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Alcohol-induced motor impairment caused by increased extrasynaptic $GABA_A$ receptor activity

H Jacob Hanchar[1,3], **Paul D Dodson**[2,3], **Richard W Olsen**[1], **Thomas S Otis**[2], and **Martin Wallner**[1]

[1] Department of Molecular and Medical Pharmacology, David Geffen School of Medicine, University of California, Los Angeles, California 90095, USA

[2] Department of Neurobiology, David Geffen School of Medicine, University of California, Los Angeles, California 90095, USA

Abstract

Neuronal mechanisms underlying alcohol intoxication are unclear. We find that alcohol impairs motor coordination by enhancing tonic inhibition mediated by a specific subtype of extrasynaptic $GABA_A$ receptor (GABAR), α6β3δ, expressed exclusively in cerebellar granule cells. In recombinant studies, we characterize a naturally occurring single-nucleotide polymorphism that causes a single amino acid change (R100Q) in α6 (encoded in rats by the *Gabra6* gene). We show that this change selectively increases alcohol sensitivity of α6β3δ GABARs. Behavioral and electrophysiological comparisons of *Gabra6*$^{100R/100R}$ and *Gabra6*$^{100Q/100Q}$ rats strongly suggest that alcohol impairs motor coordination by enhancing granule cell tonic inhibition. These findings identify extrasynaptic GABARs as critical targets underlying low-dose alcohol intoxication and demonstrate that subtle changes in tonic inhibition in one class of neurons can alter behavior.

Humans have been consuming alcohol for thousands of years, and the use of alcoholic beverages pervades human culture and society and can have substantial health effects[1]. Different mechanisms by which ethanol might depress brain function have been proposed based on ethanol's ability to modulate a wide variety of ion channels[2–4], neurotransmitter receptors[5–10] and transporters[11]. Among these diverse targets, however, GABARs are arguably the most attractive candidates. This is in part because other classes of known GABAR modulators such as benzodiazepines, barbiturates and certain anesthetics lead to behavioral effects that closely resemble ethanol intoxication. Yet despite strong evidence implicating GABARs in ethanol's action, critical details remain unclear. For instance, although it is known that native GABARs are heteropentamers assembled from 19 possible subunits[12,13], it has not been possible to link the activity of particular GABAR subunits to changes in behavioral sensitivity to ethanol.

Recent studies suggest that specific combinations of GABAR subunits (those containing α4β3δ and α6β3δ) are uniquely sensitive to ethanol, showing dose dependencies that mirror blood alcohol levels associated with intoxication in humans[9,10]. GABARs containing α4 and δ subunits are expressed in many brain regions[14,15], but α6 is found in only two types of neurons (cerebellar granule cells and granule cells in the cochlear nucleus) and is expressed together with δ only in cerebellar granule cells[14,16,17]. In granule cells α6 and δ combine with β subunits

Correspondence should be addressed to M.W. (mwallner@mednet.ucla.edu) or T.S.O. (otist@ucla.edu).
[3]These authors contributed equally to this work.

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to give rise to high-affinity[18] extrasynaptic GABARs[19–21]. These GABARs generate a tonic inhibitory conductance[20–22] that exerts strong control over granule cell firing patterns *in vivo*[23].

We set out to examine whether such extrasynaptic GABARs containing α6 and δ subunits account for behavioral effects of ethanol at moderately intoxicating doses. To link these particular GABARs to behavioral sensitivity, we first characterized a naturally occurring single-nucleotide polymorphism in the gene encoding rat α6 (*Gabra6*). This polymorphism is of interest because it has been reported to be present in alcohol-nontolerant (ANT) rats[24] and enriched in Sardinian alcohol-nonpreferring rats[25], two lines of animals selectively bred either for heightened ethanol-induced motor impairment (ANT rats) or for aversion to ethanol (Sardinian nonpreferring rats). A single nucleotide change (from a guanine to an adenine) leads to a single amino acid substitution, from arginine (R) to glutamine (Q), at amino acid position 100 in α6. The α6-R100Q polymorphism causes a marked increase in benzodiazepine impairment of motor coordination *in vivo* and has been shown to convert recombinant GABARs containing α6 and γ subunits from benzodiazepine insensitive to benzodiazepine sensitive[24]. However, because it has not been shown that this polymorphism affects ethanol sensitivity in recombinant or native GABARs, it has been suggested that other co-segregating polymorphisms might be responsible for the increased ethanol sensitivity in ANT rats[26,27].

We report here that the α6-R100Q polymorphism further enhances the ethanol sensitivity of a specific subtype of GABAR composed of α6, β3 and δ subunits. Notably, we found that the α6-R100Q polymorphism is common in outbred strains of Sprague-Dawley rats. This allowed us to obtain rats homozygous for each of the two alleles and then to test ethanol sensitivity *in situ*. We showed that behaviorally-relevant concentrations of ethanol enhance tonic inhibition mediated by extrasynaptic GABARs in cerebellar granule cells from *Gabra6*$^{100R/100R}$ rats and cause an even larger increase in tonic inhibition in granule cells from *Gabra6*$^{100Q/100Q}$ rats. Finally, in a cerebellum-dependent behavioral task we found that ethanol more severely impairs coordination in *Gabra6*$^{100Q/100Q}$ rats. Together these findings imply that small increases in a tonic GABA conductance in a single class of cerebellar neurons can account for the adverse effects of ethanol on motor coordination. The results strongly imply that similar, more widely expressed isoforms of extrasynaptic GABARs are critical components of ethanol intoxication.

