Published in final edited form as:

Alcohol Clin Exp Res. 2014 May ; 38(5): 1293–1300. doi:10.1111/acer.12362.

Third Trimester-Equivalent Ethanol Exposure does not Alter Complex Spikes and Climbing Fiber Long-Term Depression in Cerebellar Purkinje Neurons from Juvenile Rats

Paula A. Zamudio-Bulcock, Russell M. Morton, and C. Fernando Valenzuela

Department of Neurosciences, School of Medicine, University of New Mexico Health Sciences Center, Albuquerque, New Mexico, U.S.A

Abstract

Background—Studies indicate that exposure to ethanol (EtOH) during fetal development damages cerebellar Purkinje cells (PCs). PC proximal dendrites receive glutamatergic input from climbing fibers (CFs) originating at the inferior olive. CF input produces a characteristic response in PCs known as the complex spike (CS). During the first two weeks of life in rodents (equivalent to the human third trimester of pregnancy), CF-PC synapses undergo profound refinement. Here, we characterized the impact of EtOH exposure during this period on CF-evoked responses in PCs.

Methods—Using vapor chambers, neonatal rat pups and their mothers were exposed to air or EtOH for 4 hr/day between postnatal day (P) 2 and P12 (pup serum EtOH concentration, 0.16 g/dl). The function of CF-PC synapses was characterized using patch-clamp electrophysiological techniques in acute slices from the cerebellar vermis. Experiments were performed soon after EtOH withdrawal, when perisomatic CFs are still being eliminated (P15–P17) and after weaning when CF dendritic translocation is almost complete (P21–P34).

Results—Neither the baseline characteristics of the CS (Na^+ spike amplitude, area, coast line index, and after hyperpolarization amplitude) nor the type-1 metabotropic glutamate receptor (mGluR1)-mediated component of both the CS and after hyperpolarization were significantly affected by EtOH exposure at P15–P17 or P21–P34. The mGluR1-dependent long-term depression of CF-evoked excitatory postsynaptic currents was not significantly affected by EtOH exposure at P21–P34.

Conclusions—EtOH exposure during the third trimester-equivalent neither affected basal characteristics of the CS nor CF long-term depression at rat cerebellar PCs from juvenile rats.

Keywords

cerebellum; complex spike; climbing fiber; Purkinje cell; synaptic; plasticity

Introduction

Exposure to ethanol (EtOH) during development produces fetal alcohol spectrum disorders (FASDs), characterized by morphological and neurobehavioral alterations that vary depending on several factors, including timing and pattern of exposure, dose of EtOH, co-exposure to other agents (e.g., nicotine), genetic background, maternal age and nutritional status (Jones, 2011). Prenatal EtOH exposure has been shown to impair motor coordination and balance, which are known to rely on cerebellar control (Roebuck et al., 1998b, Connor et al., 2006). Autopsy and imaging studies have demonstrated reductions in cerebellar volume in fetal alcohol syndrome patients, particularly in the anterior vermis (McGee and Riley, 2006, Roebuck et al., 1998a, Astley et al., 2009, O'Hare et al., 2005). It has also been reported that the anterior vermis is displaced in fetal alcohol exposed subjects and this is negatively correlated with verbal learning and memory performance (O'Hare et al., 2005). Structural alterations in the cerebellar white matter microstructure were found to be related to math ability and oculomotor control in children with FASDs (Lebel et al., 2010, Green et al., 2013). Moreover, studies have independently demonstrated that children prenatally exposed to EtOH have impairments in eyeblink conditioning, a motor learning paradigm that is mediated by cerebellar circuits (Coffin et al., 2005, Jacobson et al., 2008). Collectively, these studies with humans indicate that the cerebellar effects induced by developmental EtOH exposure can adversely impact, not only motor coordination and motor learning, but also a number of complex cognitive functions.

