

Differential Effect of Ethanol on NMDA EPSCs in Pyramidal Cells in the Posterior Cingulate Cortex of Juvenile and Adult Rats

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Li, Qiang, Wilkie A. Wilson, and H. Scott Swartzwelder. Differential effect of ethanol on NMDA EPSCs in pyramidal cells in the posterior cingulate cortex of juvenile and adult rats. *J Neurophysiol* 87: 705–711, 2002; 10.1152/jn.00433.2001. Ethanol (EtOH) is a potent inhibitor of *N*-methyl-D-aspartate (NMDA) receptor-mediated activity in a number of brain areas, and recent studies have indicated that this inhibitory effect of ethanol is more powerful in the juvenile brain compared with the adult brain. However, previous direct developmental comparisons have been limited to studies of extracellular responses in the hippocampus. To begin an assessment of the mechanisms underlying this developmental sensitivity, we assessed the inhibitory effect of EtOH on NMDA receptor-mediated synaptic transmission in neocortical slices from adult (95–135 days old) and juvenile (28–32 days old) rats using the whole cell patch-clamp recording technique. In the presence of 6,7-dinitroquinoxaline-2,3-dione (20 μ M) and bicuculline methiodine (20 μ M), NMDA receptor-mediated excitatory postsynaptic currents were isolated from pyramidal cells of the posterior cingulate cortex (PCC). In slices from juvenile rats 5, 10, 30, and 60 mM EtOH reduced the mean amplitude of NMDA receptor-mediated EPSCs by 11, 22, 35, and 46%, respectively. However, the same concentrations of EtOH inhibited the mean amplitude of EPSCs by only 4, 8, 15, and 31% in slices from adult rats. This developmental difference in the potency of EtOH against NMDA receptor-mediated EPSCs was also observed when the holding potential of the neurons was increased to +30 mV, although the inhibitory effect of ethanol on adult neurons was diminished at that voltage. These results provide a cellular analysis of the enhanced potency of ethanol against NMDA receptor-mediated EPSCs in neocortical cells from juvenile animals compared with adults.

INTRODUCTION

The cognitive impairment that accompanies ethanol intoxication is a particular concern in young drinkers for several reasons. First, during the second decade of life people are generally engaged in educational activities that require the assimilation of large amounts of new information. Ethanol-induced compromises of learning and anterograde memory clearly compromise learning as suggested by the negative correlation between drinking and academic performance among students (Pullen 1994; Wechsler et al. 1995). In addition to memory impairment, the effects of ethanol on other higher-order cognitive functions such as judgment and response inhibition may predispose intoxicated individuals to

ward accidents and other negative sequelae of ethanol use such as fights, sexual assaults, and unprotected sex. Finally, the brain undergoes rapid development of neocortical synaptic circuitry throughout the second decade of life. This developmental change could alter neuronal responsiveness to acute doses of ethanol, and chronic exposure to ethanol during this period could potentially alter the developmental trajectory of neural circuits.

The effects of ethanol on neural signaling are varied and complex. However, it is now clear that *N*-methyl-D-aspartate (NMDA) receptor-mediated synaptic transmission is particularly sensitive to the acute (Lovinger et al. 1990; Morrisett and Swartzwelder 1993) and chronic (Snell et al. 1993) effects of ethanol. Given the linkage of NMDA receptor-mediated activity with learning and memory (Moser et al. 1998), impairment of this activity may be one mechanism whereby ethanol diminishes cognitive functions in vivo. The potency of ethanol against NMDA receptor-mediated neural activity, and its implications for the cognitive effects of ethanol, has sparked considerable interest. However, the cellular mechanisms of that potency remain obscure, and there is little information about ethanol potency at the cellular level outside the hippocampal formation.

It is now clear that the responsiveness of NMDA receptor-mediated neural activity to ethanol varies across development. The potency of ethanol as an antagonist of NMDA receptor-mediated population excitatory postsynaptic potentials (pEPSPs) (Swartzwelder et al. 1995a) and long-term potentiation (LTP) (Pyapali et al. 1999; Swartzwelder et al. 1995b) is greater in hippocampal slices taken from juvenile or adolescent rats compared with those taken from adults. In addition, hippocampally mediated spatial learning was impaired more potently by ethanol in adolescent rats compared with adults (Markweise et al. 1998). Interestingly, in humans an acute dose of ethanol has been shown to impair both verbal and figural learning more powerfully in people 21–24 yr of age than in those 25–29 yr of age (Acheson et al. 1998).

