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Ethanol Modulates Synaptic and Extrasynaptic GABA_A Receptors in the Thalamus

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Abstract

Drinking alcohol is associated with the disturbance of normal sleep rhythms, and insomnia is a major factor in alcoholic relapse. The thalamus is a brain structure that plays a pivotal role in sleep regulation and rhythmicity. A number of studies have implicated GABA_A receptors (GABA_A-Rs) in the anxiolytic, amnesic, sedative, and anesthetic effects of ethanol. In the present study, we examined the effects of ethanol on both synaptic and extrasynaptic GABA_A-Rs of relay neurons in the thalamus. We found that ethanol (≥ 50 mM) elicits a sustained current in thalamocortical relay neurons from the mouse ventrobasal thalamus, and this current is associated with a decrease in neuronal excitability and firing rate in response to depolarization. The steady current induced by ethanol was totally abolished by gabazine and was absent in relay neurons from GABA_A-R α_4 subunit knockout mice, indicating that the effect of ethanol is to enhance tonic GABA-mediated inhibition. Ethanol (50 mM) enhanced the amplitude of tonic inhibition by nearly 50%. On the other hand, ethanol had no effect on spontaneous or evoked inhibitory postsynaptic currents (IPSCs) at 50 mM but did prolong IPSCs at 100 mM. Ethanol had no effect on the paired-pulse depression ratio, suggesting that the release of GABA from presynaptic terminals is insensitive to ethanol. We conclude that ethanol, at moderate (50 mM) but not low (10 mM) concentrations, can inhibit thalamocortical relay neurons and that this occurs mainly via the actions of ethanol at extrasynaptic GABA_A-Rs containing GABA_A-R α_4 subunits.

Drinking alcohol can promote the onset of sleep, but it can also disrupt the normal sleep pattern, increase nocturnal awakenings, and reduce sleep quality (Drummond et al., 1998). Sleep disturbance caused by chronic alcohol can play a role in the progression of alcoholism, and poor sleep quality is often cited as a factor in alcoholic relapse (Brower et al., 1998; Brower, 2001). Inhibition in the thalamus plays an important role in the normal regulation of sleep cycles (Steriade, 2000; Huguenard and McCormick, 2007; Jia et al., 2007) and may, therefore, be involved in both the sedative effects of acute alcohol and in the development of alcoholism.

The inhibitory neurotransmitter GABA has long been implicated in the anxiolytic, amnesic, sedative, and anesthetic effects of alcohol. A large number of studies have investigated the interactions of alcohol with GABA_A receptors (GABA_A-Rs). The standard forms of recombinant GABA_A-Rs that are found at GABAergic synapses ($\alpha_1\beta\gamma_2$ and $\alpha_2\beta\gamma_2$ subtypes) are modulated only by >60 mM ethanol (Sigel et al., 1993; Mihic et al., 1997). Most investigators have failed to observe direct postsynaptic actions of alcohol (<60 mM) on GABA-mediated inhibitory postsynaptic currents (IPSCs) in brain slices, except at high levels

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(Ariwodola and Weiner, 2004; Weiner and Valenzuela, 2006). In several brain areas, however, ethanol has been shown to facilitate synaptic inhibition by a presynaptic mechanism, for example, in the amygdala (Roberto et al., 2003, 2004; Roberto and Siggins, 2006; Zhu and Lovinger, 2006), cerebellum (Carta et al., 2004; Hancher et al., 2005; Ming et al., 2006; Kelm et al., 2007), hippocampus (Ariwodola and Weiner, 2004; Sanna et al., 2004; Galindo et al., 2005), and nucleus accumbens (Nie et al., 2000; Crowder et al., 2002).

A novel form of “tonic inhibition” has also been described in the CNS, which is generated by the persistent activation of extrasynaptic GABA_A-Rs (Semyanov et al., 2004; Farrant and Nusser, 2005; Mody, 2005). GABAergic tonic inhibition has been shown to regulate the excitability of individual neurons and the behavior of neural networks. Tonic inhibition is most often generated by activation of GABA_A-Rs that contain δ subunits, which are normally located at extrasynaptic or perisynaptic sites (Nusser et al., 1998; Wei et al., 2003). Several laboratories have reported that extrasynaptic GABA_A-Rs containing δ subunits are sensitive to low concentrations (≤ 30 mM) of alcohol (Sundstrom-Poromaa et al., 2002; Wallner et al., 2003, 2006; Wei et al., 2004; Hancher et al., 2005, 2006; Santhakumar et al., 2006; Glykys et al., 2007); however, other laboratories have reported contradictory results (Carta et al., 2004; Borghese et al., 2006; Botta et al., 2007; Korpi et al., 2007).

