The In Vitro Ethanol Sensitivity of Hippocampal Synaptic γ-Aminobutyric Acid$_A$ Responses Differs in Lines of Mice and Rats Genetically Selected for Behavioral Sensitivity or Insensitivity to Ethanol

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ABSTRACT
Previous work has demonstrated that in the hippocampal CA1 region of Sprague-Dawley rats, there are ethanol-sensitive and ethanol-insensitive populations of GABAergic synapses on pyramidal neurons. The present experiments characterized the ethanol sensitivity of these pathways in lines of rats and mice genetically selected for sensitivity or insensitivity to the behavioral effects of ethanol. In ethanol-sensitive inbred long sleep mice, GABA$_A$ IPSCs induced by stimulation of proximal (probably somatic) synapses were enhanced by 80 mM ethanol, whereas the distal (i.e., dendritic) pathway was unaffected. Thus, the relative sensitivity of these pathways (proximal > distal) is the same in both Sprague-Dawley rats and in inbred long sleep mice. However, in the ethanol-insensitive inbred short sleep mice, neither proximal nor distal IPSCs were affected by 80 mM ethanol. The ethanol sensitivity of the proximal pathway was also examined in replicate lines of rats selected for either high ethanol sensitivity or low ethanol sensitivity. GABA$_A$ IPSCs in the high ethanol sensitivity lines were significantly enhanced by 80 mM ethanol, whereas IPSCs in the low ethanol sensitivity lines were unaffected. Thus, IPSCs evoked via the proximal pathway were enhanced by ethanol in all the sensitive mouse and rat lines, and unaffected in all the insensitive lines. These experiments demonstrate that GABA$_A$ synapses in brain differ in their sensitivity to enhancement by ethanol, and the sensitivity to such enhancement is under the control of genes that can be selected for using classical genetic selective breeding based on a behavioral phenotype.

Despite the fact that ethanol is one of the most frequently abused drugs, the molecular targets through which it exerts its actions on the nervous system are uncertain. Although previous research focused on ethanol interactions with membrane lipids, voltage- as well as ligand-gated ion channels have now been shown to be important candidates for ethanol action (Loving, 1997; Mihic, 1999). So many effects of ethanol on ion channels have been described that an important issue confronting this field is identifying which of these many actions underlie the disturbances of higher order nervous function induced by ethanol (Harris, 1999).

Because the behavioral effects of ethanol resemble those initiated by other central anesthetic compounds known to be specific modulators of GABA$_A$ receptor function, the GABA$_A$ receptor complex has been hypothesized to be an important target of ethanol action (Mihic et al., 1997; Harris, 1999). This receptor is the primary mediator of fast inhibitory neurotransmission in the central nervous system, and enhancement of the effects of GABA on these receptors would have significant inhibitory effects on neuronal activity. However, there is a great deal of variability in the reported effects of ethanol on this receptor complex. Biochemical studies in brain synaptosome and microsac preparations (Allan and Harris, 1987) and in cultured neurons (Mehta and Ticku, 1994) have reported enhancement of GABA$_A$ receptor-mediated responses by intoxicating concentrations of ethanol, as have electrophysiological studies of GABA$_A$ receptor-mediated currents (Aguayo, 1990; Reynolds et al., 1992; Weiner et al., 1997a; Soldo et al., 1998). However, even in these studies, ethanol-sensitive and ethanol-insensitive responses have been reported, and there have also been numerous studies in...
which potentiation by ethanol was not observed (Morelli et al., 1988; Osmanovic and Shefner, 1990; White et al., 1990).

To account for such variability in ethanol enhancement, a number of hypotheses have been proposed. One possibility is that differences in ethanol sensitivity may be attributable to differences in receptor subunit composition. Weiner et al. (1997a) showed that even on single hippocampal CA1 pyramidal neurons, ethanol sensitivity of GABAA receptors can differ depending on which subset of GABA\textsubscript{A} synapses is activated. 

Electrical stimulation of GABAergic afferents in the pyramidal cell layer (proximal responses) evoked inhibitory postsynaptic currents (IPSCs) that were potentiated by intoxicating concentrations of ethanol, whereas IPSCs evoked by stimulation within the stratum radiatum (distal responses) were less sensitive to all concentrations of ethanol tested. One possible explanation for these results is that different synapses have postsynaptic receptors that incorporate different GABAA receptor subunits, which leads to differences in ethanol sensitivity.