Although the developmental differences in ethanol potency are intriguing and may be of considerable value for health education, the cellular mechanisms underlying them remain virtually unexplored, and the extracellular effects have been assessed only in hippocampal tissue. The present study was

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designed to assess the potency of ethanol against NMDA receptor-mediated EPSCs in pyramidal neurons in the posterior cingulate cortex of juvenile and adult rats.

METHODS

Slice preparation

Neocortical slices were prepared from juvenile [*postnatal days* 28–32 (*P28–32*)] and adult (*P95–135*) male Sprague-Dawley rats. The rats were anesthetized with isoflurane and decapitated. The brains of juvenile rats were quickly removed from the skulls and placed in cold (4°C) artificial cerebrospinal fluid (ACSF) containing (in mM) 120 NaCl, 3.3 KCl, 1.23 NaH₂PO₄, 25 NaHCO₃, 1.2 MgSO₄, 1.8 CaCl₂, and 10 D-glucose at pH 7.3 (normal ACSF), previously saturated with 95% O₂-5% CO₂. The brains of adults were placed in a modified ACSF in which 1.8 mM CaCl₂ was replaced with 0.5 mM CaCl₂. Coronal cortical slices from both age groups containing posterior cingulate cortex (PCC) (Paxinos and Watson 1986) (300 μm thickness), were cut on a vibratome (Campden, model 752, England) and incubated in a holding chamber that contained normal ACSF that was continuously bubbled with 95% O₂-5% CO₂ at room temperature (22–24°C).

Whole cell voltage-clamp recording

Our whole cell patch-clamp techniques have been described in detail previously (Mott et al. 1999). For recording, patch pipettes were pulled from borosilicate glass capillary tubing (1.5 mm OD, 1.05 mm ID, World Precision Instruments, Sarasota, FL) on a Flaming-Brown horizontal microelectrode puller (model P-97, Sutter Instrument, Novato, CA). The pipettes were filled with an intracellular solution containing (in mM) 130 Cs-gluconate, 7 CsCl, 10 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), and 4 Mg-ATP (pH 7.25). The quaternary lidocaine derivative QX-314 (4 mM; Sigma, St. Louis, MO) was also included to suppress fast sodium currents. Osmolarity was adjusted to 280 mOsm. The pipette resistances generally were in the range of 4–7 MΩ. Biocytin (0.3 to ~0.4%; Sigma, St. Louis, MO) was also added to the intracellular solution for later visualization of the morphology of the recorded cells.

After 1 h of incubation in the holding chamber, a slice was transferred into a small submersion recording chamber at room temperature (22–24°C) and secured in place with a bent piece of platinum wire resting on the top of the slice. Individual cells were visualized using an infrared differential interference contrast (IR-DIC) Zeiss Axioskop microscopy and a ×40 water immersion objective. Tight seals (>1 GΩ) were obtained on pyramidal-shaped cells, and whole cell recordings were made after rupturing the cell membrane with gentle suction. The evoked NMDA receptor-mediated excitatory postsynaptic currents (EPSCs) were recorded continuously using an Axopatch 1D amplifier (Axon Instrument, Foster City, CA). Output current signals were DC-coupled to a digital oscilloscope (Nicolet model 410). Series resistance was monitored throughout the recording, and a cell was discarded if it changed significantly. The signal was further analyzed using Strathclyde Electrophysiology Software, Whole Cell Program (Courtesy of Dr. John Dempster) with an interface (BNC-2090, National Instruments, Austin, TX) to a PC-based computer.

Electrical stimulation and isolation of NMDA receptor-mediated EPSCs

A monopolar tungsten electrode (A-M System, Carlsborg, WA) was placed about 50 to ~70 μm from the soma of the recorded pyramidal cells. In the presence of GABA_A receptor antagonist bicuculline methiodine (BMI; 20 μM) and α-amino-3-hydroxy-5-methyl-

isoxazole-4-propionic acid (AMPA) receptor antagonist, 6,7-dinitroquinoxaline-2,3-dione (DNQX; 20 μM), NMDA receptor-mediated EPSCs were evoked by electrical stimulation. The stimulus threshold was first determined by increasing intensity of constant current rectangular wave pulses generated by an isolated stimulator (Grass S88, Grass Instrument, Quincy, MA) until detectable responses occurred. Then constant current rectangular stimulus pulses 50% higher than threshold intensity with duration of 0.1 ms and interval of 0.033 Hz were delivered through the electrode. After the baseline measurements were established in control ACSF, bath application of 5, 10, 30, and 60 mM of ethanol (EtOH) was initiated. These concentrations of ethanol were chosen because they correspond with human blood ethanol concentrations across a range of very mild to very heavy intoxication. The evoked NMDA receptor-mediated EPSCs were continuously monitored for 15 min followed by a 30-min wash out period.