Tonic inhibition also occurs in the relay neurons of the thalamus (Porcello et al., 2003; Belelli et al., 2005; Cope et al., 2005; Jia et al., 2005, 2008b; Chandra et al., 2006; Bright et al., 2007; Peden et al., 2008), where the extrasynaptic GABA_A-Rs contain α_4 , β_2 , and δ subunits (Belelli et al., 2005; Chandra et al., 2006). Thalamic extrasynaptic GABA_A-Rs have distinct pharmacological properties that differentiate them from synaptic GABA_A-Rs, consisting mainly of α_1 , β_2 , and γ_2 subunits (Pirker et al., 2000; Browne et al., 2001; Jia et al., 2005). Several studies show that hypnotics and anesthetics are much more potent at thalamic extrasynaptic GABA_A-Rs than at synaptic receptors (Belelli et al., 2005; Cope et al., 2005; Jia et al., 2005; Chandra et al., 2006). Investigating the effects of alcohol on both synaptic and extrasynaptic inhibition in the thalamus should enhance our understanding of the mechanisms underlying the interaction between alcohol and sleep. Therefore, we examined the actions of ethanol on the function of GABA_A-Rs of thalamocortical relay neurons in the mouse ventrobasal (VB) thalamus.

Materials and Methods

Electrophysiological Recordings in Brain Slices

Experiments were performed in accordance with institutional and federal guidelines, using mice between 23 and 60 days old (C57BL/6, Gabra4^{+/+}, and Gabra4^{-/-}) by methods we have described previously (Jia et al., 2005). The knockout (Gabra4^{-/-}) and wild-type (Gabra4^{+/+}) littermates used were age-matched and on the same genetic background (129 $\times$ 1/S1 $\times$ C57BL/6J hybrid; F2–F4 generations) (Chandra et al., 2006). The experimenters were blind to genotype.

Animals were anesthetized with halothane, and brains were removed and placed in ice-cold slicing solution, which contained 2.5 mM KCl, 26 mM NaHCO₃, 1.25 mM NaH₂PO₄, 220 mM sucrose, 11 mM glucose, 10 mM MgSO₄, and 0.5 mM CaCl₂, before horizontal slices (300 μ m thick) were cut on a microslicer (VT 1000S; Leica, Wetzlar, Germany). Slices were perfused with carbogenated artificial cerebrospinal fluid, which contained 124 mM NaCl, 2.5 mM KCl, 2 mM MgSO₄, 2 mM CaCl₂, 26 mM NaHCO₃, 1.25 mM NaH₂PO₄, and 10 mM glucose. Whole-cell patch-clamp recordings from visually identified thalamic neurons were performed at room temperature as described previously (Jia et al., 2005). Intracellular solution for most voltage-clamp recordings contained 140 mM CsCl, 4 mM NaCl, 1 mM MgCl₂, 0.05 mM EGTA, 2 mM ATP-Mg²⁺, 0.3 mM GTP-Na⁺, and 10 mM HEPES, pH adjusted to 7.25

with CsOH. For voltage-clamp recordings involving acamprosate, intracellular solution contained 130 mM CsCH₃SO₃, 8.3 mM NaCH₃SO₃, 1.7 mM NaCl, 1 mM CaCl₂, 10 mM EGTA, 2 mM ATP-Mg²⁺, 0.3 mM GTP-Na⁺, and 10 mM HEPES, pH adjusted to 7.2 with CsOH. Intracellular solution for current-clamp recordings contained 130 mM K⁺-gluconate, 5 mM NaCl, 2 mM MgCl₂, 10 mM HEPES, 0.5 mM EGTA, 2 mM ATP-K⁺, and 0.3 mM GTP-Na⁺, pH adjusted to 7.25 with KOH.