At least some of the differences in the ethanol sensitivity of GABAA responses appear to be under genetic control. Biochemical measures of GABAA receptor function, such as muscimol-stimulated $^{36}$Cl$^{-}$ flux, are differentially sensitive to modulation by ethanol in lines of animals that differ in their behavioral sensitivity to ethanol (Allan and Harris, 1986). If the GABAA receptor is a specific target of ethanol action that subserves at least part of the behavioral response to ethanol, and if there are forms of this receptor that differ in their ethanol sensitivity, then genetic selection experiments could result in animal lines that also differ in the effects of ethanol on synaptically mediated GABAA responses. However, the sensitivity of specific populations of GABA\textsubscript{A} synapses to ethanol has never been characterized in selected lines of animals. More specifically, the ethanol sensitivities of the proximal (ethanol-sensitive) and distal (ethanol-insensitive) subpopulations of GABAA receptors, which were initially characterized by Weiner et al. (1997a) in Sprague-Dawley rats, have not been examined in selected lines of animals.

To address this issue, the present experiments examined the effect of ethanol on IPSCs in hippocampal slices from rodents selectively bred based on their behavioral sensitivity to ethanol. We have examined proximal and distal GABAA IPSCs in six lines of animals, which include the inbred long sleep (ILS) mice and the replicate high alcohol sensitivity (HAS\textsubscript{1} and HAS\textsubscript{2}) lines of rats (all bred for ethanol sensitivity), and the low alcohol sensitivity (LAS\textsubscript{1} and LAS\textsubscript{2}) rats and inbred short sleep (ISS) mice (all ethanol insensitive), to determine whether the sensitivity of GABAergic synapses to ethanol is altered in these selected lines of animals. If such differences could be observed, this would suggest that there are genetically controlled factors that can regulate ethanol sensitivity of GABAA receptors.

**Materials and Methods**

Transverse hippocampal slices (400 $\mu$m) were prepared from 4- to 6-week-old HAS and LAS rats, and ILS and ISS mice using a Sorvall (Newtown, CT) tissue chopper. Submerged slices were incubated in a submerged chamber consisting of a grid of small, square compartments with plastic netting attached to the bottom, suspended in a 250-ml beaker and covered with a loose-fitting plastic lid. This chamber was maintained at a constant temperature of 31–33°C in aerated (95% O\textsubscript{2}, 5% CO\textsubscript{2}) artificial cerebrospinal fluid containing 126 mM NaCl, 3 mM KCl, 1.5 mM MgCl\textsubscript{2}, 2.4 mM CaCl\textsubscript{2}, 1.2 mM NaH\textsubscript{2}PO\textsubscript{4}, 11 mM glucose, and 26 mM NaHCO\textsubscript{3}. Slices were left in this chamber for at least 90 min after the dissection. For recordings, slices were transferred to a submersion recording chamber maintained at a constant temperature of 31–33°C and superfused with aerated artificial cerebrospinal fluid at 2 ml/min. Slices were allowed to equilibrate in the recording chamber for a few minutes before electrophysiological recordings were begun.

GABA\textsubscript{A} IPSCs were recorded from CA1 neurons using the whole-cell patch-clamp technique. Recording electrodes were constructed from borosilicate glass (1.5 mm o.d., 0.98 i.d.; Sutter Instrument Co., Novato, CA) and had resistances of 6 to 9 M$\Omega$ when filled with the patch pipette solution. The patch pipette solution contained 125 mM potassium-glucuronate (Fluka, Buchs, Switzerland), 5 mM KCl, 10 mM HEPES (Fluka), 0.1 mM CaCl\textsubscript{2}, 1 mM potassium-EGTA (Fluka), 2 mM MgCl\textsubscript{2}, 2 mM magnesium-ATP, and 0.2 mM Tris-GTP (pH = 7.3 adjusted with KOH; 290 mOsm) and was kept on ice until immediately before use. Series resistances ranged from 10 to 41 M$\Omega$ (average 31 ± 1.5 M$\Omega$). The average change in the series resistance for all cells from the beginning of the control to the end of the washout period was 15.6 ± 1.2%, and all cells in which the change was >25% were excluded from subsequent analysis.

All recordings were made in the presence of 20 $\mu$M 6,7-dinitroquinoxaline-2,3-dione (DNQX) and 50 $\mu$M bic(-)2-amino-5-phosphonovaleric acid (APV) to block excitatory postsynaptic currents. 

