Ethanol Acutely Modulates mGluR1-Dependent Long-Term Depression in Cerebellum

Li-Da Su, Cheng-Long Sun, and Ying Shen

Background: Acute and chronic ethanol exposure produces profound impairments in motor functioning. Individuals with lower sensitivity to the acute motor impairing effects of ethanol have an increased risk of developing alcohol dependence and abuse, and infants with subtle delays in motor coordination development may have an increased risk for subsequently developing alcoholism. Thus, understanding the mechanism by which ethanol disrupts motor functioning is very important.

Methods: Parasagittal slices of the cerebellar vermis (250 μM thick) were prepared from P17 to 20 Sprague–Dawley rats. Whole-cell recordings of Purkinje cells were obtained with an Axopatch 200B amplifier. Parallel fiber-Purkinje cell synaptic currents were sampled at 1 kHz and digitized at 10 kHz, and synaptic long-term depression (LTD) was observed in either external or internal application of ethanol for comparison.

Results: We determined whether ethanol acutely affects parallel fiber LTD using whole-cell patch-clamp recordings from Purkinje cells. Application of ethanol both externally (50 mM) and internally (17 and 10 mM) significantly suppressed mGluR-mediate slow currents. Short-term external ethanol exposure (50 but not 17 mM) during tetanus blocked mGluR-dependent parallel fiber LTD. Furthermore, internal 17 and 10 mM ethanol completely inhibited this LTD.

Conclusions: The results of the current study demonstrate that ethanol acutely suppresses parallel fiber LTD and may influence the mGluR-mediated slow current intracellularly. This study, plus previous evidence by Carta and colleagues (2006) and Belmeguenai and colleagues (2008), suggests significant actions of ethanol on mGluR-mediated currents and its dependent plasticity in brain.

Key Words: Cerebellum, Ethanol, Long-Term Depression, Metabotropic Glutamate Receptor, Purkinje Cell.

CEREBELLAR LONG-TERM DEPRESSION (LTD) is a major form of synaptic plasticity that is thought to be critical for certain types of motor learning (Bear and Linden, 2000). Previous work has shown that two forms of LTD, parallel fiber LTD and climbing fiber LTD, are expressed in the principle GABAergic Purkinje cells, where postsynaptic metabotropic glutamate receptor 1 (mGluR1) plays critical roles in LTD induction (Hansel and Linden, 2000), as indicated by evidence that activation of mGluR1 is required for parallel fiber and climbing fiber LTD induction (Bear and Linden, 2000; Hansel and Linden, 2000).

It is known that ethanol enhances γ-aminobutyric acid (GABA)-mediated currents ($GABA_A$ and $GABA_C$) (Yeh and Kolb, 1997), impairs glutamatergic transmission (Woodward, 1999), and decreases voltage-gated calcium currents (Walter and Messing, 1999). Chronic ethanol exposure produces alterations in the mature nervous system leading to permanent motor deficits (Coffin et al., 2005). In the cerebellum, evidence indicates that ethanol increases GABAergic signaling at molecular layer interneuron to Purkinje cell (Criswell and Breese, 2005) and Golgi cell to granule cell synapses (Carta et al., 2004). Carta and colleagues (2006) reported that acute ethanol application inhibits climbing fiber-Purkinje cell LTD. A recent collaborative study indicated that ethanol exposure inhibits parallel fiber-Purkinje cell LTD without affecting AMPA receptor-mediated EPSC (Belmeguenai et al., 2008). Both aforementioned studies suggested that ethanol influences LTD by inhibiting postsynaptic mGluR current (Belmeguenai et al., 2008; Carta et al., 2006). However, there exist two caveats in the observations by Belmeguenai and colleagues (2008), which make the action of ethanol on parallel fiber LTD unclear. First, two different ethanol applications were used in separate experiments. While acute ethanol exposure was used to inhibit the mGluR current, long-term

From the Department of Neurobiology/Institute of Neuroscience (LDS, CLS, YS), Key Laboratory of Medical Neurobiology of Ministry of Health of China, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China; and Neuroscience Care Unit (LDS), the Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou, Zhejiang, China.

Received for publication September 17, 2009; accepted January 29, 2010.