Spontaneous inhibitory postsynaptic currents were recorded at -65 mV and isolated by bath application of 3 to 5 mM kynurenic acid (Jia et al., 2005), and evoked IPSCs were elicited by electrical stimulation (50 μ s; 2 $\times$ threshold) with a bipolar metal electrode (FHC, Bowdoin, ME) located in the reticular thalamic nucleus. The interval between successive stimuli was long (>15 s) to prevent cumulative synaptic depression. Access resistance was monitored throughout the recording period and was less than 20 M Ω throughout.

Drugs and Data Analysis

Gabazine [4-[6-imino-3-(4-methoxyphenyl)pyridazin-1-yl]butanoic acid hydrobromide], kynurenic acid (4-oxo-1*H*-quinoline-2-carboxylic acid), baclofen [4-amino-3-(4-chlorophenyl)-butanoic acid], and ethanol were purchased from Sigma (St. Louis, MO). Acamprosate calcium (3-acetamidopropane-1-sulfonic acid calcium salt) was obtained from Toronto Research Chemicals Inc. (North York, ON, Canada). Off-line analysis was performed using MiniAnalysis 5.5 (Synaptosoft, Decatur, GA), SigmaPlot 6.0 (SPSS, Chicago, IL), and Excel 2000 (Microsoft, Redmond, WA), as described in our previous publications. Tonic currents were measured as illustrated in Fig. 2C. The holding current was calculated by averaging an IPSC-free 5-ms section from every 100 ms of record. The all-points histograms were fitted with a Gaussian curve. The difference between the peaks of these Gaussian curves in the presence and absence of drug were calculated to determine the change of holding current. Spontaneous IPSCs were detected and analyzed using MiniAnalysis as described previously (Jia et al., 2005). Numeric data are expressed as mean $\pm$ S.E.M., except where indicated. The statistical significance of results was assessed using Student's *t* test or one-way ANOVA, and a level of *p* < 0.05 was considered significant.

Results

Ethanol Decreases Excitability in Thalamic Relay Neurons via GABA_A-Rs

We began by investigating whether ethanol modulates tonic firing of action potentials in depolarized thalamocortical relay neurons (Sherman, 2001). The membrane potentials of VB neurons were maintained at around -60 mV by constant current injection. At this membrane potential, VB neurons were generally silent but displayed sustained AP firing in response to depolarizing current steps. Ethanol (50 mM) decreased the excitability of VB relay neurons (Fig. 1A) and shifted the input-output curve to the right. This effect of alcohol to inhibit neuronal excitability is dependent on GABA_A-Rs, because in the presence of gabazine (20 μ M), a specific GABA_A-R antagonist, ethanol had no effect on the input-output relationship (Fig. 1B).

To facilitate the comparison of firing rates, the amplitude of the current step (500 ms duration) was adjusted in each neuron to induce $\sim$ 10 APs, corresponding to a firing frequency of $\sim$ 20 Hz. We then compared the number of APs evoked by depolarizing current steps before and after ethanol application. Ethanol (20 mM) failed to decrease firing rate (9.6 ± 1.5 versus 9.4 ± 1.6 ; *p* > 0.05, *n* = 5; Fig. 1C). In contrast, 50 mM ethanol significantly reduced the number of evoked APs from 10.9 ± 1.4 to 8.5 ± 1.4 (*p* < 0.01, *n* = 6; Fig. 1D). Preapplication of gabazine completely prevented the inhibitory effects of 50 mM ethanol on firing (*p* > 0.05, *n* = 5; Fig. 1E). These results demonstrate that a sedative-hypnotic concentration (50 mM) of ethanol can

reduce the excitability of depolarized VB relay neurons mainly through a GABA_A-R mechanism.