Studies indicate that cerebellar alterations associated with FASDs are, in part, a consequence of damage to Purkinje cells (PCs) (Green, 2004). These specialized GABAergic neurons constitute the sole output of cerebellar cortex and make synapses with neurons located in deep cerebellar and vestibular nuclei. PCs receive glutamatergic input from parallel fibers and climbing fibers (CFs) that originate in cerebellar granule cells and inferior olivary neurons, respectively. Mature PCs receive input from a single CF, which forms numerous synapses in the proximal dendritic tree of these neurons (Ito, 2006). CFs have a high probability of glutamate release and evoke a unique response in PCs known as the complex spike (CS) that is composed of a primary spike followed by a variable number of secondary spikelets that are superimposed on a depolarization plateau (Zagha et al., 2008, Schmolesky et al., 2002). CF input to PCs causes large dendritic Ca^{2+} transients that play a central role in the induction of long-term depression (LTD) at parallel fiber-to-PC synapses. This form of synaptic plasticity may be involved in cerebellar-dependent motor learning, including eyeblink conditioning (Ito, 2006). CF-PC synapses can also undergo LTD, which is dependent on activation of type-1 metabotropic glutamate receptors (mGluR1) and can regulate the probability of synaptic plasticity at parallel fiber-to-PC synapses (Hansel and Linden, 2000, Ohtsuki et al., 2009).

CF-PC synapses undergo dramatic changes during the first weeks of life in rodents (Hashimoto and Kano, 2013). During the first three days of postnatal life, multiple CFs form weak synaptic contacts of similar strength at the soma of single immature PCs. Between postnatal day (P)3 and P7, a process of functional differentiation takes place in which a single CF is strengthened. Subsequently, the strong CF translocates to PC dendrites, where it stabilizes its synaptic contacts, a process that starts at P9 and is mostly completed by P20.

Surplus CFs are eliminated via a two-phase process that occurs between P8 and P17, with most perisomatic CF synapses being eliminated by P15 (Hashimoto and Kano, 2013). Studies have shown that postnatal EtOH exposure produces structural alterations at CF-PC synapses (Pierce et al., 2010, Pierce et al., 2011). Given that CF-PC synapses undergo significant refinement during the first 12 days of life in rodents (equivalent to the third trimester of human pregnancy), we hypothesized that EtOH exposure during this period impairs synaptic transmission at CF-PC synapses. We tested this hypothesis using a vapor chamber EtOH exposure paradigm and patch-clamp electrophysiological techniques with cerebellar slices.

Materials and Methods

EtOH Vapor Chamber Exposure Paradigm

The University of New Mexico Health Sciences Center Institutional Care and Use Committee approved all animal procedures. Pregnant Sprague-Dawley dams between gestational days 12 and 17 were received from Harlan Laboratories (Indianapolis, IN). Pregnant dams were individually housed with a plastic hut to reduce stress and *ad libitum* access to food and water. Pregnant dams were monitored daily for presence of pups and the day of birth was considered postnatal day 0. On postnatal day, 2 dams and pups were transferred to vapor chambers with *ad libitum* access to food and water at all times. Vapor chambers were built in-house, based on a previous design (Becker and Hale, 1993). Dams and pups were exposed to EtOH vapor generated by forcing air (using a Silent Air[®] pump Model 600, LMI Manufacturing Group, Lake Hopatcong, NJ) through a diffusion stone placed into liquid EtOH (190 proof) (Pharmco-AAPER, Shellbyville, KY) at room temperature. Vaporized EtOH was forced into the top of the chambers and excess air was pushed out through a small exit port in the back of the chambers that was directly vented out into the room's air exhaust system (Fig 1A). Dams and pups were exposed to EtOH for 4 hours per day from postnatal day (P) 2 to P12. At the end of the EtOH exposure, the air pumps were turned off, the doors to the chambers were opened, and food pellets were replaced. To minimize stress, animals were continuously housed in the chambers from P2 to P12. Air exposed controls were housed in identical chambers and exposed to forced room air only from P2 to P12. EtOH exposed litters were exposed to vaporized EtOH at an average concentration near 3 g/dl (Table 1). Serum EtOH concentrations (SECs) were measured with an alcohol dehydrogenase-based assay, as previously described (Galindo and Valenzuela, 2006).