Reprint requests: Ying Shen, PhD, Department of Neurobiology/Institute of Neuroscience, Zhejiang University School of Medicine, 388 YuHangTang Rd, Hangzhou, Zhejiang 310058, China. Tel: +(86)571-88208240; Fax: +(86)571-88208241; E-mail: yshen@zju.edu.cn

DOI: 10.1111/j.1530-0277.2010.01190.x

ethanol exposure was used in LTD induction (Belmeguenai et al., 2008). Second, it was not examined whether ethanol had an intracellular action on both mGluR current and LTD induction. Hence, it is of interest to investigate the acute effects of ethanol on parallel fiber LTD and the possible action site of ethanol.

In this study, by using whole-cell patch-clamp technique combined with the application of ethanol in external or internal saline, we aimed to focus on the effects of ethanol on mGluR-mediated slow current and parallel fiber LTD induction. The results show that ethanol acutely suppresses parallel fiber LTD, which might influence mGluR-mediated slow current via an intracellular pathway.

MATERIALS AND METHODS

All animal experiments were performed under the guidance of Animal Experimentation Ethics Committee of Zhejiang University. The experimental procedures used here were modified from those of Su and Shen (2009). All the experiments were specifically designed to *minimize the sacrifice* of animals. Parasagittal slices of the cerebellar vermis (250 μM thick) were prepared from P17 to 20 Sprague–Dawley rats using a vibrating tissue slicer (Leica VT1000S, Nussloch, Germany) and ice-cold standard artificial cerebrospinal fluid (aCSF) containing (in mM): 124 NaCl, 5 KCl, 1.25 NaH_2PO_4, 2 $MgSO_4$, 2 $CaCl_2$, 26 $NaHCO_3$, and 10 D-glucose, bubbled with 95 O_2 and 5% CO_2. After a recovery period of 30 minutes at 37°C, the slices were placed in a submerged chamber that was perfused at a rate of 2 ml/min with aCSF supplemented with 10 μM GABAzine to block γ-*amino*-butyric acid A ($GABA_A$) receptors. The recording electrodes with resistance of 2 to 3 MΩ were filled with a solution containing (in mM): 135 CsMes, 10 CsCl, 10 HEPES, 4 Na_2ATP, 0.4 Na_3GTP, and 0.3 EGTA (pH 7.2). Recording pipettes were typically 1.5 to 3 MΩ, and uncompensated series resistances were less than 5 MΩ.

Purkinje cells were visualized using an upright Olympus BX51 (Olympus Optical, Tokyo, Japan) with a 40X water immersion objective and equipped with infrared-differential interference contrast enhancement. All whole-cell recordings were obtained with an Axopatch 200B amplifier (Molecular Devices, Foster City, CA). Currents were filtered at 1 kHz and digitized at 10 kHz. For parallel fiber stimulation, standard patch pipettes were filled with aCSF and placed in the middle third of the molecular layer. Synaptic responses were evoked every 30 seconds using 12 to 16 μA pulses (100 μs duration). When burst stimulation was employed, the inter-pulse interval was 10 ms.

All drugs were purchased from Sigma (St Louis, MO), except for GABAzine, NBQX, and CPCCOEt (Ascent Scientific, UK). Data analysis was performed using Excel 2003 (Microsoft, Chicago, IL), Clampfit 10 (Molecular Devices), and Igor Pro 6.0 (Wavemetrics Inc, Lake Oswego, OR). All group data are shown as mean ± SEM. Student's *t*-tests were used to determine *p* values. Cells were excluded from the study if series resistance or input resistance varied by more than 15% over the course of an experiment.

RESULTS

When a Purkinje cell is held at −70 mV, stimulation of parallel fibers with a brief burst (10 pulses at 100 Hz) gives rise to a fast component predominantly mediated by AMPA receptors and a slow component mediated by mGluR1 (Tempia et al., 1998). To record mGluR1-mediated slow EPSCs, we added 10 μM NBQX to block AMPA/kainate receptors

