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Binge-pattern ethanol exposure during adolescence, but not adulthood, causes persistent changes in GABAA receptor-mediated tonic inhibition in dentate granule cells

Rebekah L. Fleming, Ph.D.^{1,2}, Qiang Li, M.D., Ph.D.^{1,2}, M.-Louise Risher, Ph.D.^{1,2}, Hannah G. Sexton¹, Scott D. Moore, M.D., Ph.D.^{1,2}, Wilkie A. Wilson, Ph.D.^{1,3}, Shawn K. Acheson, Ph.D.^{1,2}, and H. Scott Swartzwelder, Ph.D.^{1,2}

¹Durham Veterans Affairs Medical Center, Durham, NC 27705

²Department of Psychiatry, Duke University Medical Center, Durham, NC 27705

³The Center for Child and Family Policy, Social Sciences Research Institute, Duke University, Durham, NC 27705

Abstract

Background—In recent years, it has become clear that acute ethanol affects various neurobiological and behavioral functions differently in adolescent animals than in adults. However, less is known about the long-term neural consequences of chronic ethanol exposure during adolescence, and most importantly whether adolescence represents a developmental period of enhanced vulnerability to such effects.

Methods—We made whole cell recordings of GABA_A receptor-mediated tonic inhibitory currents from dentate gyrus granule cells (DGGCs) in hippocampal slices from adult rats that had been treated with chronic intermittent ethanol (CIE) or saline during adolescence, young adulthood, or adulthood.

Results—CIE reduced baseline tonic current amplitude in DGGCs from animals pre-treated with ethanol during adolescence, but not in GCs from those pre-treated with ethanol during young adulthood or adulthood. Similarly, the enhancement of tonic currents by acute ethanol exposure *ex vivo* was increased in GCs from animals pre-treated with ethanol during adolescence, but not in those from animals pre-treated during either of the other two developmental periods.

Conclusions—These findings underscore our recent report that CIE during adolescence results in enduring alterations in tonic current and its acute ethanol sensitivity and establish that adolescence is a developmental period during which the hippocampal formation is distinctively vulnerable to long-term alteration by chronic ethanol exposure.

Keywords

ethanol; extrasynaptic GABA-A receptor; tonic inhibition; dentate gyrus; adolescence

Introduction

One of the most compelling and important questions in contemporary alcohol research is whether adolescence represents a period of enhanced vulnerability to the long-term effects of repeated alcohol exposure. In the U.S., most people begin to drink alcohol (ethanol)

during adolescence, and binge drinking by adolescents is a major public health concern because this pattern of ethanol exposure is associated with increased risk of neurotoxicity and the development of alcohol abuse disorders (Crews et al., 2000; Hunt 1993; Monti et al., 2005; 2009). Studies from several laboratories have shown that ethanol affects brain function differently during adolescence than adulthood; however, it is important to note that not all reports have shown differences in ethanol sensitivity between adolescents and adults (Chin et al., 2011; Land and Spear, 2004; Markwiese et al., 1998; Sircar et al., 2009). We have consistently found that adolescent and adult rats respond differently to acute ethanol exposure: adolescent rats are more sensitive to the memory-impairing effects of ethanol than are adults (Markwiese et al., 1998), but are less sensitive than adults to the effects of ethanol on sedation (Little et al., 1996) and motor impairment (White et al., 2002a,b).

Previous behavioral studies from our laboratory have shown that in male rats, chronic intermittent ethanol (CIE) exposure during adolescence, but not adulthood, resulted in long lasting changes in sensitivity to the amnesic effects of ethanol (White et al., 2000). However, other studies have not shown similar long-term effects of CIE during adolescence (Van Skike et al., 2012). We have also recently shown that CIE during adolescence produces changes in the ethanol sensitivity of tonic inhibitory current in dentate gyrus granule cells (DGGCs) from adult rats (Fleming et al., 2012). However, that study assessed only the effects of CIE during adolescence and did not include direct comparisons with the effects of CIE during any other developmental periods. Thus a critically important question remained unanswered: is the adolescent brain uniquely vulnerable to the long-term effects of CIE on tonic inhibition in DGGCs? To answer this question, we measured tonic inhibition and its ethanol sensitivity in DGGCs from adult rats subjected to CIE during adolescence, young adulthood, and adulthood.