Electrophysiological experiments

Cerebellar vermis parasagittal slices (250 μ m-thick) were prepared from air control or EtOH exposed male offspring, as previously described (Zamudio-Bulcock et al., 2011). CS recordings were performed a few days after EtOH withdrawal, during a period where perisomatic CFs are still being eliminated (postnatal days 15–17), and after weaning when CF dendritic translocation has been mostly completed (postnatal days 21–34). Whole-cell patch-clamp recordings in the current-clamp or voltage-clamp mode were performed at 32°C using an internal solution containing (in mM): 135 K-gluconate, 10 MgCl₂, 0.1 CaCl₂, 1 EGTA, 10 HEPES (pH 7.3), and 2 Na₂-ATP. The artificial cerebrospinal fluid (ACSF) was

equilibrated with 95% O₂/5% CO₂ and contained the following (in mM): 126 NaCl, 2 KCl, 1.25 NaH₂PO₄, 1 MgSO₄, 26 NaHCO₃, 2 CaCl₂, 10 glucose, and 50 μ M picrotoxin. CF mediated responses were evoked with a concentric bipolar electrode located in the granule cell layer, approximately 150 μ m from the patched PC, using a stimulation intensity of 300–600 μ A. CF responses were confirmed by their all or none fashion and presence of paired-pulse depression. The paired-pulse ratio (PPR) was calculated as the ratio of the amplitude of the second CF-evoked excitatory postsynaptic current (EPSC) over the amplitude of the first CF-evoked EPSC. During CS recordings, the baseline membrane potential was maintained between –60 and –70 mV via current injection. CF-induced excitatory postsynaptic currents (EPSCs) were recorded in the whole-cell voltage-clamp configuration using a holding potential between –40 and –60 mV using the same internal solution described above. To improve voltage-clamp stability in recordings of CF-EPSCs, 4 mM of the blocker of voltage-gated Na⁺ channels, N-(2,6-Dimethylphenylcarbamoylmethyl)triethylammonium bromide (QX-314), was added to the internal solution and 0.5 μ M of the AMPA receptor blocker, 2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX) was added to the ACSF. Long-term depression (LTD) was induced by stimulating CFs for 30 s at 5 Hz, as previously described (Carta et al., 2006).

Data were acquired and analyzed with pClamp 10 (Molecular Devices, Sunnyvale, CA) at a filtering frequency of 2 kHz and an acquisition rate of 10 kHz. The secondary spikelet phase of the CS response was quantified by the coast line index (CLI). The CLI was calculated with an algorithm written in Matlab r2011b (Mathworks, Natick, MA). This algorithm calculated the length of a manually chosen segment of a CS, which started at the end of the sodium spike and ended 25 ms later. The units of this measurement are arbitrary. Statistical analyses of pooled data were performed with Prism 4 or 5 (GraphPad Software, San Diego, CA). Data were initially analyzed with the Kolmogorov-Smirnov normality test and if data passed this test, parametric tests were used for statistical analysis. If data did not pass the normality test, non-parametric tests were used. The level of significance was $p < 0.05$. Data are presented as mean $\pm$ standard error of the mean. Each number of determination represents the average of PC slice recordings obtained from 1 animal. For each set of data a minimum of 3 different litters were included.

Results

EtOH Vapor Chamber Paradigm

To model human EtOH consumption during the 3rd trimester of pregnancy, we exposed rat pups with their respective dams using EtOH vapor chambers (Fig 1A). Litter size at P6 and percent of pups that died during the exposure were not significantly different between the air and EtOH groups (Table 1). Peak serum EtOH concentrations in the pups were approximately twice the legal intoxication limit (Table 1; legal intoxication limit: 0.08 g/dl = 17.4 mM); therefore, this exposure procedure modeled relatively heavy drinking during the 3rd trimester of pregnancy (Burd et al., 2012). Under these exposure conditions, SECs in the dams were low (1.3 ± 0.33 mM; $n = 4$). Analysis of pup weight gain by 2-way ANOVA revealed a main effect of age [$F(12,374) = 140.2$; $p < 0.0001$] and exposure [$F(1,374) =$

11.06; $p=0.001$], but no interaction [$F(12,374) = 0.83$; $p=0.61$] (Fig 1B). A Bonferroni multiple comparisons post-hoc test did not reveal any significant effect of exposure on weights across the different ages. The membrane capacitance and membrane resistance of PCs were not significantly affected by EtOH exposure (Table 1).