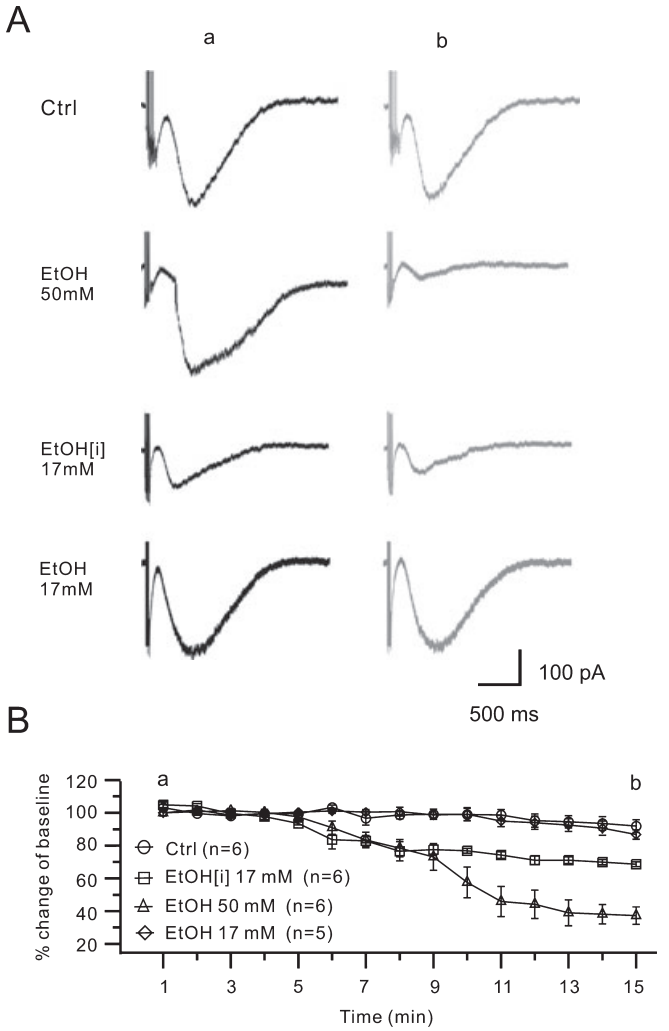

Fig. 1. Effects of ethanol on mGluR1-mediated slow currents at parallel fiber-Purkinje cell synapses. (**A**) Typical responses of a Purkinje cell evoked by burst stimuli. The responses obtained from the same cells are indicated by black (t = 0 minutes) and gray (t = 15 minutes) representing either the absence of ethanol (Ctrl) or in the presence of extracellular 50 mM ethanol (EtOH 50 mM) or intracellular 17 mM ethanol (EtOH[i]17 mM) or extracellular 17 mM ethanol (EtOH 17 mM). Note that baseline response in EtOH[i]17 mM is smaller than those in other conditions. (**B**) Time course of mGluR1-mediated slow current peak amplitudes in ctrl (n = 6), EtOH 50 mM (n = 6), EtOH[i] 17 mM (n = 6), or EtOH17 mM conditions (n = 5).

(Belmeguenai et al., 2008; Brasnjo and Otis, 2001; Kim et al., 2003), as shown by representative traces in Fig. 1*A*. The stimulus intensity was adjusted to produce a slow current amplitude at 80% of the maximum, which was blocked by bath application of the mGluR1 antagonist, 100 μM CPCCOEt (n = 4; data not shown), showing that these currents were mediated by mGluR1. Under the control condition, the mGluR1-mediated slow currents remained stable with almost peak amplitudes unchanged (91.9 ± 3.9% of baseline at 15 minutes, n = 6; Fig. 1; $p > 0.05$), in agreement with previous result (Jin et al., 2007). Several manipulations were made to clarify the role of ethanol in mGluR1-mediated slow currents. First, we applied 50 mM ethanol in the background perfusion and found that the slow currents were largely

attenuated compared with recordings under the control condition (37.3 ± 5.3% of baseline at 15 minutes, $n = 6$; $p < 0.01$; Fig. 1*B*), consistent with previous report (Belmeguenai et al., 2008). Second, we included different concentrations of ethanol (1, 10, and 17 mM) in the internal saline. The mGluR1-mediated currents with 10 and 17 mM internal ethanol were evidently smaller at $t = 0$ minutes with a mean peak amplitude of 106.9 ± 1.5 pA (10 mM; $n = 5$; Fig. S1*A*) and 98.4 ± 8.8 pA (17 mM; $n = 6$; Fig. 1*A*), compared to control 279.2 ± 22.7 pA ($n = 6$; $p < 0.01$). As with external application of ethanol, mGluR1 current decreased over time with internal ethanol (10 mM: 64.9 ± 4.2% of baseline at 15 minutes, $n = 5$; $p < 0.01$; Fig. S1; 17 mM: 68.6 ± 2.4% of baseline at 15 minutes, $n = 6$; $p < 0.01$; Fig. 1*B*). The smaller inhibition by loaded ethanol may be because of existing effects before recording period. In contrast, internal 1 mM ethanol did not exert apparent actions on mGluR currents (97.2 ± 4.4% of baseline at 15 minutes, $n = 5$; $p > 0.05$; Fig. S1). Third, as ethanol is membrane-permeable, one may argue that ethanol applied to the internal saline is able to cross the membrane to act externally. To preclude this possibility, we hereby externally applied 17 mM ethanol and found that mGluR currents did not decrease with time (86.7 ± 2.9% of baseline at 15 minutes, $n = 5$; $p > 0.05$; Fig. 1), implying that ethanol did not act externally. These data suggest that ethanol acutely reduces mGluR1 currents when applied either externally or internally, favoring potential internal effects in a dose-dependent manner.