RESULTS

α 6-R100Q polymorphism enhances ethanol sensitivity

The ethanol sensitivities of heteromeric GABARs containing the α6-100R and α6-100Q polymorphisms were tested by expressing each variant in *Xenopus laevis* oocytes together with a β subunit and either δ or a γ2 subunit. We evaluated ethanol dose-response curves for several combinations of GABAR subunits thought to exist in granule cells (α6β2δ, α6β3δ, α6β2γ2 and α6β3γ2)[16,28,29] as well as other combinations that may be present in these cells (Fig. 1 and Table 1). Consistent with published results[24], we found that introduction of the α6-R100Q polymorphism into GABARs composed of α6, β3 and γ2 gave rise to benzodiazepine-sensitive receptors (data not shown). However, the low ethanol sensitivity of α6(100R)β3γ2 GABARs was unchanged in α6(100Q)β3γ2 receptors (Table 1).

In contrast, for the highly ethanol-sensitive α6β3δ GABARs[10], we found that changing amino acid position 100 in α6 from R to Q leads to a further increase in ethanol sensitivity (Fig. 1). Notably, α6β3δ receptors were the only GABARs that showed a significant change in ethanol sensitivity in response to the α6-R100Q polymorphism (Fig. 1 and Table 1). In particular, α6β2δ receptors, another species of GABAR that may contribute to tonic GABA currents in granule cells, were unaffected.

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Ethanol enhances tonic inhibition mediated by α6β3δ GABARs

Based on these results, we hypothesized that the presence of this polymorphism in α6 should enhance ethanol sensitivity of tonic GABA conductances in cerebellar granule cells, the only class of neurons in the brain that express α6 together with β3 and δ subunits. We fortuitously discovered that the *Gabra6*^{100Q} allele is present in outbred Sprague-Dawley rats obtained from Charles River (Fig. 2). Genotyping 35 rats showed that 10 were *Gabra6*$^{100R/100R}$, 11 were *Gabra6*$^{100Q/100Q}$ and 14 were heterozygotes. Whole-cell recordings were made from granule cells in cerebellar slices prepared from rats of the two homozygous genotypes (Fig. 3). We tested ethanol concentrations that would result in moderate to severe intoxication (30 and 100 mM) and found that they enhanced tonic GABA currents in granule cells from rats of both genotypes. However, the enhancement was significantly larger in granule cells from *Gabra6*$^{100Q/100Q}$ rats (Fig. 3b,c). Consistent with a recent report[30], ethanol also enhanced the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) in granule cells, and the mean increases in frequency were larger in recordings from the mutant rats (Fig. 3d). We did not find any ethanol-induced changes in the mean amplitudes of sIPSCs nor in their decay rates. These findings demonstrate that tonic GABA current is sensitive to low concentrations of ethanol in wild-type rats and that the presence of R100Q in α6 subunits renders tonic GABA current even more sensitive to ethanol. Furthermore, the increased sIPSC frequency in *Gabra6*$^{100Q/100Q}$ compared with *Gabra6*$^{100R/100R}$ rats is likely to result indirectly from changes in granule cell excitability, as α6 is not expressed in any other cerebellar cell type[14, 16].

Low ethanol doses act directly on extrasynaptic GABARs

In recombinant studies, we have shown that wild-type GABARs containing α4 or α6 along with β3 and δ subunits are enhanced by 10 mM ethanol, a concentration below the legal maximally permitted blood alcohol level for motor vehicle operation in many countries (typically 0.08% (wt/vol) or 17.4 mM). To examine postsynaptic effects of such low doses of ethanol on tonic GABA currents, we performed experiments in the presence of 0.5 μM tetrodotoxin, 2 μM NBQX, 300 nM added GABA and 10 μM NO-711, a GABA transporter antagonist. Significant enhancement by 10 mM ethanol was observed for *Gabra6*$^{100R/100R}$ granule cells, and even larger increases were observed for *Gabra6*$^{100Q/100Q}$ granule cells (Fig. 4). Together, these results indicate that increases in tonic GABA current could be detected independent of changes in GABA release and in response to concentrations of ethanol associated with minimal intoxication.

Ethanol more severely impairs behavior in *Gabra6*$^{100Q/100Q}$ rats

To explore whether increases in granule cell tonic inhibition in response to alcohol could account for motor behaviors associated with intoxication, we compared *Gabra6*$^{100R/100R}$ and *Gabra6*$^{100Q/100Q}$ rats in cerebellar and non-cerebellar behavioral tests. In the accelerating rotarod test, a cerebellum-dependent behavioral assay, we found that low ethanol concentrations impaired *Gabra6*$^{100R/100R}$ rats in a dose- and time-dependent manner (Fig. 5a–d). Administration of identical doses of ethanol to *Gabra6*$^{100Q/100Q}$ rats led to significantly larger impairment on the rotarod at the three doses shown (0.75, 1 and 1.25 g kg^{-1}). Blood ethanol concentrations obtained in these behavioral tests 25–30 min after administration of 1g kg^{-1} ethanol (15.2 ± 2 mM in *Gabra6*$^{100R/100R}$, 16.4 ± 2 mM in *Gabra6*$^{100Q/100Q}$, $P = 0.34$, $n = 4$ and 5, respectively) confirmed that plasma concentrations of ethanol were not affected by genotype and showed that the concentrations achieved were the equivalent of mildly intoxicating doses in humans. In contrast, hypersensitivity to ethanol was not found in the loss of righting reflex (LORR) assay, a common behavioral test for sedative effects that arise principally from non-cerebellar brain areas (Fig. 5e,f).