Neonatal EtOH exposure did not affect basal parameters of CSs

In slices from air- and EtOH-exposed rats, stimulation of CFs elicited typical CSs consisting of a fast Na^+ spike followed by a variable number of secondary spikelets and an afterhyperpolarization (AHP) (Fig 2A–F). At P15–17, Na^+ spike amplitudes were 105.4 ± 8.19 mV ($n=8$) and 106.5 ± 4.0 mV ($n=5$) in the air and EtOH groups, respectively [Mann-Witney $U = 20$; sum of ranks in air group = 56; sum of ranks in EtOH group = 35; $p > 0.99$]. Similarly, at P21–34, the Na^+ spike amplitudes were 102.0 ± 6.94 mV ($n=8$) and 92.21 ± 5.4 mV ($n=8$) in the air and EtOH groups, respectively [$t(14) = 1.12$, $p = 0.28$]. As shown in Fig 2A, B, the area of the CS, measured from the onset of the event until the start of the AHP, was not significantly different between treatment groups [for P15–17: Mann-Witney $U = 15$; sum of ranks in air group = 51 ($n=8$); sum of ranks in EtOH group = 40 ($n=5$); $p = 0.52$; for P21–34: $t(14) = 1.79$; $p = 0.09$; $n=8$]. Figs 2C, D show that the amplitude of the AHP was not significantly different between the experimental groups [for P15–17, $t(11) = 1.36$; $p = 0.20$; $n=8$ and 5 for the air and EtOH groups, respectively; for P21–34: $t(14) = 1.18$; $p = 0.256$; $n=8$]. Given that the number and shape of secondary spikelets varies from cell to cell, we calculated the coastline index (CLI) of secondary spikelets rather than simply counting these (Fig 2E, F). There were no statistically significant differences in the CLI between the air and EtOH groups for either the air controls or the EtOH exposed groups [for P15–17: Mann-Witney $U = 19$; sum of ranks in air group = 57; sum of ranks in EtOH group = 34; $p = 0.88$; $n=8$ and 5 for the air and EtOH groups, respectively; for P21–34: Mann-Witney $U = 26$; sum of ranks in air group = 74; sum of ranks in EtOH group = 62; $p = 0.56$; $n=8$].

Neonatal EtOH exposure did not affect the mGluR1-dependent component of the CS

Studies from our laboratory have demonstrated that the type-1 metabotropic glutamate receptor (mGluR1) contributes to the late phase of the CS (Carta et al., 2006). We also showed that this component of the CS is acutely inhibited by EtOH in cerebellar slices from juvenile rats (Carta et al., 2006). Therefore, we tested whether the mGluR1-mediated portion of the CS was affected by 3rd trimester-equivalent EtOH exposure. Application of the mGluR1 antagonist LY367385 (50 μM) significantly reduced the area of the CS in slices from air control groups by 15–20% (Fig 3A, C). In slices from EtOH exposed rats, LY367385 application decreased the CS area by a similar extent (Fig 3B, C). There was not a statistically significant difference in the effect of LY367385 on the CS area between air control and EtOH treated groups at both postnatal ages [P15–17: $t(8) = 1.31$, $n=5$; $p = 0.22$; P21–34: $t(7) = 0.98$; $p = 0.36$, $n=4$ for air and $n=5$ for EtOH] (Fig 3C).

In addition to the previously described effect of mGluR1 on the late phase of the CS, we identified a novel action of this receptor in the AHP phase that follows this event. As shown in Fig 3A–B, application of the mGluR1 antagonist LY367385 significantly reduced the amplitude of the AHP in slices from both air- and EtOH-exposed rats by 30–40%. The effect of LY367385 on the CS AHP was not significantly different between air and EtOH-exposed

groups [P15–17: $t(8) = 0.75$; $p = 0.4761$, $n = 5$; P21–34: $t(7) = 1.22$, $p = 0.2613$, $n = 4$ for air and $n = 5$ for EtOH] (Fig 3D).