Ethanol Enhances Tonic Inhibition in VB Neurons

We made whole-cell voltage-clamp recordings to explore the modulation by ethanol of synaptic and extrasynaptic GABA_A-Rs. First, we examined whether ethanol induces any change of the tonic current mediated by extrasynaptic GABA_A-Rs. At low concentrations (10–30 mM), ethanol induced no significant change of holding current (10 mM: 0.8 ± 0.3 pA, $n = 12$; 20 mM: 1.8 ± 0.7 pA, $n = 18$; 30 mM: 2.4 ± 1.2 pA, $n = 10$), but at higher concentrations (50–100 mM), ethanol elicited a steady and sustained current shift (50 mM: 9.5 ± 1.5 pA, $n = 19$; 100 mM: 19.0 ± 3.6 pA, $n = 12$; Fig. 2, A and B). In some experiments, we applied 20 μ M gabazine following the application of 50 mM ethanol. Gabazine not only blocked the ethanol-induced current shift but also revealed the underlying tonic inhibition, indicating that the sustained currents induced by ethanol were due to enhancement of tonic inhibition mediated by GABA_A-Rs. We measured the tonic currents before and after ethanol perfusion, as shown in Fig. 2C, and the pooled data from all experiments ($n = 12$) were well fitted by a straight line ($r = 0.98$), with a slope of 1.50, indicating that 50 mM ethanol enhanced tonic currents by 50%.

Ethanol-Evoked Currents Are Absent in VB Neurons from *Gabra4*^{-/-} Mice

We have previously demonstrated that extrasynaptic GABA_A-Rs are absent from the thalamus of *Gabra4*^{-/-} mice (Chandra et al., 2006) and, therefore, investigated the actions of ethanol in VB neurons from *Gabra4*^{-/-} mice and their wild-type littermates (Fig. 3). Ethanol (50 mM) failed to induce any shift in baseline current in VB neurons from α_4 knockout mice (1.7 ± 0.9 pA, $n = 10$). In contrast, wild-type neurons showed measurable current shifts in response to 50 mM ethanol (8.6 ± 2.6 pA, $n = 6$), which were comparable to ethanol-induced currents recorded from C57BL/6 mice. This difference between the genotypes was highly significant ($p < 0.01$). These results are consistent with the idea that low concentrations of ethanol selectively enhance the activity of extrasynaptic GABA_A-Rs in VB neurons that contain α_4/δ subunits (Jia et al., 2005; Chandra et al., 2006).

Acamprosate Enhances Tonic Inhibition in VB Neurons Only at High Concentrations

Our recent work has shown that taurine is also a potent agonist for thalamic extrasynaptic GABA_A-Rs (Jia et al., 2008b). Acamprosate (3-acetamidopropane-1-sulfonic acid) is a taurine analog that has been used to treat alcohol abuse and alcoholism (De Witte et al., 2005; Gupta et al., 2005), although its mechanism of action is not yet fully understood. Given that neurons become hyperexcitable during alcohol withdrawal, we wondered whether acamprosate might reduce excitability and inhibit the neurons via extrasynaptic GABA_A-Rs in a way similar to taurine. Our recordings showed that 1 μ M acamprosate was unable to induce any change in the tonic current in VB neurons (0.1 ± 1.6 pA, $n = 6$). Tonic currents were also insensitive to 10 and 100 μ M acamprosate (0.3 ± 1.8 pA, $n = 5$, and 2.8 ± 2.0 pA, $n = 6$, respectively). At higher concentrations, 200 and 500 μ M acamprosate did elicit modest currents (14.7 ± 3.8 pA, $p < 0.05$, $n = 6$, and 42.5 ± 6.8 pA, $p < 0.01$, $n = 6$, respectively; Fig. 4). These results are consistent with a recent report (Reilly et al., 2008) that shows that acamprosate at low, clinically relevant concentrations has no effect on $\alpha_4\beta_3\delta$ GABA_A-Rs expressed in *Xenopus laevis* oocytes.

High Concentrations of Ethanol Prolong IPSC Decay Time

We investigated next whether ethanol changes the properties of IPSCs. Spontaneous inhibitory synaptic currents are readily observed in VB neurons and can be blocked by gabazine (data not shown), indicating that they are mediated by synaptic GABA_A-Rs. The averaged data from this set of experiments (Fig. 5C) show that, at all of the three concentrations that we tested (20,

50, and 100 mM), ethanol induced no significant change in the frequency (percentage change: 20 mM: $-3.2 \pm 3.6\%$, $n = 9$; 50 mM: $3.8 \pm 3.1\%$, $n = 16$; 100 mM: $7.0 \pm 6.6\%$, $n = 8$) or the amplitude of spontaneous IPSCs (percentage change: 20 mM: $-1.0 \pm 2.3\%$, $n = 9$; 50 mM: $-1.2 \pm 2.4\%$, $n = 16$; 100 mM: $1.5 \pm 4.5\%$, $n = 8$). At 100 mM, ethanol did significantly increase the decay time of spontaneous IPSCs by $8.5 \pm 2.0\%$ ($p < 0.01$, $n = 8$).

