazepam	Korpi et al. [103]
	= Chronic tolerance to motor incoordinating action of ethanol	Vekovischeva et al. [104]
	Intravenous and volatile anesthetic	
	= Immobilizing action of enflurane	Homanics et al. [36]
	= Hypnotic action of enflurane	Homanics et al. [36]
	= Hypnotic action of halothane	Homanics et al. [36]
	Ethanol	
	= Chronic ethanol withdrawal	Homanics et al. [41]
	= Acute tolerance to motor incoordinating action of ethanol	Homanics et al. [41]
	= Protracted tolerance to hypnotic action of ethanol following chronic ethanol exposure	Homanics et al. [41]
	= Hypnotic action of ethanol	Homanics et al. [36]
β1	Currently unavailable	
β2	GABA-A agonist/antagonist	
	↓ Hypnotic action of THIP	Blednov et al. [16]
	Barbiturate	
	= Hypnotic action of pentobarbital	Blednov et al. [16]
	Benzodiazepine	
	↓ Hypnotic action of flurazepam	Blednov et al. [16]
	↓ Hypnotic action of zolpidem	Blednov et al. [16]
β2	Neuroactive steroid	
	= Hypnotic action of alphaxalone	Boehm II et al., unpublished
	Intravenous and volatile anesthetic	
	↓ Hypnotic action of etomidate	Blednov et al. [16]
	= Hypnotic action of etomidate	O'Meara et al. [46]
	↓ Locomotor sedative actions of etomidate	O'Meara et al. [46]
	Ethanol	
	= Ethanol consumption	Blednov et al. [14]
	= Ethanol aversion	Blednov et al. [14]
	= Ethanol conditioned place preference	Blednov et al. [14]
	= Locomotor stimulant action of ethanol	Blednov et al. [14]
	= Chronic ethanol withdrawal	Blednov et al. [14]
	↑ Acute ethanol withdrawal	Blednov et al., unpublished
	↓ Hypnotic action of ethanol—males only	Blednov et al. [16]
β3	GABA-A agonist/antagonist	
	↓ Antinociceptive action of THIP	Ugarte et al. [105]
	Barbiturate	
	= Hypnotic action of pentobarbital	Quinlan et al. [53]
	Benzodiazepine	
	↓ Hypnotic action of midazolam	Quinlan et al. [53]
	↓ REM sleep recuding action of midazolam	Wisor et al. [106]
	Neuroactive steroid	
	Intravenous and volatile anesthetic	

Appendix A (*Continued*)

Subunit	Drug phenotype	References
	↓ Hypnotic action of etomidate	Quinlan et al. [53]
	↓ Immobilizing action of enflurane	Quinlan et al. [53]
	= Hypnotic action of enflurane	Quinlan et al. [53]
	↓ Immobilizing action of halothane	Quinlan et al. [53]
	= Hypnotic action of halothane	Quinlan et al. [53]
	Ethanol	
	= Hypnotic action of ethanol	Quinlan et al. [53]
γ2S	Benzodiazepine	
	= Motor incoordinating action of Flunitrazepam (Tg)	Wick et al. [57]
	Ethanol	
	↓ Acute tolerance to motor incoordinating action of ethanol (Tg)	Wick et al. [57]
γ2L	Barbiturate	
	= Hypnotic action of pentobarbital	Quinlan et al. [107]
	Benzodiazepine	
	↑ Hypnotic action of midazolam	Quinlan et al. [107]
	↑ Hypnotic action of zolpidem	Quinlan et al. [107]
	= Motor incoordinating action of flunitrazepam (Tg)	Wick et al. [57]
	Intravenous and volative anesthetic	
	= Hypnotic action of etomidate	Quinlan et al. [107]
	Ethanol	
	= Anxiolytic action of ethanol	Homanics et al. [58]
	= Acute tolerance ot motor incoordinating action of ethanol	Homanics et al. [58]
	= Chronic ethanol withdrawal severity	Homanics et al. [58]
	= Locomotor stimulant action of ethanol	Homanics et al. [58]
	= Hypnotic action of ethanol	Homanics et al. [58]
	↓ Acute tolerance to motor incoordinating action of ethanol (Tg)	Wick et al. [57]
	= Acute ethanol withdrawal (Tg)	Wick et al. [57]
	= Hypnotic action of ethanol (Tg)	Wick et al. [57]
δ	GABA-A agonist/antagonist	
	↑ Pentylenetetrazol-induced siezure susceptibility	Spigelman et al. [68]
	↓ Hypnotic action of THIP	Boehm II et al., unpublished
	Barbiturate	
	= Hypnotic action of pentobarbital	Mihalek et al. [62]
	Benzodiazepine	
	= hypnotic action of midazolam	Mihalek et al. [62]
	Neuroactive steroid	
	↓ Hypnotic action of alphaxalone	Mihalek et al. [62]
	↓ Hypnotic action of pregnanolone	Mihalek et al. [62]
	↓ Anxiolytic action of ganaxolone	Mihalek et al. [62]
	↓ Ganaxolone prolongation of pentylenetetrazol-induced seizure	Mihalek et al. [62]
	Intravenous and volatile anesthetic	
	= Hypnotic action of etomidate	Mihalek et al. [62]

Appendix A (*Continued*).

Subunit	Drug phenotype	References
	= Immobilizing action of enflurane	Mihalek et al. [62]
	= Hypnotic action of enflurane	Mihalek et al. [62]
	= Immobilizing action of halothane	Mihalek et al. [62]
δ	Intravenous and volatile anesthetic	
	= Hypnotic action of halothane	Mihalek et al. [62]
	Ethanol	
	↓ Ethanol consumption	Mihalek et al. [69]
	↓ Chronic ethanol withdrawal severity	Mihalek et al. [69]
	↓ Anticonvulsant action of ethanol	Mihalek et al. [69]
	= Anxiolytic action of ethanol	Mihalek et al. [69]
	= Hypothermic action of ethanol	Mihalek et al. [69]
	= Chronic tolerance to hypnotic action of	Mihalek et al. [69]
	Ethanol	
	= Acute tolerance to motor incoordinating action of ethanol	Mihalek et al. [69]

Results are from knock-out mice unless otherwise indicated. Results from transgenic (over-expressing) mice are designated by (Tg).

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