Histological identification of PCC pyramidal neurons

During recording, neurons were filled with biocytin. After the end of the recording, the slices remained in the recording chamber for an additional 10–20 min to allow biocytin transport within the axon. The slices were then placed overnight in 4% paraformaldehyde, 0.05% glutaraldehyde in 0.1 M phosphate buffer saline (PBS). They were then washed thoroughly in PBS and incubated in 0.1 M Tris-buffered saline (TBS) containing 1% H₂O₂ for 30 min. The slices were then incubated with avidin-biotin-peroxidase complex (ABC kit, Vector Labs, Burlingame, CA) in TBS containing 0.05% Triton X-100 overnight at 4°C, rinsed three times in PBS, and then reacted in a solution containing DAB (DAB kit, Vector Labs, Burlingame, CA).

The slices were then cleared and mounted. The morphology of the biocytin-filled pyramidal cells was examined under light microscope, and neurons were drawn using a camera lucida.

Statistical analysis of data and drug applications

The data were analyzed off-line using the Strathclyde Electrophysiological Software. Two-way analyses of variance (ANOVA) were used to assess age and dose effects, and Student's *t*-tests were used post hoc where appropriate. The significance level was set to $P < 0.05$ for all statistical tests. All grouped data are presented as means ± SE in the figures. All drugs were applied via bath superfusion. DNQX and BMI were purchased from Sigma (St. Louis, MO). D-(–)-2-Amino-5-phosphonovaleric acid (APV) was from ACROS (Geel, Belgium).

RESULTS

NMDA receptor-mediated EPSCs

Whole cell recordings were obtained from 45 morphologically identified pyramidal cells in the PCC. The recorded PCC pyramidal cells were located in the layers II–V. Among these cells, 25 neurons were from slices from juvenile rats, and 20 were from slices from adults. The resting membrane potentials (determined immediately after rupturing the cell membrane) of PCC pyramidal cells recorded from juvenile (-68.7 ± 0.63 mV, mean ± SE, $n = 13$) and adult (-67.9 ± 0.55 mV, $n = 12$) rats were not significantly different. In addition, current-voltage (*I*-*V*) curves constructed from PCC pyramidal cells in the presence of 1.2 mM Mg²⁺ indicated that NMDA receptor-mediated EPSCs reversed at 4.4 ± 0.6 mV in juvenile rats ($n = 10$) and at 5.1 ± 0.57 mV in adult rats ($n = 8$), similar to those observed in the somatosensory cortex of rats (Kim et al. 1995) and the hippocampus of mice (Kirson and Yaari 1996). There was no significant difference between these reversal potentials,

indicating that there is no developmental change in EPSC reversal potential in slices from juvenile compared with adult rats.

Figure 1 shows evoked NMDA receptor-mediated EPSCs recorded from two PCC pyramidal cells from juvenile (28–32 days old) rats. In the presence of DNQX and BMI, electrical stimuli evoked a slow inward current in pyramidal cells that were held at -30 mV (Fig. 1A, left panel). The evoked currents in pyramidal cells were completely abolished by bath application of 50 μ M APV in all cells recorded from juvenile rats, indicating these currents were mediated by NMDA receptors. Furthermore, the evoked NMDA EPSCs became outward