We next investigated whether acute ethanol exposure inhibits its parallel fiber-Purkinje cell synaptic LTD. Following a stable 10-minute baseline recording period, parallel fibers were tetanized with a train of 5 pulses at 100 Hz, which was accompanied by a 100 ms-long depolarization of the Purkinje cell to 0 mV (Steinberg et al., 2006). These bursts were repeated 30 times at an inter-burst interval of 2 seconds. During the tetanus, 50 mM ethanol was applied. Slices from the control condition showed robust LTD (52.5 ± 4.8% of baseline at $t = 30$ minutes, $n = 6$; $p < 0.01$), while LTD could not be induced in the presence of ethanol (98.5 ± 3.8% of baseline at $t = 30$ minutes, $n = 6$; $p > 0.05$; Fig. 2). Application of ethanol did not change AMPA current amplitude before tetanus (data not shown) and paired-pulse facilitation (Fig. 2*B*), consistent with previous reports (Belmeguenai et al., 2008; Carta et al., 2006).

mGluR currents were acutely blocked by internal ethanol (Fig. 1), we proposed that internal ethanol may also be able to block mGluR-dependent parallel fiber LTD. With supplemental of 17 mM ethanol in pipette solution, we recorded parallel fiber EPSCs for a baseline period (10 minutes) and then delivered the same parallel fiber LTD induction protocol. This stimulation failed to induce parallel fiber LTD in Purkinje cells because the EPSC amplitude did not changed (90.0 ± 5.1% of baseline, $n = 6$; $p > 0.05$) at the time point of 30 minutes (Fig. 3). Likewise, internal ethanol did not change the AMPA current amplitude before tetanus (data