We also compared evoked inhibitory synaptic currents before and after ethanol applications. Gabazine completely blocked evoked IPSCs (data not shown), suggesting that they were mediated by GABA_A-Rs, and ethanol (20–100 mM) did not enhance the amplitude of evoked IPSCs (percentage change: 20 mM: $1.7 \pm 1.4\%$, $n = 7$; 50 mM: $2.3 \pm 3.1\%$, $n = 8$; 100 mM: $0.3 \pm 2.5\%$, $n = 13$); only 100 mM ethanol increased the decay time of evoked IPSCs (percentage change: 20 mM: $-0.5 \pm 1.5\%$, $n = 7$; 50 mM: $5.8 \pm 2.6\%$, $n = 8$; 100 mM: $13.6 \pm 2.5\%$, $p < 0.001$, $n = 13$; Fig. 6, A and B). Ethanol (≤ 50 mM) does not appear to modulate the function of synaptic GABA_A-Rs in VB neurons.

Ethanol Has No Presynaptic Effect on IPSCs in VB Neurons

In many parts of the CNS, alcohol alters synaptic inhibition via a presynaptic mechanism, often by an increase in frequency. These presynaptic effects have been reported in the amygdala and cerebellum, for example (Siggins et al., 2005; Breese et al., 2006; Roberto et al., 2006; Weiner and Valenzuela, 2006). As mentioned above, we recorded evoked IPSCs of VB neurons and used the well described paired-pulse stimulation protocol (Zalutsky and Nicoll, 1990), which is widely used to detect a change in transmitter release from presynaptic terminals. In response to paired stimuli (separated by 150 ms), IPSCs in VB relay neurons show substantial paired-pulse depression. At all of the concentrations we tested (20–100 mM), ethanol had no effect on the paired-pulse ratio. In contrast, baclofen (5 μ M), a GABA_B receptor agonist that acts by decreasing GABA release from the presynaptic terminal, significantly increased the paired-pulse ratio from 0.59 ± 0.04 to 0.97 ± 0.17 ($p < 0.05$, $n = 7$). These results suggest that ethanol does not modulate synaptic GABA release in the thalamus.

Discussion

Alcohol is one of the most widely abused drugs. Blood alcohol levels between 5 and 20 mM reduce anxiety and produce mild sedation; these levels are commonly associated with light to moderate intoxication associated with social drinking. Blood ethanol (20–50 mM) typically elicits profound sedation, cognitive impairment, amnesia, and loss of motor coordination. Higher concentrations of ethanol (100 mM) in normal individuals cause general anesthesia, decreased ventilation, and risk of death (Deitrich and Harris, 1996; Little, 1999). All of these effects are less pronounced in chronic alcoholics, who routinely tolerate extraordinary high levels of the drug. Many of the pharmacological properties of ethanol are shared by drugs, such as the benzodiazepines and barbiturates, that have long been known to achieve their effects via regulation of the GABA_A-Rs, and a large body of evidence implicates GABA_A-R as an important target for ethanol in the CNS (Martz et al., 1983; Grobin et al., 1998). However, the mechanisms by which ethanol enhances GABAergic transmission are unclear and may vary substantially among brain regions (Weiner and Valenzuela, 2006).

The main findings of this study are as follows. i) Ethanol (50 mM), but not 20 mM ethanol, reduces firing rate of depolarized VB neurons via GABA_A-Rs; ii) ethanol (≥ 50 mM) enhances tonic inhibition mediated by extrasynaptic GABA_A-Rs; iii) ethanol (100 mM), but not 20 to 50 mM ethanol, exerts a postsynaptic action to prolong IPSCs on VB neurons; iv) enhancement of tonic currents by ethanol is absent in VB relay neurons from α_4 subunit knockout mice; and v) ethanol has no presynaptic action at inhibitory synapses made by reticular thalamic nucleus neurons on to VB neurons.