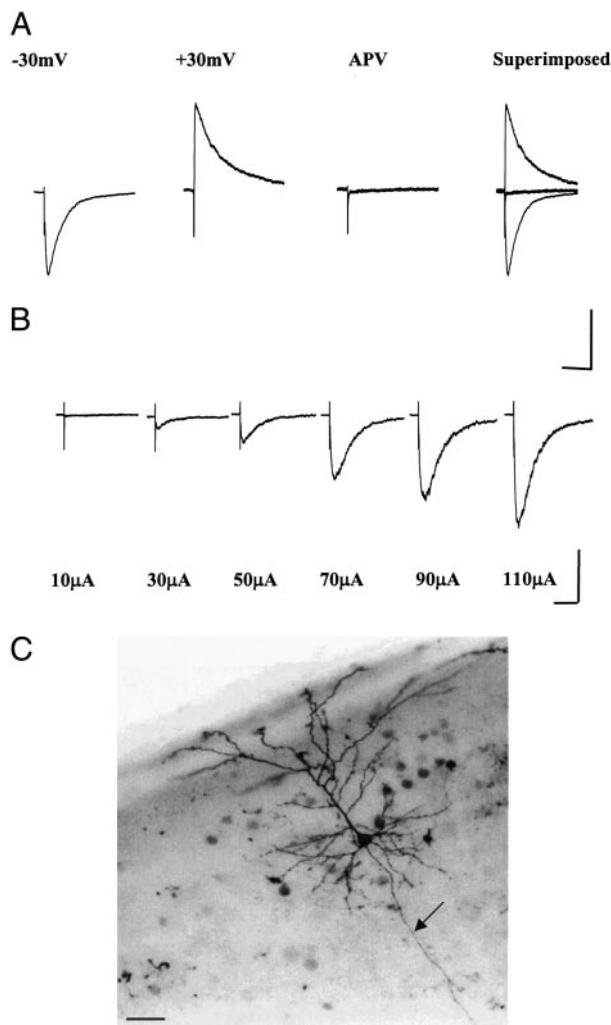


FIG. 1. *N*-methyl-D-aspartate (NMDA) receptor-mediated excitatory postsynaptic currents (EPSCs) isolated from posterior cingulate cortex (PCC) pyramidal cells in slices from juvenile rats. *A*: in the presence of 6,7-dinitroquinoline-2,3-dione (DNQX; 20 μ M) and bicuculline methiodine (BMI; 40 μ M), a single pulse evoked an inward current from a PCC pyramidal cell from a 29-day-old rat at a holding potential of -30 mV. The current became outward when the holding potential was raised to $+30$ mV. The slow current was completely blocked by bath application of 50 μ M D-(-)-2-amino-5-phosphonvaleric acid (APV). Calibration bars, 200 mS and 100 pA. *B*: in another PCC cell, gradual increases of electrical stimulus intensity result in a graded increase in the amplitude of NMDA receptor-mediated EPSCs. Evoked EPSCs were recorded when cell was held at -30 mV. Calibration bars, 200 ms and 100 pA. *C*: microphotograph showing the morphology of the cell whose electrophysiology was shown in *A*. Axon is indicated by an arrow. Scale bar, 30 μ m.