Materials and Methods

Animals and Chronic Intermittent Ethanol Exposure

The animal research reported in this study was conducted according to protocols that were approved by the Durham VAMC and Duke University Institutional Animal Care and Use Committees. Male Sprague-Dawley rats were obtained from Charles River (Raleigh, NC). The rats arrived at the Durham VAMC vivarium 5 or 6 days before the beginning of ethanol exposure and were acclimated to handling for 3 days. They were housed 2 animals per cage in room with a 12 hr on 12 hr off reversed light / dark cycle and provided with *ad libitum* access to food and water for the duration of the study. Three age groups were exposed to a chronic intermittent ethanol (CIE) regimen; adolescent rats began the regimen at 30 days of age, young adults at 50 days of age, and adults at 70 days of age. The CIE regimen consisted of 10 doses by gavage of 5 g/kg ethanol (35% v/v in 0.9% saline) in a two-days-on, two-days-off sequence such that the animals received single gavage exposures on days 1 and 2, 5 and 6, 9 and 10, 13 and 14, and 17 and 18 of the regimen. Control rats received 18.12 ml/kg saline by gavage. After the last ethanol exposure, the rats were housed in the vivarium for another 23 days until the adolescent CIE group had reached adulthood at 70 days of age; by this time, the young adult CIE group had reached 90 days of age, and the adult CIE group had reached 119 days of age. Although patterned slightly differently, this CIE exposure paradigm closely parallels that of our previous behavioral studies (White et al., 2000; 2002a) as well as recent studies of the effects of CIE during adolescence on hippocampal pyramidal cell function (Tokunaga et al., 2006). The electrophysiology was conducted between 40 and 54 days after the start of the CIE regimen. Thus, all *ex vivo* electrophysiological recording was carried out in brain slices from adult rats. The exposure group, ethanol or saline, used for each slice preparation was pseudo-randomized, and the electrophysiologist was blind to the pre-exposure group of the animals during the slice preparation, data acquisition, and analysis phases of the experiment.

Brain slice preparation

The rats were deeply anesthetized using either isoflurane or a ketamine/acepromazine/xylozine cocktail and were transcardially perfused with an ice-cold cutting solution that consisted of (mM) 115 N-Methyl-D-glucamine, 3.3 KCl, 25 NaHCO₃, 1.23 NaH₂PO₄, 15 D-Glucose, 3 Myo-Inositol, 2 Na Pyruvate, 0.4 Na Ascorbate, 0.2 CaCl₂, 12 MgCl, pH 7.4 and was equilibrated with a carboxygen gas mixture of 95% O₂ - 5% CO₂ (Peca et al., 2011; Zhao et al., 2011). Hippocampus slices (350 μm) were cut using a Compressstome (Precisionary Instruments, San Jose, CA) and incubated at 32°C for 15 min; then, the slices were transferred to a holding chamber containing room temperature (RT: 21–23°C) carboxygenated artificial cerebrospinal fluid (aCSF) that consisted of (mM) 120 NaCl, 3.3 KCl, 25 NaHCO₃, 1.23 NaH₂PO₄, 15 D-Glucose, 3 Myo-Inositol, 2 Na Pyruvate, 0.4 Na Ascorbate, 2.0 CaCl₂, 1.3 MgCl. The slices were allowed to equilibrate in carboxygenated aCSF for at least 1 hour before electrophysiological recording.