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Our finding that this single amino acid difference is sufficient for increased ethanol sensitivity of motor coordination suggests that it makes an important contribution to the ANT phenotype. Moreover, the α6-R100Q polymorphism enhances ethanol sensitivity only in GABARs composed of α6, β3 and δ subunits. As receptors of this molecular makeup are found only in extrasynaptic membrane of granule cells, the tonic GABA current carried they carry must reduce the excitability of this specific class of cerebellar neurons to account for the hypersensitive phenotype.

DISCUSSION

Mechanisms underlying the effects of ethanol

Two general hypotheses have been put forward to explain how ethanol depresses neuronal activity. One proposes an indirect influence on ion channels and receptors through nonspecific interactions with membrane lipids. An alternative hypothesis is that ethanol interacts with specific, saturable binding sites on receptor or ion channel proteins. Many targets have been proposed including potassium channels[2–4], glutamate-gated channels[6], glycine-gated[7] channels and $GABA_A$ receptors[5,8–10]. However, not all of these channels respond to the low concentrations of ethanol associated with mild intoxication, and it is unclear how ethanol acting at these various sites alters behavior. Our results suggest that extrasynaptic GABARs are important targets for mildly intoxicating concentrations of ethanol. This is dramatically demonstrated by the *Gabra6*$^{100Q/100Q}$ rats, in which a single amino acid change in an extrasynaptic GABA receptor subtype expressed in one class of cerebellar neurons (granule cells) leads to the predicted enhancement of ethanol sensitivity of granule cell tonic inhibition and of cerebellum-dependent behavior.

Link between tonic inhibition and behavior

Tonic inhibition mediated by extrasynaptic, δ subunit–containing GABARs has been identified in a number of neurons[31–35] and is thought to regulate neuronal excitability; however, the role of tonic inhibition in shaping behavior is unclear. The results reported here were obtained from cerebellar granule cells, a cell type for which there is strong evidence that receptors containing α6 and δ subunits are extrasynaptic[16,19,36] and are required for tonic GABA current[20,21]. We demonstrate that tonic current is facilitated by low ethanol concentrations and, in addition, identify a variant of α6 (α6-100Q) that renders tonic current carried by α6β3δ GABARs much more sensitive to ethanol. Behavioral analysis suggests that enhancement of this specific extrasynaptic GABAR subtype in granule cells results in motor impairment. How do modest changes in tonic inhibition of these neurons lead to such pronounced behavioral differences? First, granule cells have extremely high input resistance; only small changes in conductance are required to affect excitability. Second, tonic inhibition seems to influence the gain of the input-output relationship in addition to shifting the amount of excitation required for granule cell firing[37]. Ethanol is not the only compound reported to selectively increase tonic inhibition in granule cells. Tonic currents have also been shown to be highly sensitive to endogenous neuroactive steroids[21], raising the possibility that these compounds could similarly affect motor behavior. Future studies using such specific endogenous and exogenous modulators should provide insight into the role of extrasynaptic GABARs in influencing information processing in the granule cell layer.

Gabra6^{100Q} allele and behavioral sensitivity to ethanol

Through selective breeding, several lines of rodents that show ethanol hypersensitivity have been generated. In two such lines, the ANT and the Sardinian nonpreferring rats, the *Gabra6*^{100R} and *Gabra6*^{100Q} alleles have been found to segregate into ethanol-hyposensitive and -hypersensitive groups, respectively. For the Sardinian nonpreferring rats, it is a partial segregation[25], whereas for the alcohol tolerant (AT)/alcohol non-tolerant (ANT) line, almost

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all of the ANT rats are homozygous for the *Gabra6*^{100Q} allele[24]. However, because the α6-R100Q polymorphism had not been shown to affect ethanol sensitivity of recombinant receptors[24] and because backcrosses of ANT/AT rats had led researchers to question the correlation between this polymorphism and the ethanol-hypersensitive phenotype, it has been concluded that genetic differences other than the one causing the α6-R100Q polymorphism were important[27,38].

It remains to be determined whether the α6 polymorphism accounts for all aspects of the ANT phenotype and whether it contributes to complex behaviors that do not obviously involve the cerebellum (such as alcohol preference in the Sardinian line or anxiety in ANT rats[39]). In these selectively bred lines, control and experimental groups of rats are typically isolated by more than 40 generations of breeding, a strategy likely to segregate multiple genetic loci. In the present study, control (*Gabra6*$^{100R/100R}$) and experimental (*Gabra6*$^{100Q/100Q}$) groups were identified only by genotype with respect to this one gene. As is the case for all knockout and 'knock-in' animal studies, we cannot completely exclude the possibility that other alleles near the genetic locus of interest cosegregate and contribute to cellular and behavioral phenotypes. However, considered with the positive evidence provided here that α6-100Q markedly increases ethanol sensitivity of recombinant α6β3δ receptors and tonic current in cerebellar granule cells, it seems very likely that the α6-100Q polymorphism is responsible for the additional ethanol-induced motor impairment.

Our findings suggest that mice deficient in the *Gabra6* gene product might show less ethanol-induced motor impairment. High doses of ethanol (2 g kg^{-1}) have been reported to similarly impair wild-type and *Gabra6*$^{-/-}$ mice in rotarod tests[40], and at ethanol doses of 3.5 g kg^{-1}, no significant differences have been observed between wild type and *Gabra6*$^{-/-}$ in a LORR test[41,42]. At such high ethanol doses, we also find no change for *Gabra6*$^{100R/100R}$ and *Gabra6*$^{100Q/100Q}$ rats in rotarod performance (data not shown) or in LORR (Fig 5e,f). It is possible that *Gabra6*$^{-/-}$ mice will show less impairment on the rotarod in a lower range of ethanol doses, but there are reasons to believe that ethanol sensitivity of knockout mice may be complicated. First, the expression of GABAR subunits α1, β2, γ2 and δ is changed markedly in *Gabra6*$^{-/-}$ mice[19,36,43], and other potential ethanol targets such as two-pore domain K^+ channels[20] show increased function in granule cells, making it difficult to rule out compensatory changes in *Gabra6*$^{-/-}$ mice. Second, differences in cerebellum-dependent behavior at moderate ethanol doses may be obscured in the *Gabra6*$^{-/-}$ mouse by other more abundant and widely expressed ethanol targets. These and other high-affinity ethanol targets may make it more difficult to detect behavioral changes in animals lacking the α6 gene than in animals having the ethanol-hypersensitive polymorphism, in which very low doses of ethanol selectively impair motor behavior (as in Fig. 5b).