Synchronous stimulation was delivered using a bipolar twisted nichrome wire electrode (0.2-ms pulses of 7–30 V) positioned within 250 $\mu$m of the recording pipette and placed directly over stratum pyramidale, with an interstimulus interval of 30 to 60 s, as previously described (proximal stimulation; Weiner et al., 1997a). All cells were clamped to −65 mV (after correction for the liquid junction potential) and recorded in the voltage-clamp mode. After superfusion with DNQX and APV to block the glutamatergic components of the synaptic current, the strength of the stimulation pulse was adjusted so that the peak amplitude of the residual GABAergic component (GABA\textsubscript{A} IPSC) was about 50 to 100 pA.

All drugs were purchased from Sigma (St. Louis, MO) unless otherwise indicated. Drugs applied to slices were made up as 100-fold concentrates and added to the superfusion buffer via calibrated syringe pumps (Razel Scientific Instruments, Stamford, CT). A 4 M solution of ethanol (Aaper, Shelbyville, KY; diluted in deionized water) was prepared immediately before each experiment from a 95% stock solution kept in a glass storage bottle.

Drug effects were quantified as the percentage of change in amplitude or area under the curve of IPSCs relative to the mean of control and washout values. Statistical analyses of drug effects were carried out using two-tailed Student’s paired and unpaired $t$ tests, or two-way ANOVAs as indicated, with a level of significance of $P < .05$.

**Results**

Recordings were made from CA1 pyramidal neurons in hippocampal slices from ILS and ISS mice, and from the replicate lines of HAS\textsubscript{1} and HAS\textsubscript{2}, LAS\textsubscript{1} and LAS\textsubscript{2} rats. In most cases, the responses evoked in the presence of DNQX + APV were mediated primarily via GABA\textsubscript{A} receptors, but in some instances there was a small, late component on the falling phase of the IPSC (Fig. 1A) that could be blocked by superfusion with the GABA\textsubscript{B} receptor antagonist CGP 35348 (data not shown). However, this secondary component did not overlap significantly with the peak of the GABA\textsubscript{A} response, so it was not blocked pharmacologically in the instances where it was observed. To examine the ethanol sensitivity of GABA\textsubscript{A} responses, slices were superfused with 80 mM ethanol, and changes in the amplitude and area of the IPSC response were determined. This concentration of ethanol was
used because this is the mean blood ethanol concentration at regain of righting reflex in the heterogeneous stock of animals from which the HAS and LAS rat lines were originally selected (Draski et al., 1992). At generation 12 of selection, the LAS and HAS animals regained their righting reflexes with mean blood ethanol concentrations of 87 and 75 mM, respectively (Draski et al., 1992).

Under these experimental conditions, bath superfusion with ethanol did not significantly change either the holding current or the input resistance of CA1 pyramidal neurons in any of the lines of animals tested (Table 1). However, 80 mM ethanol significantly enhanced the GABA_A IPSC in both of the lines of rats selected for behavioral sensitivity to ethanol (HAS_1, HAS_2; Fig. 1, A and C) compared with the baseline (Table 1). The falling phases of the IPSCs with no GABA_B component were fitted to single exponential functions, and the time constants for the decay were compared for control and ethanol responses. Ethanol had no significant effect on the time constants in any of the individual lines tested, but across all the animals there was a significant 22 ± 7.9% (n = 23; P < .02) increase in the average time constant, suggesting that ethanol did prolong the IPSCs to a limited extent.

The time course of the effect of bath superfusion with ethanol on GABA_A responses in HAS animals was similar to what we have previously reported in Sprague-Dawley rats, i.e., it usually took 5 to 10 min of superfusion with ethanol to achieve the maximal enhancement of the IPSC (Fig. 2A). There did not appear to be any short-term tolerance occurring during the superfusion with ethanol, and recovery to baseline was observed within 5 to 10 min of ethanol washout (Fig. 2). The enhancement of GABA_A IPSCs induced by ethanol was also repeatable, and in some slices, potentiation was observed with as many as three successive applications of ethanol with no apparent decrement in the ethanol enhancement (Fig. 2B). Unlike the HAS rats, GABA_A IPSCs elicited in hippocampal slices from the two independent strains of rats with low behavioral sensitivity to ethanol (LAS_1, LAS_2) showed no significant change in response to ethanol superfusion (Fig. 1, B and D). In addition to there being no effect on the peak amplitude of the IPSC, this concentration of ethanol had no effect on the area under the curve for the IPSC as well (Table 1). When the changes in the peak IPSC induced by ethanol were compared between the pooled HAS and LAS animals, there was a highly significant difference between the ethanol-sensitive and ethanol-insensitive groups of rats (LAS: 107 ± 4.7%, n = 29; HAS: 141 ± 9.6%, n = 30; P < .01).