Electrophysiology

Individual DGGCs were visually identified using a Zeiss Axioskop (Carl Zeiss Microscopy, LLC, Thornwood, NY) equipped with IR-DIC videomicroscopy and a 40X water immersion objective. While in the recording chamber, the slices were perfused with RT carboxygenated aCSF at a rate of 2–4 ml/min. Microelectrodes with a tip resistance of 5–10 MΩ when filled were pulled from thin-walled borosilicate glass capillaries (World Precision Instruments, Inc. or Garner Glass Company) using a Sutter Instrument Co. (Novato, CA) P-2000 puller. The electrode solution consisted of (mM) 130 CsCl, 10 HEPES, 4 NaCl, 0.2 EGTA, 10 Na₂CreatinePO₄, 4 MgATP, 0.3 TrisGTP, 6 QX-314; pH 7.2, osm 290. Whole-cell voltage-clamp recordings were performed using an Axopatch 200B amplifier (Molecular Devices, LLC, Sunnyvale, CA). Signals were low-pass filtered at 2 kHz and digitized at 10 kHz using an Axon Instruments Digidata 1440A and Clampex 10.2 (Molecular Devices, LLC, Sunnyvale, CA).

Tonic and phasic inhibitory currents were measured as previously described (Fleming et al., 2007). Briefly, granule cells were voltage clamped at –80 mV, and the holding current was recorded during 3 periods: (1) baseline, aCSF only; (2) during bath application of 30 mM ethanol; and (3) during application of 200 μM picrotoxin. To avoid repeat exposures to ethanol, only one cell was obtained per slice.

Data Analysis and Statistics

Data analysis was performed on data records that had been stripped of exposure and age group information. Tonic and phasic current determination was performed using an in-house function written for MATLAB (Mathworks, Natick, MA) that is based on the method used by Glykys and Mody (2007). For each recording period, all-point histograms were generated for 60 sec of data, and the Gaussian function $f(x) = A \cdot \exp(-(x-\mu)^2/2\sigma^2)$ was fitted to the part of each histogram that was not skewed by synaptic events. The center of this distribution (μ) represents the mean holding current, while σ represents the root-mean-square (RMS) noise over the 60 sec interval. For each cell, the GABA_A receptor-mediated tonic current was determined by calculating the difference between the centers of the fitted Gaussian functions obtained for the baseline and picrotoxin conditions ($\mu_{\text{picrotoxin}} - \mu_{\text{baseline}}$). The tonic noise was determined by calculating the difference in RMS noise between the two conditions ($\sigma_{\text{baseline}} - \sigma_{\text{picrotoxin}}$).

Mean phasic current was determined by subtracting μ from the raw data trace for each interval, which brought the mean of the Gaussian to 0 pA, and computing the cumulative sum of the data, which was then divided by the number of points in the trace. Thus, the noise in the holding current summed to 0 pA, and the tail generated by the IPSCs summed to yield

the mean phasic current (phasic I_{mean}). The GABA_AR-mediated contributions to phasic I_{mean} were computed by subtracting the values obtained under the picrotoxin condition, e.g., the baseline mean phasic current equaled $I_{\text{mean/baseline}} - I_{\text{mean/picrotoxin}}$.

Spontaneous inhibitory postsynaptic currents (sIPSCs) were also analyzed using the MiniAnalysis program (Synaptosoft, Inc., Decatur, GA). First, individual sIPSCs were detected using parameters that were based on the recommended settings for detecting GABAergic events and optimized using a subset of the available recordings. Once the final parameters were chosen, all the recordings were analyzed using the same settings. After sIPSC detection, a burst analysis was conducted; the minimum number of events in a burst was set to 3, and the maximum interevent interval within a burst was 75 ms. For each recording period, the overall sIPSC frequency, the number of bursts per minute, and the % of IPSCs within bursts were calculated.

Inferential statistics included paired t-tests to compare pre-drug baseline recordings to recordings made under drug conditions. During the analysis of the effect of acute ethanol exposure on tonic current, some neurons were found to be statistical outliers, i.e. > two standard deviations from the group mean, and these cells were eliminated from all further analysis. Comparisons across groups were made using 2-way ANOVA, one-way ANOVA, or t-test, as appropriate. The criterion for significance was set at $p < 0.05$; all data are presented as mean $\pm$ standard error.