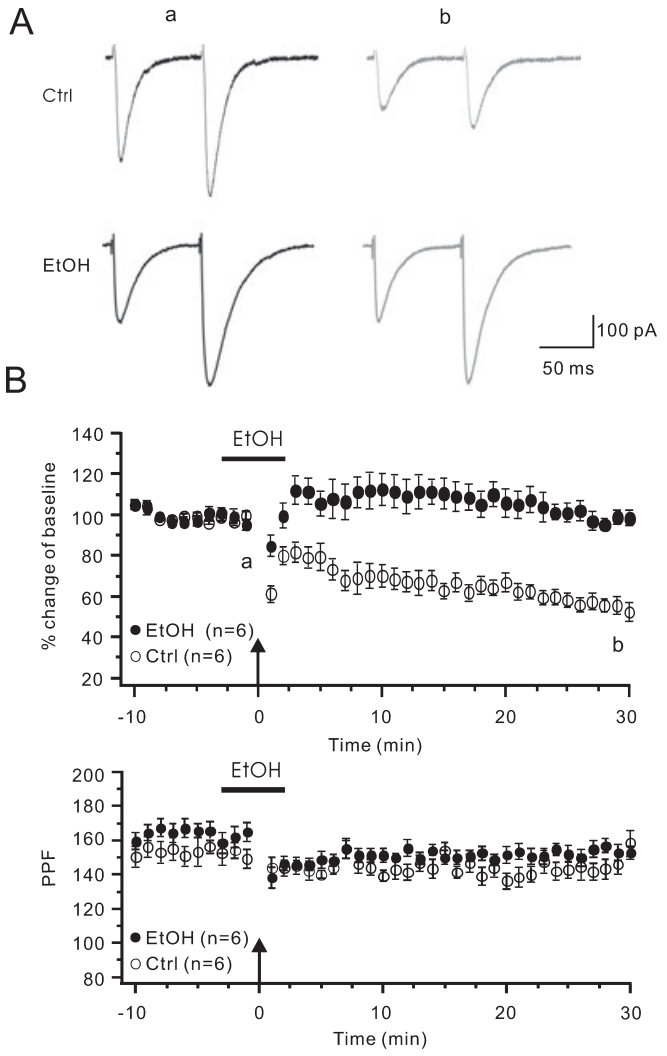

Fig. 2. Acute application of ethanol inhibited parallel fiber long-term depression (LTD). (**A**) Example traces before and after low frequency stimuli to induce parallel fiber-Purkinje cell LTD. Sample currents indicated by a or b were collected at the times indicated on the panel B, which illustrates the effects of two manipulations: control (Ctrl) and 50 mM ethanol (EtOH). (**B**) Comparison of two groups of neurons recorded. Mean peak amplitudes of parallel fiber-evoked EPSCs are displayed versus time of control (unfilled circles, $n = 6$) and 50 mM ethanol (filled circles, $n = 6$). Following a baseline recording period, LTD was induced at $t = 0$ minutes. Ethanol was added 3 minutes prior to induction and immediately washed out after the induction time window. Therefore, 3-minute recordings of EPSCs before and after the induction were discarded to ensure that they were not contaminated by ethanol. Tetanic stimulation is indicated by the upward arrow.

now shown) and paired-pulse facilitation (Fig. 3*B*). To preclude the possibility that internal 17 mM ethanol may act externally, we applied 17 mM ethanol in a prolonged duration before and during the tetanus in external saline. Slices from this condition showed robust LTD (54.9 ± 3.5% of baseline at $t = 30$ minutes, $n = 5$; $p < 0.01$), with paired-pulse facilitation not changed too (Fig. 3), implying that internal 17 mM ethanol leak is not sufficient to block LTD induction. Furthermore, we observed action of 10 mM or 1 mM internal ethanol on LTD induction. As shown in Fig. S2, while 10 mM ethanol effectively blocked LTD (91.0 ± 2.5% of baseline at $t = 30$ minutes, $n = 5$;

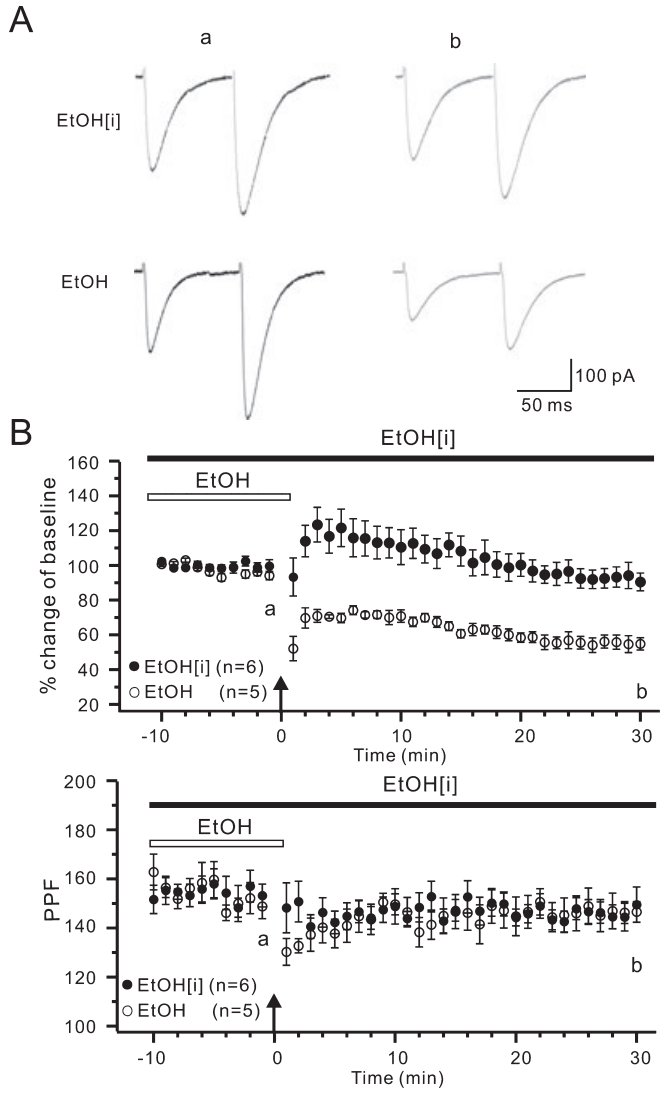

Fig. 3. Internal 17 mM ethanol inhibited parallel fiber-Purkinje cell long-term depression. (**A**) Example traces before and after stimulation. Sample currents indicated by a or b were collected at the times indicated on panel B with group treatments as same as in Fig. 2. (**B**) Comparison of two groups of neurons recorded in either external 17 mM ethanol (EtOH) or internal 17 mM ethanol (EtOH[i]). Upper panel: time courses of mean peak amplitudes of parallel fiber-evoked EPSCs. Following a baseline recording period, a tetanus was delivered at $t = 0$ minutes. Lower panel: time courses of paired–pulse facilitation (PPF) of EPSCs. To ensure the inhibition of mGluR currents, external ethanol (17 mM) was added after establishing of whole-cell recording until end of induction and immediately washed out afterward. Tetanic stimulation is indicated by the upward arrow.