Because it is not known whether there are proximal and distal GABA_A responses in mice such as we have previously described in rats (Weiner et al., 1997a), all of the experiments in ILS and ISS mice were conducted with two independent stimulating electrodes positioned to activate each of these pathways selectively. IPSC responses evoked by stimulation in stratum radiatum (distal stimulation) were unaffected by 80 mM ethanol in both lines of mice. The amplitude of the IPSCs in ethanol were 109 ± 13% (n = 17) of control in ISS mice, and 95 ± 6.8% (n = 17) of control in ILS mice. As far as the proximal pathway was concerned, the ethanol-insensitive mouse strain (ISS) did not show any change either in the amplitude or the area of the hippocampal GABA_A IPSC during ethanol superfusion (Fig. 1F; Table 1), but IPSCs in the ethanol-sensitive ILS line were significantly enhanced rela-

<table>
<thead>
<tr>
<th>Animal Line</th>
<th>IPSC Amplitude</th>
<th>IPSC Area under the Curve</th>
<th>Input Resistance</th>
<th>Δ Holding Current</th>
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<tbody>
<tr>
<td></td>
<td>% control</td>
<td>% control</td>
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<tr>
<td>HAS_1 rats</td>
<td>127 ± 11* (18)</td>
<td>133 ± 12* (18)</td>
<td>99 ± 3.4 (14)</td>
<td>-16 ± 10 (18)</td>
</tr>
<tr>
<td>HAS_2 rats</td>
<td>164 ± 15* (12)</td>
<td>158 ± 8.8* (12)</td>
<td>103 ± 2.7 (12)</td>
<td>-4.0 ± 4.9 (12)</td>
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<td>ILS mice</td>
<td>124 ± 6.6* (17)</td>
<td>121 ± 6.3* (17)</td>
<td>104 ± 6.1 (17)</td>
<td>2.2 ± 4.3 (17)</td>
</tr>
<tr>
<td>LAS_1 rats</td>
<td>112 ± 7.1 (16)</td>
<td>117 ± 11 (16)</td>
<td>102 ± 5.1 (16)</td>
<td>-18 ± 13 (15)</td>
</tr>
<tr>
<td>LAS_2 rats</td>
<td>101 ± 5.7 (13)</td>
<td>105 ± 9.0 (13)</td>
<td>101 ± 3.3 (13)</td>
<td>15 ± 7.9 (13)</td>
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<tr>
<td>ILS mice</td>
<td>100 ± 5.5 (17)</td>
<td>103 ± 5.2 (17)</td>
<td>104 ± 3.7 (17)</td>
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* P < .05 versus ethanol baseline (average of pre- and washout responses).
Discussion

Ethanol Effects in Selected Lines of Rodents. Ethanol activates or inhibits the activity of a number of different ligand-gated ion channels, and a key issue in understanding the cellular mechanisms of ethanol action is to determine which of these effects underlie specific pharmacological actions of ethanol. One approach to making such determinations is to use animals developed using classical selective breeding approaches to segregate genes that are associated with specific behavioral phenotypes, such as ethanol sensitivity. Comparisons of ethanol responses in animals developed by this approach can then be used to identify the specific molecular targets of ethanol that are linked to the behavioral phenotype.

One model that has been used to explore the basis for ethanol sensitivity differences is the long sleep (LS) and short sleep (SS) mice, which were selected based on the duration of loss of righting reflex in response to ethanol (Heston et al., 1973). Martz et al. (1983) proposed based on behavioral experiments with LS and SS mice that the sensitivity of GABA_α responses to ethanol might be one factor responsible for genetic differences in ethanol sensitivity. Subsequent biochemical studies showed that ethanol potentiates muscimol-stimulated chloride flux in LS mice, but has no effect in preparations from SS mice (Allan and Harris, 1986; Harris and Allan, 1989). A similar potentiating effect of ethanol has been demonstrated in cortical and cerebellar microsacs, as well as membrane vesicles from whole brain in HAS rats, but not in LAS rats (Allan et al., 1988, 1991; Liu and Deitrich, 1998). However, the ethanol sensitivity of synaptically mediated GABA_α IPSPs has never been determined in these lines of animals. Because the ethanol enhancement of GABA_α receptor-mediated ⁸⁶⁴⁴Cl⁻ flux is primarily observed at low concentrations of GABA, and is negligible at high GABA concentrations (Mihic et al., 1994), this suggests that ethanol might not affect synaptic responses, because of the high concentrations of synaptic GABA after release.