CF-LTD induction was not affected by developmental EtOH exposure

Chronic prenatal EtOH exposure has been shown to block LTD and reduce glutamate release at the parallel fiber-to-PC synapse (Servais et al., 2007). In this study, we tested whether EtOH exposure during the human third trimester-equivalent affected LTD at CF-to-PC synapses in P21–P34 rats. This form of synaptic plasticity is dependent on mGluR1 activation (Hansel and Linden, 2000). In agreement with previous reports, tetanic stimulation of the CF for 30 s at 5 Hz induced a long-lasting decrease of ~15% in the amplitude of CF-evoked EPSCs (Carta et al., 2006, Hansel and Linden, 2000) (Fig 4A–B). We found that there was no significant difference in the magnitude of LTD in slices from the air and EtOH groups [Mann-Whitney U: 13; sum of ranks for air = 38, sum of ranks for EtOH = 28; $p = 0.74$] (Fig 4A,B). To evaluate changes in glutamate release, we calculated the PPR of two EPSCs evoked at an inter-event interval of 60 ms. We found no difference in the PPR of controls vs EtOH treated groups [$t(9) = 0.26$; $p = 0.79$] (Fig 4C). Additionally, we compared the current densities of CF-EPSC1 and found no significant difference between the two experimental groups [Mann-Whitney U: 14; Sum of ranks for air = 35, Sum of ranks for EtOH: 31; $p = 0.90$].

Discussion

In this study, neonatal rats were exposed to relatively high levels of EtOH during P2–P12, a developmental period where CF-PC synapses are significantly refined. We found that all-or-none CS responses of similar characteristics could be evoked in PCs from control and EtOH exposed rats, suggesting that refinement of CF-PC synapses during the third trimester equivalent was either not significantly affected by EtOH exposure or was only transiently affected. This finding is somewhat surprising given that some of the mediators of the process of CF differentiation and elimination (e.g., voltage-gated Ca^{2+} channels, mGluR1, and NMDA receptors) have been shown to be modulated by EtOH in PCs (Hashimoto and Kano, 2013, Carta et al., 2006, Belmeguenai et al., 2008, Gruol and Parsons, 1994, He et al., 2013, Gruol and Parsons, 1996, Idrus et al., 2011). However, the effects of EtOH on these targets in developing PCs and inferior olivary neurons have not been fully characterized. It is possible that these developing neurons are able to engage compensatory mechanisms that normalize their activity during repeated cycles of EtOH exposure and withdrawal, ultimately resulting in normal synaptic transmission at CF-PC synapses.

We found that EtOH exposure neither affected the basal electrophysiological properties of the CS nor the CF-evoked EPSCs that trigger the CS response. The primary spike of the CS is mediated by voltage-activated Na^+ channels while the secondary spikelets and slow depolarization plateau are thought to be generated by non-inactivating and resurgent Na^+ currents, dendritic Ca^{2+} conductances, and Kv3 K^+ channels (Zagha et al., 2008, Schmolesky et al., 2002, Hurlock et al., 2008). Therefore, our findings suggest that chronic neonatal EtOH exposure does not induce a persistent alteration in these mediators of the CS response. In addition, the CF-EPSCs are primarily mediated by the AMPA receptor subtype

of ionotropic glutamate receptors under our experimental conditions and the amplitude of these events was not significantly different between treatment groups. This finding suggests that the levels and the function of AMPA receptors at CF-PC synapses are normal in our EtOH treated animals. In addition, paired-pulse plasticity of CF-EPSCs was not affected in slices from these animals, indicating that glutamate release from CFs was not altered either. Our findings are in general agreement with those of Servais et al. (2007) who reported that chronic prenatal EtOH exposure (peak blood alcohol levels = 0.07 g/dl) does not affect CF responses; however, Servais et al. found that this EtOH exposure paradigm reduces parallel fiber-evoked EPSCs in PCs by decreasing glutamate release. These results would suggest that synaptic transmission at parallel fiber-PC synapses is more sensitive to developmental EtOH exposure than transmission at CF-PC synapses. It should be kept in mind, however, that the exposure paradigm used by Servais et al. (2007) involved administration of a solution of 18% EtOH in water as the only source of fluid. It has previously been shown that mice exposed to this paradigm have significantly lower fluid and food intake, suggesting that results may have been, in part, a consequence of dehydration and nutritional deficits (Servais et al., 2005). *In vivo* electrophysiological recordings failed to demonstrate any alterations in synaptic transmission at parallel fiber-PC synapses in adult rats that were exposed to EtOH in a binge-like manner during P4–P10 (Backman et al., 1998). Interestingly, the study of Backman et al. also demonstrated a significant reduction in CS activity in PC of EtOH-exposed rats, although CS bursts were of normal characteristics in these animals. These findings suggest that inferior olivary neurons are an important target of EtOH exposure during the third trimester-equivalent.