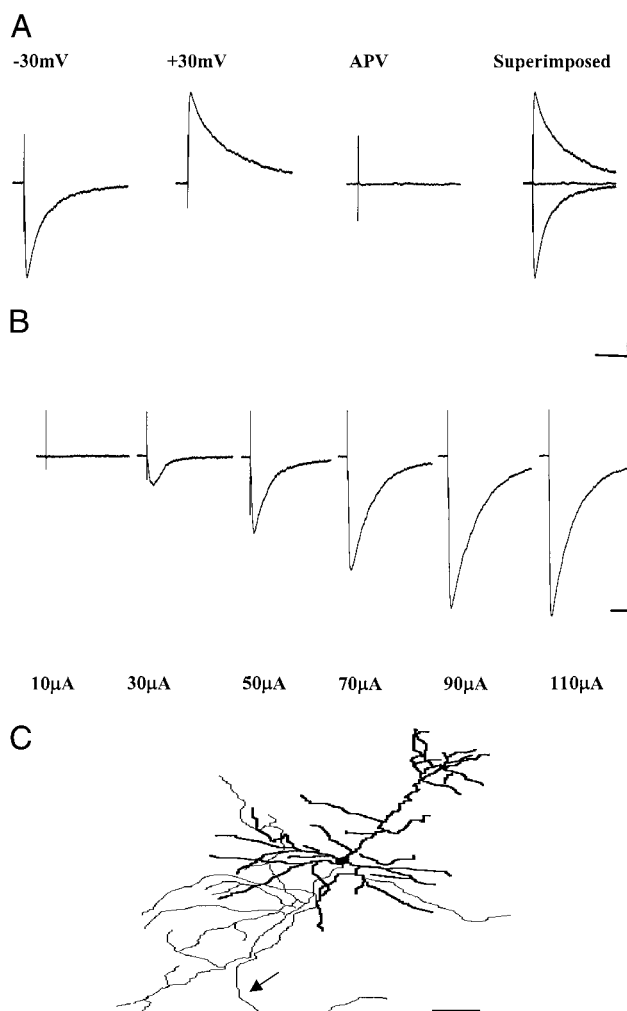


FIG. 2. NMDA receptor-mediated EPSCs isolated from PCC pyramidal cells in slices from adult rats. *A*: in the presence of DNQX (20 μ M) and BMI (20 μ M), a single pulse evoked an inward current from a PCC pyramidal cell from a 129-day-old rat at a holding potential of -30 mV. The current became outward when the holding potential was raised to $+30$ mV. The slow current was completely blocked by bath application of 50 μ M APV. Calibration bars, 200 mS and 100 pA. *B*: in another PCC cell, gradual increases of electrical stimulus intensity result in a graded increase in the amplitude of NMDA receptor-mediated EPSCs. Evoked EPSCs were recorded when cell was held at -30 mV. Calibration bars, 200 ms and 100 pA. *C*: camera lucida reconstruction of biocytin-filled neuron whose electrophysiology was shown in *A*. Axon is indicated by an arrow. Scale bar, 30 μ m.

when the cell was held at $+30$ mV. At the holding potential of -30 mV, increases of the stimulus intensity from 10 to 110 μ A evoked EPSCs of increasing amplitude in another pyramidal cell (Fig. 1*B*). Figure 1*C* shows the morphology of the biocytin-filled pyramidal cell that generated the EPSCs shown in Fig. 1*A*.

Figure 2 shows NMDA receptor-mediated EPSCs recorded from two PCC pyramidal cells in slices from adult (95–135 days old) rats. Similar to those recorded from the juvenile rats, the evoked NMDA receptor-mediated EPSCs were completely blocked by D-APV (50 μ M), and electrical stimuli elicited an inward and outward current when the cell was held at -30 and $+30$ mV, respectively (Fig. 2*A*). A similar input-output curve was observed for the EPSCs across the range of increasing electrical stimulus intensities described above (Fig. 2*B*). Figure

2C shows a camera lucida reconstruction of the pyramidal cell whose physiological responses were shown in Fig. 2A.

Effects of EtOH on NMDA receptor-mediated EPSCs

Ethanol inhibited NMDA receptor-mediated EPSCs in a concentration-dependent manner ($F_{[3]} = 144.34$, $P < 0.0009$), and did so more potently in pyramidal cells from juvenile rats than in those from adults ($F_{[1]} = 14.47$, $P = 0.002$). There was also a significant age by concentration interaction ($F_{[3]} = 4.26$, $P = 0.01$), indicating that the concentration-response effects varied with the age of the animals from which slices were taken. Post hoc comparisons indicated that the inhibitory potency of ethanol was significantly greater in PCC neurons from juveniles compared with adults at each of the EtOH concentrations tested. However, there was no significant difference between the amplitude of EPSCs across age groups either at baseline or after wash out of ethanol.

In the presence of DNQX, BMI and 1.2 mM Mg^{2+} , the effects of EtOH on NMDA receptor-mediated EPSCs were assessed when the cells were held at -30 mV. Local electrical stimulation evoked an inward EPSC in PCC pyramidal cells in both age groups. Figure 3 shows the inhibitory effects of a range of EtOH concentrations on NMDA receptor-mediated EPSCs recorded from a PCC pyramidal cell from a juvenile rat (*top panel*) and an adult rat (*bottom panel*). In these experiments, after a 10-min baseline recording period, EtOH concentrations of 5–60 mM were sequentially bath-applied, and the effects of each concentration of EtOH was observed for 15 min. In cells from juvenile rats, the EPSC amplitude began to decrease after application of 5 mM EtOH, and the decreases were concentration-dependent across the range of concentrations applied. At concentrations of 5, 10, 30, and 60 mM EtOH, this cell's peak EPSC amplitude decreased by 11, 20, 35, and 47%, respectively, relative to baseline. The inhibitory effect of EtOH on NMDA receptor-mediated EPSCs was reversible after a 30-min wash out with normal ACSF. The EPSCs recorded from the adult neuron shown in Fig. 3 (*bottom panel*) were markedly less inhibited by ethanol across the range of concentrations tested. In this neuron concentrations of 5, 10,

30, and 60 mM EtOH decreased the peak amplitude of the NMDA EPSC by 4, 9, 24, and 36%, respectively relative to baseline. Again, the inhibitory effect was reversed after wash out. In addition, the pyramidal cell from the juvenile rat in the *top panel* of Fig. 3 shows a graded reduction in the amplitude with successive application of each concentration of EtOH. In contrast, the EPSC from the pyramidal cell of an adult rat (Fig. 3, *bottom panel*) showed a marked decrease in peak amplitude only after bath application of 30 mM EtOH. A further reduction was observed after 60 mM EtOH was bath applied.