The observation that a specific combination of GABAR subunits forms an important ethanol target in cerebellum is similar to recent results showing that certain anesthetics and benzodiazepines act on heteromeric GABARs composed of particular subunits. Some of these studies make use of mice engineered to carry single amino acid changes in positions homologous to the R100Q site in α6. Point mutations at these sites, which are known to form part of a high-affinity binding pocket for benzodiazepines at the α-γ2 interface in the pentameric receptor, render GABARs containing those particular subunits insensitive to benzodiazepines. The resulting 'knock-in' mice show specific behavioral insensitivities in response to diazepam[13], implying that important clinical properties of diazepam such as sedation[44,45], amnesia[44] and anxiolysis[46] are attributable to GABARs containing particular α subunits. A similar strategy involving mice carrying a point mutation in β3 shows that this subunit is necessary for the anesthetic actions of etomidate[47]. The naturally occurring α6-R100Q mutation (polymorphism) in rats allowed us to examine the roles of α6 subunits and of granule cells in ethanol-induced impairment of cerebellar function. Like the mice with experimentally

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introduced ('knock-in') point mutations discussed above, these rats should prove to be useful tools for examining the role of α6 in certain features of ethanol and benzodiazepine intoxication.

Ethanol sensitivity of other extrasynaptic GABARs

Extrasynaptic GABARs composed of α4, β3 and δ subunits are likely to be found in neurons located in the thalamus[3], dentate gyrus[21], striatum[14,15] and cerebral cortex[15]. As such GABARs are also sensitive to low ethanol concentrations[9,10,48], we predict that ethanol-induced increases in tonic inhibition in these neuronal populations may contribute to sedative-hypnotic and anxiolytic effects and to the depressant actions of this drug on higher cognitive functions.

METHODS

Electrophysiology

Standard methods were used for isolation, injection and recordings from *X. laevis* oocytes and for preparation of cRNA[10]. For brain slice experiments, 300 μm parasagittal slices of cerebellum were prepared from 24- to 42-day-old Sprague-Dawley rats using standard techniques[20,21] with the exceptions that slicing solution consisted of (in mM) 250 sucrose, 26 $NaHCO_3$, 10 glucose, 4 $MgCl_2$, 3 myoinositol, 2.5 KCl, 2 sodium pyruvate, 1.25 NaH_2PO_4, 0.5 ascorbic acid, 0.1 $CaCl_2$, and 0.001 D,L-APV. Slice storage and recording solutions were saturated with 95% O_2/5% CO_2 and consisted of (in mM) 119 NaCl, 26 $NaHCO_3$, 11 glucose, 2.5 KCl, 2.5 $CaCl_2$, 1.3 $MgCl_2$, and 1 NaH_2PO_4; in addition, storage solution contained 0.001 D,L-APV. All procedures were in accordance with a protocol approved by the University of California at Los Angeles (UCLA) Chancellor's Animal Research Committee. For voltage-clamp recordings (holding potential −70 mV, 20–23 °C), whole-cell pipettes contained (in mM) 140 CsCl, 10 HEPES, 1 EGTA, 4 magnesium ATP, 0.4 GTP, titrated to pH 7.3 with CsOH. Recording pipettes had a bath resistance of 6–10 MΩ.

Whole-cell data were filtered at 5 kHz and acquired at a sampling rate of 20 kHz. Analysis was conducted using customized routines written in IGOR Pro 4.0 (Wavemetrics). Tonic GABAR-mediated current was defined as the steady-state current blocked by 10 μM SR95531; its magnitude was calculated by plotting all-point histograms of relevant 30-s segments of data (as in Figs. 3,4). These data were fit to the Gaussian equation, constraining fits to values 2 bins more negative than the peak. This ensured that the tail of higher amplitude values (representing sIPSCs) did not influence the fit. The effects of 10 mM ethanol on tonic current (Fig. 4) were compared with changes in tonic current observed over otherwise identical sham perfusion periods.

Genotyping

After isolation of genomic DNA from ear snips, the exon coding for the α6-100 position was amplified with primers designed to be located in introns flanking the region of interest (to avoid amplification of mRNA). The PCR fragment was sequenced using standard fluorescent dye sequencing.

Behavior

Rats were housed with food and water ad libitum in a 12 h/12 h light/dark cycle. Homozygous male and female rats (*Gabra6*$^{100R/100R}$ and *Gabra6*$^{100Q/100Q}$, > P55) were used for the rotarod (MedAssociates) and sleep time (LORR) studies. These rats were either obtained directly from a breeding colony at Charles River Laboratories or bred at UCLA. In the accelerating rotarod test, the speed of rotation increases at a constant rate from 4 to 40 r.p.m. over 5 min. All rats used in the rotarod tests were naive to ethanol and were used to test only one condition. Blood

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samples (20–50 μl) were taken from the tail and serum ethanol concentration was determined with an Analox enzymatic blood alcohol analyzer.