Nevertheless, the present studies demonstrated a direct relationship between the behavioral sensitivity to ethanol in selectively bred rodent strains, and the in vitro ethanol sensitivity of hippocampal GABAergic synapses. This constitutes the first evidence for differences in the sensitivity of GABA_α receptor-mediated synaptic potentials that correspond to the behavioral sensitivity of selected lines of animals. The present data suggest that there is a robust association between synaptic sensitivity to ethanol and behavioral sensitivity, based on the occurrence of such differences in six selected lines of animals from two different species. Using different lines of selectively bred rodents reduces the probability that the differences between lines of animals with high and low ethanol sensitivity are due to random fixation of genes associated with GABA_α receptor sensitivity. Although the differences in synaptic GABA_α receptor sensitivity are correlated with the behavioral phenotype, it seems unlikely that hippocampal sensitivity to ethanol is causally linked with the behavior because the hippocampus probably has little influence over the loss of the righting reflex. Nevertheless, the gene(s) that regulates the sensitivity of these hippocampal synapses to ethanol is likely to affect the sensitivity of other GABA_α receptors in other brain regions that may play such a role.

In the present studies, there was essentially no effect of 80 mM ethanol on GABA_α responses in the ISS/LAS animals. This might only reflect a relative difference in sensitivity, in that the threshold for ethanol effects in these lines might be higher than 80 mM. However, because behavioral effects of

Fig. 2. Time course and repeatability of ethanol responses. A. illustrates the time course of ethanol action on GABA_α IPSCs evoked from cells from a HAS animal and a LAS rat. The rate of onset and washout of the effect in the slice from the HAS animal was comparable with what we have previously observed in Sprague-Dawley rats (Weiner et al., 1997a; Fig. 3). As shown in these examples, there was no significant effect of ethanol on the LAS IPSC, but a significant enhancement of the HAS IPSC. B. shows the repeatability of the ethanol response in a single cell from a HAS animal that was superfused repeatedly with the same concentration of ethanol. Although there was some decline in the absolute magnitude of the GABA_α response over time, the relative enhancement of the IPSC by ethanol was maintained throughout the experiment.

Fig. 3. Effects of ethanol on peak IPSC amplitude in selected lines of mice and rats. The average effect of 80 mM ethanol is illustrated on the peak amplitude of the IPSC evoked by proximal stimulation for each of the six lines of animals tested. Although the magnitude of the enhancement in the ILS and HAS animals was somewhat less than in the HAS rats, the effect of ethanol was statistically significant in each of the ethanol sensitive lines, whereas it was not in any of the lines selected for ethanol insensitivity (*, indicates P < .05 compared with control/washout responses in the same line of animals, paired t test).
ethanol (such as motor incoordination) are clearly apparent in all of these lines of animals at blood ethanol concentrations of 80 mM (Draski et al., 1992), the lack of any effect on synaptic potentials at this concentration demonstrates that there are GABA_4_ synapses where concentrations of ethanol sufficient to produce a loss of the righting reflex have little effect. The behavioral effects that are observed in these insensitive lines of animals must depend either on GABA_4_ receptors in other brain regions that have greater ethanol sensitivity, or on receptors for other neurotransmitters that are more sensitive to ethanol. Previous studies have estimated that approximately six to eight genes play a significant role in the heritable component of ethanol sensitivity (Dudek and Abbott, 1984), whereas quantitative trait loci studies have identified at least five quantitative trait loci that are associated with the sleep time phenotype (Bennett et al., 1994; Markel et al., 1997). Although some of these genes may be related to GABA_4_ receptors, it seems likely that others are associated with other neurotransmitter systems.