Ethanol (5–60 mM) reduced NMDA receptor-mediated EPSC amplitudes in an age- and concentration-dependent manner. Figure 4 shows the averaged inhibitory effects of different concentrations of EtOH on NMDA receptor-mediated EPSCs from juvenile and adult neurons. In slices from juvenile rats, 5, 10, 30, and 60 mM EtOH reduced the mean amplitude of NMDA receptor-mediated EPSCs by $12 \pm 2.0\%$ ($n = 12$), $23 \pm 3.5\%$ ($n = 12$), $36 \pm 3.7\%$ ($n = 11$), and $46 \pm 2.7\%$ ($n = 13$), respectively, relative to baseline. However, the same concentrations of EtOH inhibited the responses by only $4 \pm 0.9\%$ ($n = 8$), $8 \pm 1.2\%$ ($n = 7$), $15 \pm 3.1\%$ ($n = 8$), and $31 \pm 4.1\%$ ($n = 8$) in slices from adult rats relative to baseline ($P < 0.05$). When the depressant effects caused by the same concentration of EtOH was compared between the two age groups, there are significant differences in the amplitude reduction of NMDA receptor-mediated EPSCs at each concentration tested, but not at baseline or after the wash out of ethanol.

To determine whether the effects of higher ethanol concentrations were related to either a cumulative inhibitory effect, or to acute tolerance, we compared the effects of a single application of 60 mM EtOH with the effects of 60 mM EtOH when presented at the end of a series of ethanol concentrations as described above. There were no significant differences between the effects of 60 mM EtOH presented in these ways. In juvenile rats, a single dose of 60 mM EtOH decreased the amplitude of NMDA EPSCs by $46.2 \pm 2.7\%$ ($n = 12$) compared with a reduction of $45.9 \pm 1.9\%$ ($n = 7$) observed after sequential application of 5, 10, and 30 mM EtOH prior to 60 mM EtOH. There was no

Juveniles

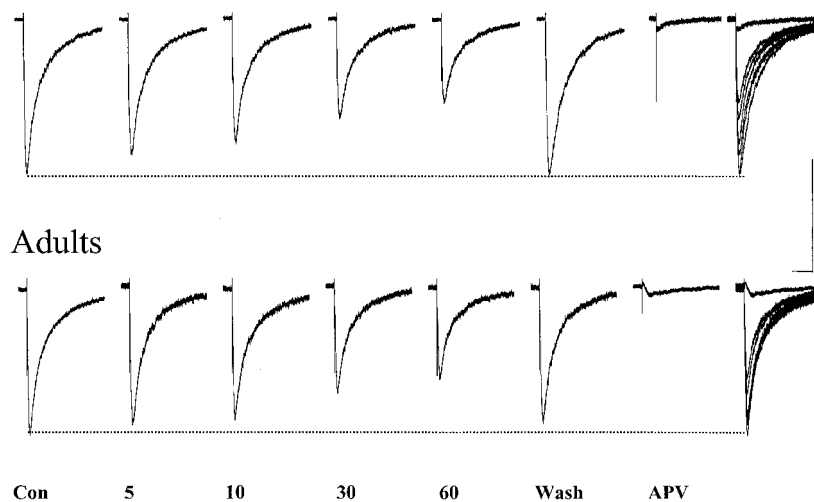


FIG. 3. Inhibitory effect of a range of EtOH concentrations on NMDA receptor-mediated EPSCs in PCC neurons from juvenile (*top panel*) and adult (*bottom panel*) rats. The inhibitory effects were reversed after wash out of EtOH from the artificial cerebrospinal fluid (ACSF). The holding potential was -30 mV for all recordings. Calibration bars, 200 ms and 100 pA.