Statistics

Values are reported as mean ± s.e.m. unless noted otherwise. To evaluate the effect of ethanol dose and time in the rotarod experiments, we used a general linear model (GLM) with repeated measures. The Wilk's lambda multivariate test was used to test the effect of the ethanol dose–time interaction. These statistical analyses were conducted using SPSS for Windows version 12. All other statistical comparisons were conducted using paired and unpaired Student's *t*-tests as appropriate.

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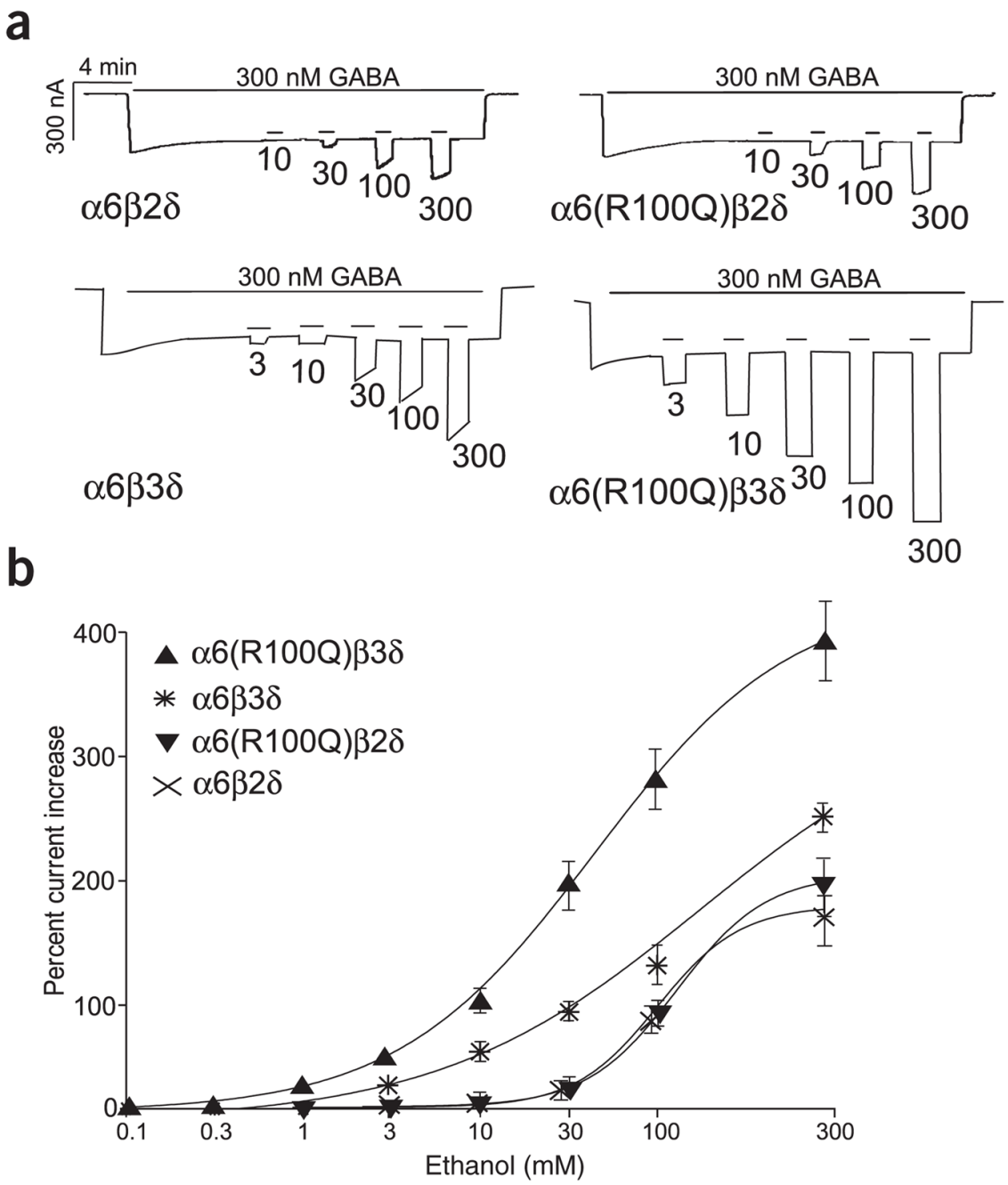

Figure 1.

The α6-R100Q polymorphism leads to a marked increase in ethanol sensitivity when expressed with β3 and δ subunits. (**a**) GABARs of the indicated subunit compositions were expressed in *X. laevis* oocytes and activated by steady-state GABA (300 nM ~EC_{30}). Brief applications of ethanol at the indicated concentrations (in mM) enhance the current in a dose-dependent manner. (**b**) Dose-response curves for ethanol showing the peak enhancement of GABA current. Shown are wild-type and mutant versions of subunit combinations that are likely to be responsible for tonic GABA current in granule cells: α6β2δ (cross, *n* = 6), α6β3δ (asterisk, *n* = 10), α6(R100Q)β2δ (inverted triangles, *n* = 7) and α6(R100Q)β3δ (upright triangles, *n* = 8). Note that the β3 and δ subunits are required for the R100Q mutation to exert an effect and

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that replacement of β2 with β3 leads to an almost tenfold increase in ethanol sensitivity. Other combinations of subunits expressed in granule cells are summarized in Table 1.