$p > 0.05$), 1 mM ethanol did not show inhibition of LTD induction (59.7 ± 3.8% of baseline at $t = 30$ minutes, $n = 5; p < 0.01$).

DISCUSSION

This study demonstrates that acute ethanol exposure inhibits the mGluR-mediated slow current and its-dependent LTD at parallel fiber-Purkinje cell synapses, which might operate through an internal pathway in ethanol dose-dependent manner.

Climbing fiber and parallel fiber-Purkinje cell synaptic LTD are important to cerebellar circuit function and motor coordination (Hansel et al., 2001; Linden, 2003). Yuan and colleagues (2007) show that mGluR currents enhance local calcium signaling and facilitate cerebellar LTD induction. Therefore, it is possible that ethanol exposure suppresses mGluR-dependent cerebellum-mediated reflexes, such as eyelid conditioning. Prior to this study, two individual studies have reported that ethanol influences climbing fiber and parallel fiber-Purkinje cell synaptic transmission (Belmeguenai et al., 2008; Carta et al., 2006). The evidence include (i) ethanol largely decreases mGluR currents and Ca^{2+} currents, (ii) ethanol does not affect AMPA currents, (iii) ethanol exposure blocks climbing fiber and parallel fiber LTD, and (iv) ethanol exposure does not affect parallel fiber long-term potentiation.

The mechanisms by which ethanol achieves its short- and long-term effects on the brain are largely unknown, though ion channels and neurotransmitter receptors have become the targets of intense inquiry (Worst and Vrana, 2005). Existing data suggest that action of ethanol on neurotransmitter receptors or in neurons could be either acute (Brust, 2002) or chronic (Servais et al., 2005). Several lines of evidence indicates that the action sites of acute ethanol modulation on membrane receptors and channels, including $GABA_A$ receptors (Hanchar et al., 2005, 2006; Mihic et al., 1997; Wallner et al., 2006; Yeh and Kolb, 1997), glutamate receptors (Lovinger et al., 1989; Woodward, 1999), voltage-gated calcium channels (Walter and Messing, 1999), A-type potassium channels (Alekseev et al., 1997), are near the extracellular portions.

Compared to acknowledged effects of ethanol on these receptors and channels, it is poorly understood how ethanol modulates mGluRs and its-dependent cerebellar LTD, except the evidence from Carta and colleagues (2006) and Belmeguenai and colleagues (2008). Nevertheless, these two reports did not address an important question where action of ethanol happens in Purkinje cells. While water moves into the cell quickly through aquaporin, ethanol and methanol are considered to be able to penetrate the membrane as well, but at unclear penetration rates. Pignataro and colleagues (2007) reported that 10 or 60 mM extracellular ethanol treatment rapidly increases α4 protein expression via Heat Shock Factor 1 within tens of minutes in a dose-dependent manner, suggesting that extracellular ethanol may influence intracellular protein activities and gene transcription and translation in a short time window. Therefore, it is important to determine the sensitivity of mGluRs to ethanol and action site of ethanol on mGluRs.

Several recent investigations showed that chronic alcohol consumption produces robust increases in mGluR/Homer2/PI3K expression within the nucleus accumbens (NAC) and amygdala (Cozzoli et al., 2009; Obara et al., 2009; Szumlinski et al., 2003, 2008). In the present work, we first demonstrated that internal 17 mM (but not external 17 mM or internal 1 mM) ethanol could block LTD induction, suggesting that ethanol might acutely inhibit mGluR-dependent LTD

intracellularly in a dose-dependent manner. Thus, our work adds another potential pathway for the modulatory function of ethanol on receptor and channels, different from its extracellular modulation on $GABA_A$ or NMDA receptors. In future, the action of ethanol on Homer/IP3/PI3K that is tightly relevant to mGluR-mediated internal calcium increase is worthwhile to be studied.

Despite the previous ethanol studies focused on separate receptors and channels, it should be noted that Nie and colleagues (2000) reported in NAC neurons that ethanol potentiation of GABA currents may involve mGluRs, perhaps via a phosphorylation mechanism that regulates the ethanol sensitivity of GABA receptors. These data suggest that the effects of ethanol may operate through interacting signaling pathways in ataxia and motor learning deficits, the well known "fetal alcohol syndrome" caused by exposure of fetuses or neonates to alcohol during brain development (Goodlett et al., 2005). Therefore, integrative studies should be used in alcohol-relevant motor learning deficits and other brain disorders.