In the present study, we demonstrate that ethanol (≤ 50 mM) does not change the amplitude or decay time of spontaneous IPSCs or evoked IPSCs in VB relay neurons. In contrast, 100 mM ethanol significantly prolongs both spontaneous IPSCs and evoked IPSCs. This finding is consistent with most previous studies on recombinant “synaptic” GABA_A-Rs ($\alpha_1\beta\gamma_2$ and $\alpha_2\beta\gamma_2$ subtypes) heterologously expressed in cultured cells (Sigel et al., 1993; Mihic et al., 1997) and native synaptic GABA_A-Rs in slices of different brain regions (Weiner and Valenzuela, 2006), which suggests that synaptic GABA_A-Rs may not act as the direct target of ethanol at subanesthetic concentrations (≤ 50 mM).

An indirect action of ethanol on GABA_A-Rs via presynaptic sites has been observed in many brain regions, including the amygdala (Roberto et al., 2003, 2004; Roberto and Siggins, 2006; Zhu and Lovinger, 2006), the cerebellum (Carta et al., 2004; Hanchar et al., 2005; Ming et al., 2006; Kelm et al., 2007), the hippocampus (Ariwodola and Weiner, 2004; Sanna et al., 2004; Galindo et al., 2005), and the nucleus accumbens (Nie et al., 2000; Crowder et al., 2002). However, we were unable to detect any change of spontaneous IPSC frequency or paired-pulse ratio of evoked IPSCs by ethanol (20–100 mM) in VB relay neurons, which suggests that presynaptic GABA release in the thalamus is insensitive to ethanol.

Recently, GABA_A-Rs have been shown to be present at extrasynaptic sites as well as subsynaptic sites (Farrant and Nusser, 2005; Mody, 2005). Several groups have begun to look at the potential for alcohol action at extrasynaptic GABA_A-Rs. First of all, two groups reported that recombinant $\alpha_4\beta_2\delta$ (Sundstrom-Poromaa et al., 2002) and $\alpha_{4/6}\beta_3\delta$ (Wallner et al., 2003) GABA_A-Rs are extremely sensitive to alcohol, with enhancement of function noted at alcohol concentrations as low as 3 mM. However, the data in these two studies are inconsistent in terms of the dose-dependent ethanol response at $\alpha_4\beta_2\delta$ subtype, and two other studies failed to observe the low concentration ethanol effects at recombinant $\alpha_4\beta\delta$ GABA_A-Rs (Borghese et al., 2006; Yamashita et al., 2006). Enhancement of tonic currents mediated presumably by $\alpha_6\beta\delta$, $\alpha_4\beta\delta$, or $\alpha_1\beta\delta$ GABA_A-Rs has also been observed in hippocampal or cerebellar brain slices (Wei et al., 2004; Hanchar et al., 2005; Glykys et al., 2007). These alcohol effects on $\alpha_6\beta\delta$ GABA_A-Rs are further exaggerated by a mutation (R100Q) in the α_6 subunit of the GABA_A-Rs (Hanchar et al., 2005). Contrasting observations on native extrasynaptic GABA_A-Rs have been reported by other groups (Carta et al., 2004; Valenzuela et al., 2005; Borghese et al., 2006; Botta et al., 2007; Korpi et al., 2007). The reasons for these discrepancies are still elusive.

In thalamocortical relay neurons and dentate granule cells, $\alpha_4\beta_2\delta$ GABA_A-Rs have been shown to be located at extrasynaptic sites and to mediate tonic inhibition (Belelli et al., 2005; Jia et al., 2005; Chandra et al., 2006; Herd et al., 2008). Small, but measurable, tonic currents have been recorded from β_2 knockout mice (Belelli et al., 2005; Herd et al., 2008). The residual tonic currents suggest the possible contribution of the $\alpha_4\beta_3\delta$ subtype or an unknown subunit compensation induced by gene knockout. Thalamic extrasynaptic GABA_A-Rs have also been implicated in the action of hypnotics and anesthetics (Belelli et al., 2005; Cope et al., 2005; Chandra et al., 2006; Jia et al., 2008a).