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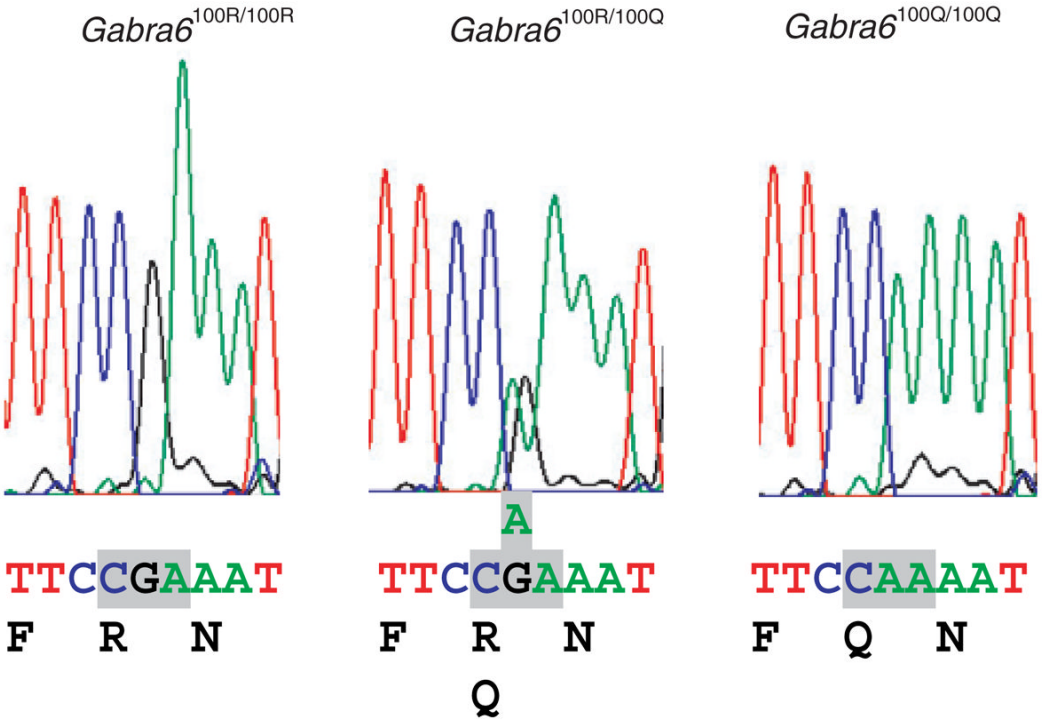

Figure 2.

A single-nucleotide polymorphism (a guanine-to-adenine substitution) in the gene encoding the rat $GABA_A$ receptor α6 subunit (*Gabra6*) is common in Sprague-Dawley rats obtained from Charles River Laboratories. Shown from left to right are the sequence chromatograms from a homozygous *Gabra6*$^{100R/100R}$, a heterozygous *Gabra6*$^{100R/100Q}$ and a homozygous *Gabra6*$^{100Q/100Q}$ rat. The lower panel shows the sequence of each *Gabra6* genotype along with the corresponding amino acid translation (bottom). Gray boxes denote the codon for amino acid position 100.

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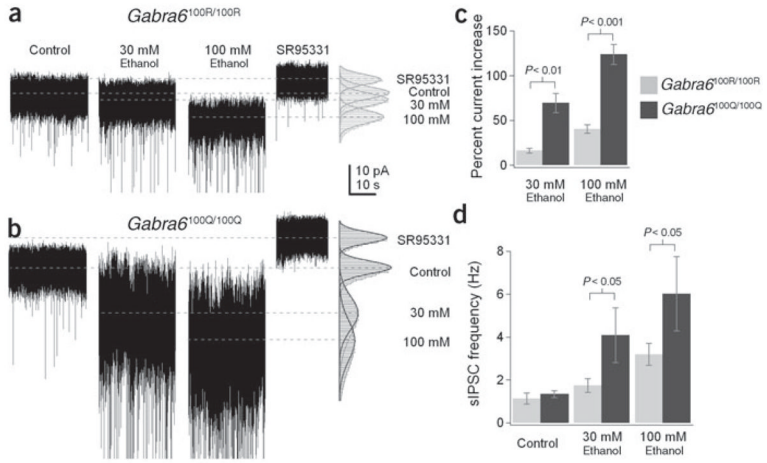

Figure 3.

Ethanol enhances granule cell tonic GABA current, and the enhancement is larger in *Gabra6*$^{100Q/100Q}$ rats. (**a**,**b**) Tonic GABA currents in granule cells recorded from *Gabra6*$^{100R/100R}$ (**a**) or *Gabra6*$^{100Q/100Q}$ (**b**) slices in the presence of indicated concentrations of ethanol or in a saturating concentration (10μM) of the GABAR antagonist SR95531. To the right are histograms of all points in each segment. Gaussian functions have been fit to each condition and are superimposed. The dashed lines indicate the mean current from these fits. (**c**) Summary of the mean ± s.e.m. percentage change in tonic current amplitude caused by 30 or 100 mM ethanol in the two genotypes (wild type, $n = 5$; mutant, $n = 7$ granule cells). Ethanol caused a significantly larger enhancement of mutant versus wild-type currents. Mean tonic GABA current under control conditions was −9.7 ± 1.7 pA and did not differ significantly between the two genotypes ($P > 0.4$). (**d**) Ethanol-induced increases in sIPSC frequency are significantly larger in mutant granule cells (*Gabra6*$^{100R/100R}$, $n = 5$; *Gabra6*$^{100Q/100Q}$, $n = 7$ granule cells). No significant changes were observed in either the amplitude (*Gabra6*$^{100R/100R}$, 112.43 ± 12.57% of control, $P > 0.49$, $n = 5$; *Gabra6*$^{100Q/100Q}$, 101.55 ± 2.42% of control, $P > 0.7$, $n = 7$) or the decay of sIPSCs (*Gabra6*$^{100R/100R}$, 90 ± 12% of control, $P > 0.42$, $n = 4$; *Gabra6*$^{100Q/100Q}$, 96 ± 18% of control, $P > 0.77$, $n = 6$) in ethanol (data not shown).