ACKNOWLEDGMENTS

We are grateful for helpful comments from members of Shen laboratory. This work was supported by grants from the National Foundation of Natural Science of China (No. 30600168, to Y. S.), the Zhejiang Provincial Foundation of Natural Science (No. R206018, to Y. S.), the Program for New Century Excellent Talents in University of Ministry of Education of China (to Y. S.), and the National Basic Research Program (973) of the Ministry of Science and Technology of China (No. 2009CB941402, to Y. S.).

REFERENCES

Alekseev SI, Alekseev AS, Ziskin MC (1997) Effects of alcohols on A-type K + currents in Lymnaea neurons. J Pharmacol Exp Ther 281:84–92.

Bear MF, Linden DJ (2000) The mechanisms and meaning of long-term synaptic depression in the mammalian brain, in The Synapse (Cowan WM, Sudhof T, Stevens CF eds). pp. 455–516. Johns Hopkins University Press, Baltimore.

Belmeguenai A, Botta P, Weber JT, Carta M, De Ruiter M, De Zeeuw CI, Valenzuela CF, Hansel C (2008) Alcohol impairs long-term depression at the cerebellar parallel fiber-Purkinje cell synapse. J Neurophysiol 100: 3167–3174.

Brasnjo G, Otis TS (2001) Neuronal glutamate transporters control activation of postsynaptic metabotropic glutamate receptors and influence cerebellar long-term depression. Neuron 31:607–616.

Brust JC (2002) Neurologic complications of substance abuse. J Acquir Immune Defic Syndr Hum Retrovirol 31:S29–S34.

Carta M, Mameli M, Valenzuela CF (2004) Alcohol enhances GABAergic transmission to cerebellar granule cells via an increase in Golgi cell excitability. J Neurosci 24:3746–3751.

Carta M, Mameli M, Valenzuela CF (2006) Alcohol potently modulates climbing fiber– > Purkinje neuron synapses: role of metabotropic glutamate receptors. J Neurosci 26:1906–1912.

Coffin JM, Baroody S, Schneider K, O'Neill J (2005) Impaired cerebellar learning in children with prenatal alcohol exposure: a comparative study of eyeblink conditioning in children with ADHD and dyslexia. Cortex 41: 389–398.

Cozzoli DK, Goulding SP, Zhang PW, Xiao B, Hu JH, Ary AW, Obara I, Rahn A, bou-Ziab H, Tyrrel B, Marini C, Yoneyama N, Metten P, Snelling C, Dehoff MH, Crabbe JC, Finn DA, Klugmann M, Worley PF, Szumlinski KK (2009) Binge drinking upregulates accumbens mGluR5-Homer2-PI3K signaling: functional implications for alcoholism. J Neurosci 29:8655–8668.

Criswell HE, Breese GR (2005) A conceptualization of integrated actions of ethanol contributing to its GABAmimetic profile: a commentary. Neuropsychopharmacoly 30:1407–1425.

Goodlett CR, Horn KH, Zhou FC (2005) Alcohol teratogenesis: mechanisms of damage and strategies for intervention. Exp Biol Med (Maywood) 230:394–406.

Hanchar HJ, Chutsrinopkun P, Meera P, Supavilai P, Sieghart W, Wallner M, Olsen RW (2006) Ethanol potently and competitively inhibits binding of the alcohol antagonist Ro15-4513 to alpha4/6beta3delta $GABA_A$ receptors. Proc Natl Acad Sci USA 103:8546–8551.

Hanchar HJ, Dodson PD, Olsen RW, Otis TS, Wallner M (2005) Alcohol-induced motor impairment caused by increased extrasynaptic $GABA_A$ receptor activity. Nat Neurosci 8:339–345.

Hansel C, Linden DJ (2000) Long-term depression of the cerebellar climbing fiber–Purkinje neuron synapse. Neuron 26:473–482.

Hansel C, Linden DJ, D'Angelo E (2001) Beyond parallel fiber LTD: the diversity of synaptic and non-synaptic plasticity in the cerebellum. Nat Neurosci 4:467–475.

Jin Y, Kim SJ, Kim J, Worley PF, Linden DJ (2007) Long-term depression of mGluR1 signaling. Neuron 55:277–287.