Results

The effects of CIE on tonic current

We performed *ex vivo* slice electrophysiology and recorded tonic inhibitory currents in DGGCs from adult rats that had been subjected to a CIE regimen during adolescence, young adulthood, or mature adulthood (Figure 1). Rats that received saline rather than ethanol during the CIE regimen served as controls for any stress effects associated with the gavage procedure. The baseline levels of tonic inhibitory current measured in each of these 6 groups (2 pre-treatment conditions $\times$ 3 age groups) are shown in Figure 2. A 2 $\times$ 3 ANOVA revealed a significant effect of age, [$F(2,72) = 3.364$, $p = 0.04$], no effect of exposure condition, and no interaction between age and exposure condition. However, visual inspection of the data strongly suggested an ordinal interaction between age and exposure condition. Therefore, separate one-way ANOVAs were run on the CIE groups and the saline controls. These analyses revealed a significant main effect of age among the saline control groups: baseline tonic current was highest in the adolescent exposure group and lowest in the adult exposure group [$F(2,33) = 3.280$, $p = 0.05$]; no effect of age was found for the CIE exposure groups. Additional follow-up testing using 1-tailed Student's t-tests revealed significantly less tonic current in adolescent CIE animals compared to their age-matched controls [$t(26) = 1.942$, $p = 0.032$]. No such differences were found for the young adult and adult exposure groups. There were no significant effects of age or exposure condition on tonic noise (Figure 2B).

Acute exposure to 30 mM ethanol in the recording chamber increased the size of the holding current in DGGCs from all 6 groups. To control for the variability in the size of the tonic current among cells, ethanol enhancement of tonic current was calculated for each cell as the increase in holding current during bath ethanol application as a percentage of the tonic current in the absence of ethanol: $100 \cdot (\mu_{\text{baseline}} - \mu_{\text{ethanol}}) / (\mu_{\text{picrotoxin}} - \mu_{\text{baseline}})$. A 2 $\times$ 3 ANOVA revealed no significant effects of age or exposure condition and no interaction. However, similarly to the tonic current results, visual inspection of the data shown in Figure 3 strongly suggested an ordinal interaction between age and exposure condition. Therefore, separate one-way ANOVAs were also run on the CIE groups and the saline controls. There

was a significant main effect of age among the saline control groups [$F(2,33) = 6.366$, $p = 0.005$]; ethanol enhancement of tonic current changed across age, with the adolescent exposure group showing the lowest ethanol enhancement and the adult exposure group showing the greatest enhancement. No effect of age was found for the CIE exposure groups. In addition, 1-tailed Student's *t*-test revealed significantly more ethanol enhancement of tonic current in adolescent CIE animals compared to their age-matched controls [$t(26) = 2.503$, $p = 0.01$]. No such differences were found for the young adult and adult exposure groups.

The effects of CIE on phasic current and bursting of sIPSCs

To assess the effect of CIE on phasic inhibition, we also calculated the mean phasic current (phasic I_{mean}) for each recording interval of each cell. Baseline phasic I_{mean} did not differ among the 6 groups (Figure 4A). Acute exposure to 30 mM ethanol in the recording chamber did not alter phasic I_{mean} in most of the groups. However, in the young adult saline group, 30 mM ethanol significantly increased the phasic I_{mean} by 68.15% from 3.33 ± 0.40 pA to 5.29 ± 0.91 pA [paired samples $t(11) = 2.411$, $p = 0.035$] (Figure 4B). As the data in Figure 4B suggest, there was a substantial amount of cell-to-cell variability in the effect of acute ethanol exposure on phasic I_{mean} . Visual inspection of several current traces revealed that many of the recordings contained bursts of overlapping sPSCs. Therefore, to determine if either CIE or acute ethanol exposure altered bursting in the slices, individual sPSCs were detected and grouped into bursts using MiniAnalysis (Synaptosoft, Inc., Decatur, GA).

In our slice preparation, spontaneous inhibitory postsynaptic currents (sIPSCs) were not isolated by adding glutamatergic antagonists to the aCSF during recording. However, in the dentate gyrus slice preparation, spontaneous firing of excitatory neurons is typically low, and the majority of sPSCs are IPSCs. In the present study, this assumption is supported by the fact that picrotoxin eliminated nearly all of the phasic current in our recordings (Figure 1). Therefore, the individual sPSCs were detected using MiniAnalysis parameters similar to those recommended for GABAergic synaptic events. Under baseline conditions, the mean number of bursts / min did not differ among the 6 groups. As previously seen in the phasic I_{mean} analysis, for most of the groups, acute ethanol (30 mM) exposure *ex vivo* did not significantly alter the overall sIPSC frequency, the number of bursts per minute, or the % of IPSCs within bursts. However, in the young adult saline group, all these parameters were significantly increased by ethanol (Table 1).