**Ethanol Effects on Synaptic GABA_4_ Responses.** Studies of ethanol effects on GABA_4_ responses in nonselected lines of rodents have found considerable variability in ethanol sensitivity, even in studies of what would seem to be the same receptors in the same population of cells (Proctor et al., 1992a,b; Peoples and Weight, 1994; Wan et al., 1996; Weiner et al., 1997a; Peoples and Weight, 1999). This suggests that ethanol sensitivity must depend on a rather specific combination of factors. One such variable is the subpopulation of GABA_4_ receptors activated by synaptic stimulation. Previous work by Pearce and colleagues has demonstrated that there are populations of GABA_4_ synapses on CA1 pyramidal neurons that differ in a variety of respects, including their kinetic properties, and sensitivity to pharmacological agents such as furosemide (Pearce, 1993). Our studies in Sprague-Dawley rats have shown that distal GABA_4_-mediated IPSCs are less sensitive to ethanol action than are proximal IPSCs (Weiner et al., 1997a). This study demonstrated such differential ethanol sensitivity of GABA_4_ receptors even in single hippocampal pyramidal neurons, confirming that the differences in sensitivity were not merely due to unknown variables in the experimental approach. The present studies found that proximal GABA_4_ IPSCs are sensitive to ethanol in the HAS_1_ and HAS_2_ lines of rats, and in the ILS mice. Further support for the conclusion that somatically located GABA_4_ receptors are particularly sensitive to ethanol comes from local application experiments in which GABA was applied either directly to the somata of CA1 pyramidal cells, or to their dendrites in stratum radiatum. Ethanol enhanced GABA_4_ responses mediated by the somatic receptors, while having no effect on dendritic currents (W. R. Proctor, unpublished data). Similarly, Soldo et al. (1998) concluded based upon local application experiments that ethanol had a selective effect on somatic GABA_4_ receptors in cortical neurons, whereas dendritic receptors showed little or no sensitivity. These experiments support the conclusion that at least a part of the action of ethanol is postsynaptic, either on the receptor itself, or on other cellular constituents that can modulate GABA_4_ receptor sensitivity.

Identifying the factors that are responsible for the differences in the sensitivity of proximal and distal GABA_4_ receptors to ethanol is clearly a critical issue, but one that will be difficult to resolve with electrophysiological approaches. One possibility is that these differences are due to varying subunit composition of the receptors, although expression studies to date have not established a clear subunit dependence in ethanol sensitivity. The furosemide sensitivity of proximal responses (Pearce et al., 1995) suggests that these receptors incorporate the α4 subunit of the GABA_4_ receptor because furosemide selectively antagonizes responses mediated by GABA_4_ receptors incorporating the α6, which is not found in hippocampus, and α4 subunits (Wafford et al., 1996). An association between the α4 subunit and ethanol sensitivity could also explain why ethanol appears to have no effect on [36Cl] flux in hippocampus (Proctor et al., 1992a) because the α4 subunit is thought to be a relatively uncommon constituent of GABA_4_ receptors in this brain region (Wisden et al., 1992), and thus such receptors would make a relatively small contribution to overall [36Cl] flux in this brain region. Another possibility is that there are differences in post-translational mechanisms such as phosphorylation, which might differ in different parts of the cell. Phosphorylation by PKC appears to positively modulate the interaction between ethanol and GABA_4_ receptors, and might play a role in regulating such sensitivity (Weiner et al., 1997b). Possibly related to this mechanism, blockade of GABA_4_ receptors in hippocampal slices sensitizes distal GABA_4_ responses to ethanol, and this has been proposed to be due to a GABA_4_-mediated reduction of PKA and/or PKC activation (Wan et al., 1996). However, in our own studies we have found that a GABA_4_ antagonist affects the sensitivity of the distal but not the proximal pathway (W. Poelchen and T. V. Dunwiddie, unpublished data), so it seems unlikely that this mechanism could account for differences in the sensitivity of the proximal pathway reported here. Finally, differences in proteins that interact with GABA_4_ receptors, such as gephyrin (Sassone-Poggetto et al., 1999), GABA_4_ receptor-associated protein (Wang et al., 1999), and the receptor for activated C kinase (Brandon et al., 1999), might also account for differences in sensitivity.

In summary, the present experiments demonstrate that synaptically evoked GABA_4_ responses in the rat and mouse hippocampus are enhanced by superfusion with the same concentrations of ethanol that produce behavioral responses in vivo. Moreover, the sensitivity of these synapses is under the control of genes that can be selected for by classical genetic selection techniques. We hypothesize that these genes regulate the sensitivity of GABA_4_ receptors in the hippocampus, a brain region that is unlikely to be involved in the loss of righting reflex, as well as in other brain regions that are directly involved in the regulation of the ethanol-sensitive or -insensitive behavioral phenotype. Although the GABA_4_ receptor is unlikely to be the only receptor involved in determining behavioral sensitivity (DeFries et al., 1989), the fact that the ethanol sensitivity of these GABA_4_ synapses is reduced in three lines of independently selected “insensitive” animals suggests that GABA_4_ receptors must play a significant role in this behavior.

**References**


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