Structural alterations at CF-PC synapses have been documented in several studies. Mohamed et al. (1987) found that exposure of pregnant rats to a liquid EtOH diet delayed CF maturation in lobules II/III, as revealed by the presence of CF-PC somatic synapses at P14. Pierce et al. (2010, 2011) found evidence indicating that binge-like exposure to a high dose (4.5 g/kg; blood alcohol level = 0.36 g/dl) but not a lower dose (3.6 g/kg; blood alcohol level = 0.24 g/dl) of EtOH delivered via intragastric intubation on P4 or P4–P6 decreases the numbers of CF-PC synapses at P14, an effect that is still evident at P40. Importantly, these investigators ruled out that the reduction in CF-PC synapse number was simply a consequence of decreased PC numbers. In general agreement with the results of Pierce et al. (2010), obtained using animals exposed to a blood alcohol level of 0.24 g/dl, we found no functional evidence consistent with a decrease in CF-PC synapses in neonates exposed to a blood alcohol level of 0.16 g/dl. It is possible that we would have seen effects of EtOH on the CS if we had exposed the neonates to a higher dose; however, the *in vivo* study of Backman et al. (1998) suggests that this response is unaffected even after repeated neonatal binge-like exposures to higher EtOH levels.

We previously demonstrated that mGluR1 mediates a portion of the late phase of the CS sustained depolarization in rats 20–30 days old (Carta et al., 2006). Here, we found that the mGluR1-mediated portion of the CS is also present during the second postnatal week and, importantly, that 3rd trimester-equivalent EtOH exposure did not affect this component of the CS response. We also demonstrated that mGluR1 receptor blockade reduces the amplitude of this AHP. The conductances that mediate the CS AHP are not fully understood.

On the basis of their functional properties, small conductance Ca^{2+} activated K^+ (SK) channels are candidates to be one of the mediators of the AHP (Schmolesky et al., 2002). Assuming that these channels are involved in the AHP, it is possible that an increase in intracellular Ca^{2+} levels, triggered by the activation of G_q -coupled mGluR1 during the late phase of the CS, contributes to the activation of SK channels (Carta et al., 2006). In support of this possibility, mGluR1 activation has been shown to induce SK channel-mediated membrane potential hyperpolarization in hippocampal and medial prefrontal cortical neurons (El-Hassar et al., 2011, Hagenston et al., 2008). Moreover, application of (S)-3,5-dihydroxyphenylglycine, a selective agonist of mGluR1, was shown to elicit Ca^{2+} transients that coincided with SK channel-mediated fast membrane potential hyperpolarizations in immature cultured PCs (Netzeband and Gruol, 2008). Thus, it is possible that intracellular Ca^{2+} evoked by CF-mediated activation of mGluR1 in PCs activates a pool of SK channels that mediate a portion of the CS AHP. Interestingly, the mGluR1-dependent component of the CS and the AHP that follows this response, as well as mGluR1-dependent LTD, were all unaffected by EtOH exposure, suggesting that mGluR1-dependent signaling pathways are not significantly affected in the EtOH exposed animals.

In conclusion, we found that exposure to relatively high levels of EtOH during the third trimester equivalent neither affected the basal electrophysiological characteristics of the CS nor CF LTD. Future studies should determine whether exposure to higher doses of EtOH using a binge-like pattern of exposure significantly affects synaptic transmission and/or plasticity at CF-PC synapses.