Kim SJ, Kim YS, Yuan JP, Petralia RS, Worley PF, Linden DJ (2003) Activation of the TRPC1 cation channel by metabotropic glutamate receptor mGluR1. Nature 426:285–291.

Linden DJ (2003) Neuroscience: from molecules to memory in the cerebellum. Science 301:1682–1685.

Lovinger DM, White G, Weight FF (1989) Ethanol inhibits NMDA-activated ion current in hippocampal neurons. Science 243:1721–1724.

Mihic SJ, Ye Q, Wick MJ, Koltchine VV, Krasowski MD, Finn SE, Mascia MP, Valenzuela CF, Hanson KK, Greenblatt EP, Harris RA, Harrison NL (1997) Sites of alcohol and volatile anaesthetic action on $GABA_A$ and glycine receptors. Nature 389:385–389.

Nie Z, Madamba SG, Siggins GR (2000) Ethanol enhances gamma-aminobutyric acid responses in a subpopulation of nucleus accumbens neurons: role of metabotropic glutamate receptors. J Pharmacol Exp Ther 293:654–661.

Obara I, Bell RL, Goulding SP, Reyes CM, Larson LA, Ary AW, Truitt WA, Szumlinski KK (2009) Differential effects of chronic ethanol consumption and withdrawal on homer/glutamate receptor expression in subregions of the accumbens and amygdala of P rats. Alcohol Clin Exp Res 33: 1924–1934.

Pignataro L, Miller AN, Ma L, Midha S, Protiva P, Herrera DG, Harrison NL (2007) Alcohol regulates gene expression in neurons via activation of heat shock factor 1. J Neurosci 27:12957–12966.

Servais L, Bearzatto B, Delvaux V, Noel E, Leach R, Brasseur M, Schiffmann SN, Cheron G (2005) Effect of chronic ethanol ingestion on Purkinje and Golgi cell firing in vivo and on motor coordination in mice. Brain Res, 1055:171–179.

Steinberg JP, Takamiya K, Shen Y, Xia J, Rubio ME, Yu S, Jin W, Thomas GM, Linden DJ, Huganir RL (2006) Targeted in vivo mutations of the AMPA receptor subunits GluR2 and its interacting protein PICK1 eliminate cerebellar long-term depression. Neuron 49:845–860.

Su LD, Shen Y (2009) Blockade of glutamate transporters facilitates cerebellar synaptic long-term depression. Neuroreport 20:502–507.

Szumlinski KK, Ary AW, Lominac KD, Klugmann M, Kippin TE (2008) Accumbens Homer2 overexpression facilitates alcohol-induced neuroplasticity in C57BL/6J mice. Neuropsychopharmacology 33:1365–1378.

Szumlinski KK, Toda S, Middaugh LD, Worley PF, Kalivas PW (2003) Evidence for a relationship between Group 1 mGluR hypofunction and increased cocaine and ethanol sensitivity in Homer2 null mutant mice. Ann N Y Acad Sci 1003:468–471.

Tempia F, Miniaci MC, Anchisi D, Strata P (1998) Postsynaptic current mediated by metabotropic glutamate receptors in cerebellar Purkinje cells. J Neurophysiol 80:520–528.

Wallner M, Hanchar HJ, Olsen RW (2006) Low-dose alcohol actions on alpha4beta3delta $GABA_A$ receptors are reversed by the behavioral alcohol antagonist Ro15-4513. Proc Natl Acad Sci USA 103:8540–8545.

Walter HJ, Messing RO (1999) Regulation of neuronal voltage-gated calcium channels by ethanol. Neurochem Int 35:95–101.

Woodward JJ (1999) Ionotropic glutamate receptors as sites of action for ethanol in the brain. Neurochem Int 35:107–113.

Worst TJ, Vrana KE (2005) Alcohol and gene expression in the central nervous system. Alcohol 40:63–75.

Yeh HH, Kolb JE (1997) Ethanol modulation of GABA-activated current responses in acutely dissociated retinal bipolar cells and ganglion cells. Alcohol Clin Exp Res 21:647–655.

Yuan Q, Qiu DL, Weber JT, Hansel C, Knöpfel T (2007) Climbing fiber-triggered metabotropic slow potentials enhance dendritic calcium transients and simple spike firing in cerebellar Purkinje cells. Mol Cell Neurosci 35:596–603.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Effects of lower internal ethanol on mGluR1 currents.

Fig. S2. Internal 10 mM but not 1 mM ethanol inhibited parallel fiber-Purkinje cell LTD.

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.