Discussion

The principle findings of this study are that CIE exposure during adolescence, but not young adulthood or adulthood, resulted in decreased baseline tonic inhibitory current and increased the sensitivity of this tonic current to enhancement by ethanol. Thus, this study represents the first demonstration that adolescence is a distinctively vulnerable period during which CIE produces enduring changes in hippocampal cellular function. We had previously shown that CIE during adolescence alters the sensitivity of tonic current to acute ethanol during adulthood (Fleming et al., 2012). The present findings add to that study in two important ways: first, by including a direct comparison of the enduring effects of CIE exposure during three distinct developmental periods, we were able to assess the distinctive vulnerability of hippocampal neuronal function to CIE during adolescence, and secondly by quantifying IPSC frequency and bursting as an index of phasic inhibition, the findings provide compelling evidence suggesting that ethanol enhances tonic inhibition via a mechanism that is independent of changes in GABA release. More generally, these findings also suggest that extrasynaptic GABA_A receptors, particularly δ GABA_ARs, may play a significant role in mediating some of the long-term consequences of binge pattern ethanol exposure during adolescence.

In addition to the principle findings, the data obtained from the saline control groups contain two findings that merit further investigation. First, the levels of baseline tonic current and its ethanol sensitivity continue to change even after rats reach an age that is typically considered fully adult. We have previously shown that, in ethanol-naïve animals, baseline levels of tonic inhibition increase and the ethanol sensitivity of the tonic current decreases between adolescence (30–40 days of age) and adulthood (70–80 days of age) (Fleming et al., 2007). The results of this study suggest that rather than remaining stable after animals reach 70 days of age, baseline levels of tonic inhibition may peak just as animals reach adulthood and then decline. However, one caveat must be considered when comparing our previous results in ethanol-naïve adult animals to the present study. In the previous study, the animals experienced only the routine handling necessary for animal husbandry, but in this study, the saline controls were subject to a gavage procedure. Thus, it is possible that long-term effects of stress, and not the effects of normal aging, are responsible for the decreases in tonic current that we observed as the animals aged.

Second, acute exposure to 30 mM ethanol increased mean phasic current, total sIPSC frequency, number of sIPSC bursts/min, and the % of sIPSCs in bursts only in the young adult saline group. This was a surprising finding because we had previously shown a small but statistically significant increase in phasic current in control animals subjected to the CIE regimen during adolescence (Fleming et al., 2012); that slight increase was not replicated in this study. However, in the young adult saline group, acute ethanol exposure *ex vivo* increased phasic I_{mean} by 68.15%, and this change was associated with increases in the amount of sIPSC bursting in these slices. At present, the mechanisms, underlying this effect of ethanol are unclear, but we speculate that the stress induced by the gavage procedure during young adulthood, but not adolescence, may have produced long-term changes in function of inhibitory interneurons in the dentate. This finding is potentially important because it suggests that while by 50 days of age, the rat brain appears to be relatively invulnerable to the long-term effect of CIE on tonic inhibition, during this “young adult” period, the rat brain may be particularly sensitive to the long-term effects of stress, and these stress effects may alter how DG interneurons respond to ethanol exposure once full maturity has been reached. More work is needed to confirm this effect and determine the mechanisms involved in producing this change.

Tonically active extrasynaptic GABA_ARs decrease the membrane resistance of neurons and thereby produce shunting inhibition that decreases the membrane depolarization during excitatory synaptic transmission (Semyanov et al., 2004; Staley and Mody, 1992). In DGGCs, ethanol-sensitive δ GABA_A receptors mediate a tonic inhibitory current that strongly influences their function (Nusser and Mody, 2002; Wallner et al., 2003). Knockout of the δ subunit enhances trace fear conditioning in mice, providing evidence that tonic inhibition mediated by extrasynaptic GABA_A receptors in the dentate gyrus suppresses hippocampus-mediated learning (Wiltgen et al., 2005). Therefore, greater ethanol enhancement of δ GABA_ARs on DGGCs after adolescent CIE is consistent with the increased sensitivity to ethanol’s amnesic effects that occurred after CIE (White et al., 2000).