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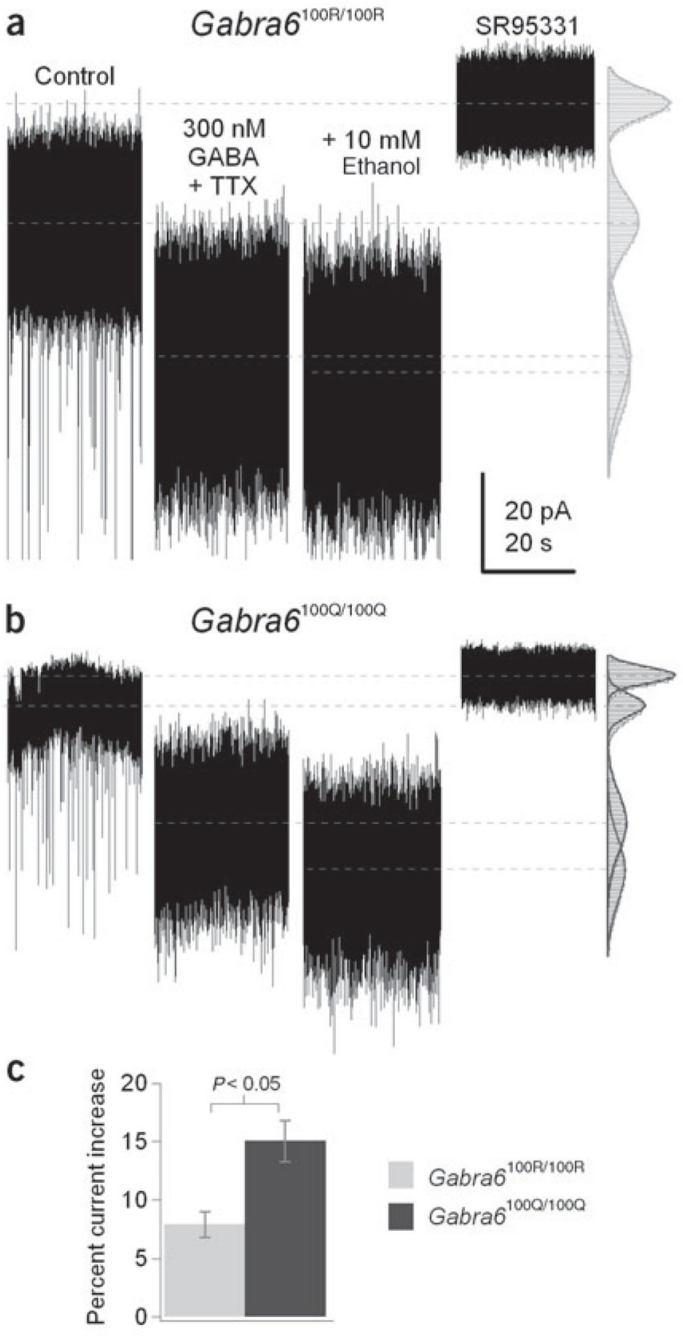

Figure 4.

Tonic GABA current in granule cells is enhanced by low concentrations of ethanol in *Gabra6*$^{100R/100R}$ and *Gabra6*$^{100Q/100Q}$ rats. (**a**,**b**) Tonic currents recorded from *Gabra6*$^{100R/100R}$ (**a**) or *Gabra6*$^{100Q/100Q}$ (**b**) granule cells showing that 10 mM ethanol enhances the tonic current in the continuous presence of 300 nM GABA, TTX, NBQX and NO-711. (**c**) Summary data indicating that in response to 10 mM ethanol, significant increases in tonic current are observed in *Gabra6*$^{100R/100R}$ granule cells ($P < 0.05$, $n = 4$) and larger enhancements are found in *Gabra6*$^{100Q/100Q}$ granule cells ($P < 0.05$, $n = 4$).

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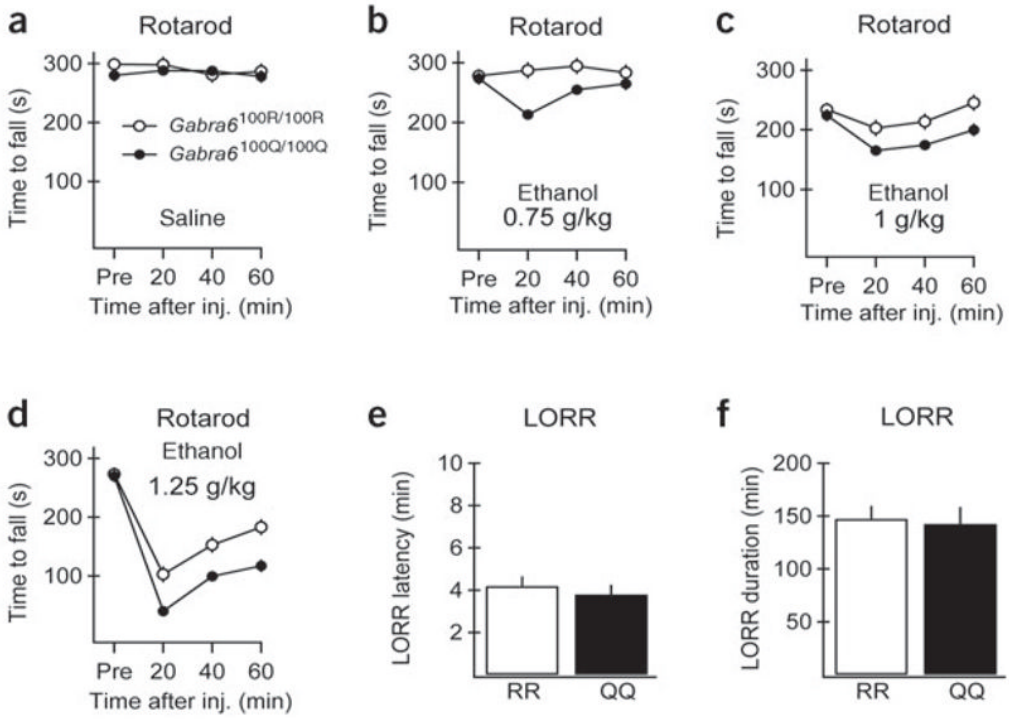