In this study, we found that tonic currents are not significantly enhanced by ethanol at low concentrations (10–30 mM) associated with social alcohol drinking. It seems that extrasynaptic GABA_A-Rs (mainly $\alpha_4\beta_2\delta$ subtype) expressed in VB relay neurons are not as sensitive to ethanol as recombinant GABA_A-Rs expressed in *X laevis* oocytes (Sundstrom-Poromaa et al., 2002; Wallner et al., 2003) or the extrasynaptic GABA_A-Rs of dentate granule cells (Wei et al., 2004). However, VB relay neurons are sensitive to sedative or anesthetic concentrations of ethanol (≥ 50 mM). At 50 mM, ethanol enhances tonic currents by approximately 50% and decreases the excitability of the relay neurons. The reasons for the discrepancies in this literature are still elusive. They may arise from methodological issues, for example, the differences in expression systems, tissue preparation, or recording temperatures, and one must

also consider the possibility that ethanol might indirectly modulate the activity of extrasynaptic GABA_A-Rs. For example, ethanol might enhance tonic inhibition by stimulating the nonvesicular release of taurine (Olive, 2002), a potent agonist of these receptors (Jia et al., 2008b).

We have shown previously that gabazine, an antagonist of GABA_A-Rs, has little effect on the holding current in VB relay neurons from Gabra4^{-/-} mice (Chandra et al., 2006), which is consistent with a loss of these extrasynaptic receptors. Likewise, 50 mM ethanol failed to evoke any significant current shift in VB neurons from Gabra4^{-/-} mice. At this concentration, ethanol does not change the properties of IPSCs from wild-type and Gabra4^{-/-} mice either. Therefore, ethanol (~50 mM) selectively enhances the activity of extrasynaptic GABA_A-Rs containing α_4 subunits. Similar ablation of ethanol-induced tonic currents has also been shown in dentate gyrus neurons from Gabra4^{-/-} mice (Liang et al., 2008).

Tonic inhibition mediated by extrasynaptic GABA_A-Rs plays a crucial role in regulating excitability at the level of individual neurons and within neuronal networks (Semyanov et al., 2004). In the present study, we show that the tonic firing in VB relay neurons was decreased by 50 mM ethanol but not by 20 mM. In addition, preapplied gabazine occluded the inhibition of tonic firing by 50 mM ethanol, which indicates that GABA_A-Rs are critical for the inhibitory effects of ethanol in the thalamus.

Alcohol not only has sedative effects that can promote onset of sleep but also causes abnormal sleep patterns (Kubota et al., 2002). After drinking alcohol, the time spent in deep (slow-wave) sleep is increased, whereas the time spent in the dreaming state (rapid eye movement sleep) is decreased. There can be little argument concerning the pivotal role of the thalamus in controlling the sleep-wake transition and in sensory transmission. The thalamocortical circuit represents an important potential target for alcohol, as reflected in the electroencephalogram changes that occur during drinking. A previous *in vivo* study demonstrates that 4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol, a hypnotic, works through extrasynaptic GABA_A-Rs that contain the δ subunits (Winsky-Sommerer et al., 2007). Our results indicate that extrasynaptic GABA_A-Rs may also play a role in the action of sedative concentrations of ethanol (~50 mM) in the thalamus. Given that sleep disturbances have been suggested to play a reciprocal role in the progression of alcoholism (Brower et al., 1998; Brower, 2001), the extrasynaptic GABA_A-Rs in the thalamus may be a potential therapeutic target for the treatment of alcoholism.

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Abbreviations

GABA_A-R(s)	GABA _A receptor(s)
IPSC	inhibitory postsynaptic current
AP	action potential
CNS	central nervous system
VB	ventrobasal thalamic nucleus

Fig. 1.

Ethanol (50 mM) decreases the excitability of VB neurons via GABA_A-Rs. A, representative current clamp traces demonstrate AP firing evoked by current steps (40–160 pA, duration 500 ms) in a VB neuron. After ethanol (50 mM) perfusion, AP firing decreased, and the input-output curve shifted rightwards. B, representative current clamp traces demonstrating AP firing evoked by current steps in the presence of gabazine (20 μ M) in another VB neuron. Ethanol (50 mM) perfusion failed to change the input-output curves when GABA_A-Rs were blocked. C, exemplar current trace demonstrating that 20 mM ethanol induced no change on firing rate evoked by depolarized current steps. On average, ethanol (20 mM) also makes no change on the numbers of APs (9.6 ± 1.5 versus 9.4 ± 1.6 , $p > 0.05$, $n = 5$). N.S., not significant. D, pooled

data show that 50 mM ethanol significantly reduced the number of evoked action potentials from 10.9 ± 1.4 to 8.5 ± 1.4 (**, $p < 0.01$, $n = 6$). E, when GABA_A-Rs are blocked by gabazine, 50 mM ethanol fails to inhibit tonic action potential firing (12.5 ± 1.7 to 12.4 ± 1.9 , $p > 0.05$, $n = 5$).