In this study, we also found that CIE during adolescence decreased the amount of baseline tonic current in DGGCs. In our previous CIE study in adolescents only, we observed a non-significant trend towards a decrease in baseline tonic current (Fleming et al., 2012); thus it is likely that the differences between our previous study and this one can be attributed to differences in statistical power. We have also previously shown that baseline tonic current in DGGCs is lower in ethanol-naïve adolescent rats than in adults, and GABA uptake by the GAT-1 transporter plays a role in maintaining this lower baseline tonic current in adolescent rats (Fleming et al., 2007; 2011). Therefore, it is possible that two distinct mechanisms,

changes in extrasynaptic GABA_AR expression or function and changes in extracellular GABA concentration, interact to produce the changes in tonic inhibition and its ethanol sensitivity that we observe after adolescent CIE.

Long-term alterations of extrasynaptic GABA receptor-mediated currents after CIE have been observed previously when the ethanol exposure occurred during young adulthood and adulthood (Liang et al., 2009; Liang et al., 2007). In these studies, there was a long-lasting reduction of extrasynaptic GABA_AR-mediated current in CA1 neurons after a CIE paradigm that involved 60 doses across 120 days, beginning at about postnatal day 50. Liang et al. also reported long-lasting down-regulation of GABA receptor δ subunit protein in hippocampus after CIE (2007). Because δ GABA_ARs are thought to be the mostly highly ethanol sensitive extrasynaptic GABA_ARs, the findings reported by Liang et al. are inconsistent with the finding of an increase in ethanol sensitivity of tonic inhibition after adolescent CIE that we report here (also see Fleming et al., 2012). However, the studies reported by Liang et al. (2004; 2009; 2007; 2006) used higher doses of ethanol (6 g/kg) for a much longer duration to create a model of alcohol withdrawal and dependence. In contrast, the CIE used in this study is designed to model a more transient exposure similar to the patterns of binge drinking observed in human adolescents. Therefore, it is likely that differences in the ethanol exposure regimens contribute to the discrepancies between the results reported by Liang et al. and our studies.

Furthermore, the data we present here suggest that the effects of CIE in “young adult” rats of 50 days of age are substantially different from the effects of CIE that starts at 30 days of age during adolescence. Our previous work on differences in ethanol sensitivity of tonic current in ethanol-naïve adolescent and adult rats has suggested that in addition to GABA_AR subunit composition, other factors, such as changes in ambient GABA concentration, also regulate ethanol’s effect on tonic inhibition (Fleming et al., 2007; 2011). Changes in intracellular signaling and Cl⁻ reversal potential could also play roles in the effects of CIE on tonic inhibition (Smith et al., 2009; Weiner and Valenzuela, 2006; Yamashita et al., 2006). More work is needed to elucidate the mechanisms that underlie the persistent changes in tonic inhibition that occur after CIE and to understand why these changes do not occur in older animals.

Because pharmacokinetic differences have been observed between adolescent and adult rats, it is important to consider the possibility that they may have contributed to the long-term effects of CIE that we report. For example, we have previously shown that adolescent male Sprague-Dawley rats achieve lower serum ethanol concentrations than do adults after intraperitoneal (i.p.) administration of 2 g/kg or 4 g/kg ethanol (Little et al., 1996). However, in contrast Walker and Ehlers (2009) found no differences in blood ethanol concentrations in adolescent and adult Wistar rats after 3 g/kg ethanol was administered i.p. or by gavage. Thus, although there is some evidence of pharmacokinetic differences between adolescent and adult rats, when differences have been observed, adolescents achieve lower, not higher ethanol concentrations. This would not appear likely to account for the greater effects that we have observed of CIE during adolescence compared to adulthood.