Figure 5.

Rats homozygous for the α6-100Q polymorphism show increased alcohol-induced motor impairment as compared with *Gabra6*$^{100R/100R}$ rats. Motor coordination was assessed by testing *Gabra6*$^{100R/100R}$ (open circles) and *Gabra6*$^{100Q/100Q}$ (filled circles) rats in an accelerating rotarod test. (**a**–**d**) Performance was measured as the latency to fall from the rotating rod before ('Pre') and 20, 40 and 60 min after intraperitoneal injection of saline (**a**; $n = 5$ rats each group), 0.75 g kg^{-1} (**b**, $n = 7$ and 8 rats for *Gabra6*$^{100R/100R}$ and *Gabra6*$^{100Q/100Q}$, respectively), 1 g kg^{-1} (**c**; $n = 6$ and 7 rats for *Gabra6*$^{100R/100R}$ and *Gabra6*$^{100Q/100Q}$, respectively) or 1.25 g kg^{-1} ethanol (**d**; $n = 8$ rats each group). Tests on the three postinjection data points yielded the following statistics: saline controls, *Gabra6*$^{100R/100R}$ versus *Gabra6*$^{100Q/100Q}$: $F_{2,7} = 0.50$, $P = 0.63$; 0.75 g kg^{-1} ethanol, *Gabra6*$^{100R/100R}$ versus *Gabra6*$^{100Q/100Q}$: $F_{2,12} = 6.66$, $P = 0.011$; 1 g kg^{-1} ethanol, *Gabra6*$^{100R/100R}$ versus *Gabra6*$^{100Q/100Q}$: $F_{2,10} = 5.41$, $P = 0.026$; 1.25 g kg^{-1} ethanol, *Gabra6*$^{100R/100R}$ versus *Gabra6*$^{100Q/100Q}$: $F_{2,13} = 135.6$, $P < 0.001$. (**e**,**f**) Latency (**e**) and duration (**f**) of LORR was determined after intraperitoneal injection of 3 g kg^{-1} ethanol ($n = 9$ for each group) and did not differ in *Gabra6*$^{100Q/100Q}$ versus *Gabra6*$^{100R/100R}$ rats ($P = 0.51$ for LORR latency, $P = 0.81$ for LORR duration).

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Table 1

Ethanol and GABA sensitivity for GABARs of different subunit combinations[a]

			Percent enhancement by ethanol			
Receptor	**GABA EC_{50} (*n*)**	***n***	**10 mM ethanol**	**30 mM ethanol**	**100 mM ethanol**	**300 mM ethanol**
α6(R100Q)β3δ	0.68 ± 0.1 (5)	8	99.3 ± 15.0	180.1 + 28.2	275.3 ± 32.4	389.2 ± 65.0
α6β3δ	0.70 ± 0.4 (6)	10	41.2 ± 4.3	92.5 ± 9.0	125.3 ± 20.5	245.0 ± 33.6
α6(R100Q)β2δ	0.51 ± 0.09 (5)	7	0	24.5 ± 10.7	97.0 ± 11.2	199.0 ± 38.1
α6β2δ	0.50 ± 0.03 (5)	6	0	23.1 ± 7.9	88.4 ± 15.6	175.0 ± 35.8
α6(R100Q)β1δ	0.62 + 0.04	8	0	24.1 ± 4.0	50.3 ± 7.8	185.2 ± 9.4
α6β1δ	0.56 + 0.07	9	0	21.2 ± 3.3	52.0 ± 5.6	167.9 ± 10.0
α6(R100Q)β2/3$\gamma2_{L/S}$	19 ± 3.5 (6)	16	0	0	40.3 ± 7.8	167.5 ± 14.2
α6β2/3$\gamma2_{L/S}$	19 ± 0.5 (6)	10	0	0	39.0 ± 7.5	182.6 ± 11.3
α1β3δ	0.56 ± 0.05 (4)	8	32.5 ± 5.4	88.2 ± 7.0	117.5 ± 10.6	295.3 ± 19.7
α1β2/3$\gamma2_{L}$	6.8 ± 0.8 (5)	9	0	0	34.4 ± 9.9	147.3 ± 12.9
α1β2/3$\gamma2_{S}$	8.8 ± 1.1 (6)					

[a]For each indicated subunit combination, GABA dose-response relationships were determined by fitting the Hill equation (see Methods). Current enhancement by ethanol was measured by co-application of ethanol and GABA (~EC_{30}). Reported values are mean (±s.d.) percent increases above responses to GABA alone, zeroes represent measurements of no change in current, and *n* indicates the number of oocytes used to determine the average ethanol dose response curves. In cases where the identities of β or γ2 subunits did not lead to differences, results have been pooled.