Fig. 2.

Ethanol (≥ 50 mM) enhances tonic currents mediated by extrasynaptic GABA_A-Rs. A, typical voltage-clamp recordings of four VB neurons in response to the applications of different concentrations (10–100 mM) of ethanol. Ethanol (≥ 50 mM) induced substantial current-shifts. B, the averaged current-shifts elicited by ethanol are dose-dependent (10 mM: 0.8 ± 0.3 pA, $n = 12$; 20 mM: 1.8 ± 0.7 pA, $n = 18$; 30 mM: 2.4 ± 1.2 pA, $n = 10$; 50 mM: 9.5 ± 1.5 pA, $n = 19$; 100 mM: 19.0 ± 3.6 pA, $n = 12$). C, gabazine (20 μ M) occluded the enhancement of tonic currents by 50 mM ethanol and revealed the background tonic current. The dotted trace and Gaussian fittings were made from the raw trace as described under *Materials and Methods*. Tonic currents before and after ethanol application in this case are 43.4 and 62.2 pA, respectively. D, each point corresponds to the tonic currents before (x-axis) and after (y-axis) ethanol application from individual experiment similar to the one shown in C. The points analyzed from twelve experiments were fitted by a straight line pretty well ($r = 0.98$). The slope of the fitted line is 1.50, well above the unitary line ($y = x$, the gray dashed line).

Fig. 3.

Ethanol-induced current-shift is absent in VB neurons from mice lacking the GABA_A-R α_4 subunit. A, ethanol (50 mM) evoked a holding current shift (~ 10 pA) in a VB neuron from a wild-type mouse. In contrast, ethanol produced no current shift in a VB neuron from a Gabra4^{-/-} mouse. WT, wild-type; KO, knockout. B, bar graph demonstrates that ethanol (50 mM) induced current shifts in wild-type, but not α_4 knockout, VB neurons (knockout: 1.7 ± 0.9 pA, $n = 10$; wild type: 8.6 ± 2.6 pA, $n = 6$; **, $p < 0.01$).

Fig. 4.

The tonic currents evoked by acamprosate at high concentrations. A, a typical recording of a VB neuron in response to 1 μM acamprosate. B, pooled data show that 200 and 500 μM, but not 1 to 100 μM, acamprosate elicits significant current-shift in VB neurons.

Fig. 5.

The effects of ethanol on spontaneous IPSCs. A, a typical recording of spontaneous IPSCs in a VB neuron in the absence and presence of 50 mM ethanol. Averaged spontaneous IPSC traces before (black) and after (gray) ethanol application are superimposed to illustrate the similarity in amplitude and decay time. B, a representative experiment shows that the frequency, amplitude, and decay time of spontaneous IPSCs did not change during the ethanol application for more than 20 min. C, pooled data demonstrate that spontaneous IPSCs are largely insensitive to ethanol. Only 100 mM ethanol significantly increases the decay time of spontaneous IPSCs (**, $p < 0.01$).

Fig. 6.

The effects of ethanol on evoked IPSCs and paired-pulse depression. A, exemplar evoked IPSC traces demonstrating that 100 mM ethanol increase the decay time, but not the amplitude of evoked IPSCs. B, average data show that evoked IPSCs are insensitive to ethanol less than 100 mM. Only 100 mM ethanol increased the decay time of evoked IPSCs significantly (***, $p < 0.001$). C, sample traces showing paired-pulse responses before (in dark) and after 100 mM ethanol (in gray) application. The superimposed traces clearly show the similar degree of paired-pulse depression. D, bar graph demonstrates that paired-pulse ratio is insensitive to ethanol (20–100 mM), which indicates that the presynaptic GABA release probability is not modified by ethanol.