The present finding that CIE during adolescence, but not young adulthood or adulthood, results in increased ethanol sensitivity of tonic current in adult DGGCs could be of mechanistic significance for explaining previous studies that showed enhanced sensitivity to the memory-impairing effects of ethanol during adulthood after CIE in adolescence (White et al., 2000). Given the close similarities between the CIE paradigms used in that study and this one, it is possible to suggest that the enhanced sensitivity to the acute memory-impairing effects of ethanol observed after CIE in adolescence by White et al (2000) could be related to enhanced ethanol potentiation of tonic inhibition in DGGCs. Along with other studies that

demonstrate long-term memory-related cellular effects of CIE during adolescence (Tokunaga et al., 2006), these results could represent a conceptual step forward in understanding the mechanisms that underlie the adolescent brain's vulnerability to alcohol.

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Figure 1.

Ethanol increased GABA_A receptor-mediated tonic inhibition in dentate granule cells from mature rats exposed to ethanol as adolescents or young adults and age-matched controls. Left: Representative voltage-clamp traces from a neuron from an adult rat that had been submitted to a chronic intermittent ethanol (CIE) regimen (B,D) or a saline control regimen (A,C) during adolescence (A,B) or young adulthood (C,D). Holding current was recorded for 3 periods: a no-drug baseline and during bath application of 30 mM ethanol application and 200 μ M picrotoxin. Right: All-point histograms and fitted Gaussian functions for the whole-cell current data shown at left. To facilitate comparison among cells, the data are plotted relative to the center of the distribution for the picrotoxin condition. Data from the adult exposure groups are not shown because they are similar to the data for animals pre-treated during young adulthood.

Figure 2. Chronic intermittent ethanol exposure (CIE) during adolescence, but not later, decreased GABA_AR-mediated tonic inhibition in adult dentate granule cells. (A) Tonic current and (B) tonic noise recorded from neurons from adult rats that had been submitted to a CIE regimen or a saline (control) regimen during adolescence, young adulthood, or adulthood. CIE during adolescence reduced tonic noise in adult neurons [$t(26) = 1.942$, $*p = 0.032$].

Figure 3.

Chronic intermittent ethanol exposure (CIE) during adolescence, but not later, increased ethanol enhancement of tonic current in adult dentate granule cells. CIE during adolescence increases the ethanol (30 mM) enhancement of tonic current in adult neurons [$t(26) = 2.503$, $*p = 0.01$].

Figure 4.

(A) Chronic intermittent ethanol exposure (CIE) did not alter phasic inhibitory current measure in the absence of ethanol. (B) Acute ethanol (30 mM) exposure *ex vivo* significantly increased phasic currents only in animals pre-treated with saline during young adulthood. * $p < 0.05$.

Table 1

Acute ethanol alters sIPSC frequency and bursting in rats pre-treated with saline during young adulthood.

Group	Baseline			Ethanol		
	sIPSCs/s	Bursts/min	% in bursts	sIPSCs/s	Bursts/min	% in bursts
Adolescent: S	2.9 (0.4)	5.9 (1.4)	9.6 (1.9)	3.1 (0.4)	7.5 (2.1)	10.8 (2.7)
Adolescent: CIE	3.1 (0.5)	8.4 (2.9)	10.9 (2.6)	3.2 (0.5)	7.9 (2.1)	11.4 (2.1)
Young adult: S	2.1 (0.3)	2.8 (0.8)	6.3 (1.3)	3.6* (0.6)	12.3* (4.5)	18.8* (4.8)
Young adult: CIE	2.3 (0.4)	4.9 (1.8)	10.2 (2.2)	2.5 (0.5)	7.3 (3.5)	11.8 (3.7)
Adult: S	2.7 (0.5)	5.3 (1.8)	8.7 (2.9)	2.8 (0.5)	6.7 (1.9)	10.7 (2.6)
Adult: CIE	1.7 (0.3)	2.1 (0.7)	5.3 (1.5)	2.0 (0.3)	4.3 (1.3)	10.5 (3.2)

* p < 0.05 by paired t-test comparing baseline and ethanol conditions.