Acknowledgments

We would like to thank Chessa Scullin, Ph.D. and Devin Jelinek for writing and editing, respectively, the algorithm in Matlab that allowed us to analyze the CLI. This work was supported by NIH grant R01-AA014973.

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Figure 1. Schematic representation of vapor chambers

(A) Ambient air mixed with vaporized EtOH is forced into the vapor chamber, where dams and pups are housed in their home cages with *ad libitum* access to food and water. Rats in the control group are exposed in an identical chamber to air only (not shown). (B) Average pup weight gain was not significantly affected by the EtOH exposure paradigm.

Figure 2. Neonatal EtOH exposure does not affect baseline parameters of complex spikes (CSs)

(A) Average sample traces and summary of results for CS area recordings obtained in slices from postnatal day (P)15–P17 rats exposed to air or EtOH during the 3rd trimester equivalent. (B) Same as above but for recordings obtained in slices from P21–P34 rats. (C) Average sample traces and summary of results for the amplitude of the CS after hyperpolarization (AHP) recorded in slices from P15–P17 rats exposed to air or EtOH during the 3rd trimester equivalent. (D) Same as above but for recordings obtained in slices from P21–P34 rats. (E) Traces illustrating the coast line index quantification of secondary spikelets (in arbitrary units) from CS responses obtained in slices from P15–P17 rats exposed to air or EtOH during the 3rd trimester equivalent. (F) Same as above but for recordings obtained in slices from P21–P34 rats. Scale bars: 20 mV, 50 ms.

Figure 3. Repeated neonatal EtOH exposure does not affect the mGluR1-mediated component of complex spikes (CSs)
 Sample traces of CSs illustrating the impact of mGluR1 inhibition on the late phase of the CS and on the after hyperpolarization (AHP) amplitude in the air control (A) and EtOH treatment (B) groups. Dotted traces represent results obtained in the presence of LY367385 (50 μ M). Scale bars: 20 mV, 50 ms. (C) Summary of the effect of mGluR1 blockade on the area of CS in slices from P15–P17 and P21–P34 rats exposed to air or EtOH during the 3rd trimester equivalent. (D) Same as above but for the effect of mGluR1 blockade on the CS AHP amplitude.

Figure 4. Repeated neonatal EtOH exposure neither affects climbing fiber (CF) excitatory postsynaptic currents (EPSCs) nor CF long-term depression (LTD) in slices from P21-P34 rats

(A) *Top panel:* sample traces illustrating pairs of CF-EPSCs evoked at an interpulse interval of 60 ms for the air control and EtOH exposed groups (scale bars: 200pA/ 50 ms). The solid lines represent the average of baseline events and the dotted lines the average of events recorded after induction of LTD. *Bottom panel:* summary graph illustrating the time course of CF-LTD for both treatment groups. The arrow represents the time of LTD induction. (B) Summary graph illustrating the lack of effect of EtOH exposure on CF-LTD. (C) Summary graph illustrating that EtOH exposure did not significantly affect the paired-pulse ratio of CF-EPSCs. (D) Summary graph illustrating that EtOH exposure did not significantly affect the current densities (i.e., amplitude/membrane capacitance) of the first CF-EPSC recorded during the paired-pulse stimulation experiments.

Table 1

EtOH Vapor Chamber exposure characterization.

	Air	EtOH
# of pups per litter	9.61 $\pm$ 0.71; n=18	11.28 $\pm$ 0.67; n=18
Mortality (%)	12.63 $\pm$ 4.69; n=15	10.94 $\pm$ 5.27; n=13
Vapor chamber EtOH concentration (g/dl)	N/A	3.0 $\pm$ 0.048; n=16
Serum EtOH concentration (mM)	N/A	36.09 $\pm$ 4.88; n=8
PC-Capacitance (pF)	782.1 $\pm$ 58.30; n=19	853.8 $\pm$ 51.32; n=20
PC-Membrane Resistance (M Ω)	97.32 $\pm$ 14.43; n=18	83.28 $\pm$ 11.69; n=18