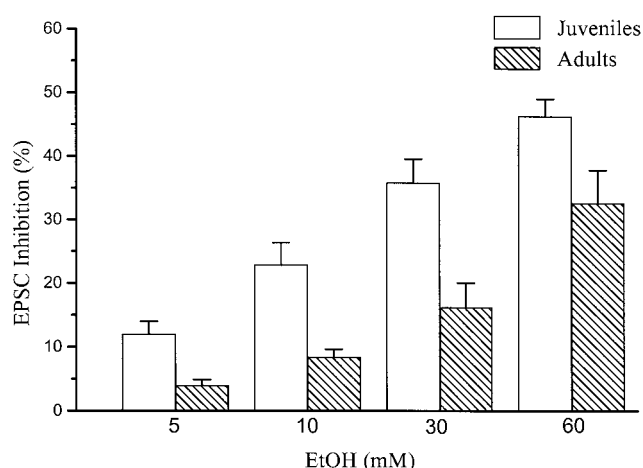


FIG. 4. Differential inhibitory effects of a range of EtOH concentrations on NMDA receptor-mediated EPSCs in PCC neurons from juvenile and adult rats. Bars represent the mean (\pm SE) percent of inhibition relative to baseline EPSC amplitude at 5–60 mM EtOH in neurons from juvenile (open bars) and adult (filled bars) rats. All recordings were made at -30 mV.

significant difference in the averaged amplitude of NMDA EPSCs under these conditions. Similarly, in slices from adult rats, a single dose of 60 mM EtOH reduced the amplitude of NMDA EPSCs by $32.6 \pm 5.1\%$ ($n = 9$) compared with $32.7 \pm 3.7\%$ ($n = 6$) from the cells exposed to sequential applications of 5, 10, and 30 mM EtOH prior to 60 mM EtOH. There was also no significant difference in the averaged amplitude of NMDA EPSCs under these conditions in the slices from adult rats (unpaired t -test, $P > 0.05$).

Previous studies of extracellular recordings have indicated that the antagonism of NMDA receptor-mediated potentials in hippocampal slices by ethanol is increased in the presence of Mg^{2+} (Morrisett et al. 1991). Thus it is possible that differential regulation of the NMDA channel by Mg^{2+} could account for the different potency of ethanol against NMDA receptor-mediated EPSCs. As an initial assessment of this possibility, we measured the effects of EtOH on NMDA receptor-mediated EPSCs while holding the neurons at $+30$ mV to remove the blockade of the NMDA receptor channel by Mg^{2+} . As shown in Fig. 5, 60 mM EtOH still caused a greater decrease in EPSC amplitude in pyramidal cells from juvenile rats (Fig. 5, A and C) compared with those from adults (Fig. 5, B and C). At the holding potential of $+30$ mV, 60 mM EtOH reduced the amplitude of NMDA receptor-mediated EPSCs by $43.7 \pm 5.4\%$ ($n = 6$) relative to baseline in neurons from juvenile rats (Fig. 5C), whereas the same concentration of EtOH only inhibited the amplitude of NMDA EPSCs by $21.6 \pm 4.6\%$ ($n = 5$) relative to baseline in cells from adult rats (Fig. 5C). This difference was statistically significant ($t_{19} = 3.00$, $P = 0.01$). As expected, relief of the Mg^{2+} block of the NMDA channel diminished the inhibitory effect of ethanol in neurons from adult animals. At -30 mV the percent inhibition produced by 60 mM EtOH was 32.6%, whereas at $+30$ mV the percent inhibition was 21.6% ($t_{12} = 2.47$, $P = 0.03$). However, there was no change in the inhibitory effect of ethanol against NMDA receptor-mediated currents in neurons from juveniles at $+30$ mV.

DISCUSSION

There are several findings from this study. First, NMDA receptor-mediated EPSCs were more sensitive to the inhibitory effect of ethanol in neocortical neurons from juvenile rats than in those from adults. Although we observed concentration-dependent inhibitory effects of ethanol on EPSCs in cells from both age groups, the inhibitory potency of ethanol was significantly greater among cells from juvenile animals. In addition, the difference in inhibitory potency did not appear to be mediated by either sensitization or acute tolerance, and increasing the holding potential to $+30$ mV did not alter the difference in inhibitory potency of a high concentration of ethanol, suggesting that it did not depend on an interaction with Mg^{2+} within the NMDA receptor channel. Finally, from a purely developmental perspective, we found that there was no difference in the reversal potential of NMDA receptor-mediated EPSCs in cells from juvenile animals compared with those from adults.

To our knowledge this is the first demonstration of an age-dependent difference in ethanol potency against NMDA

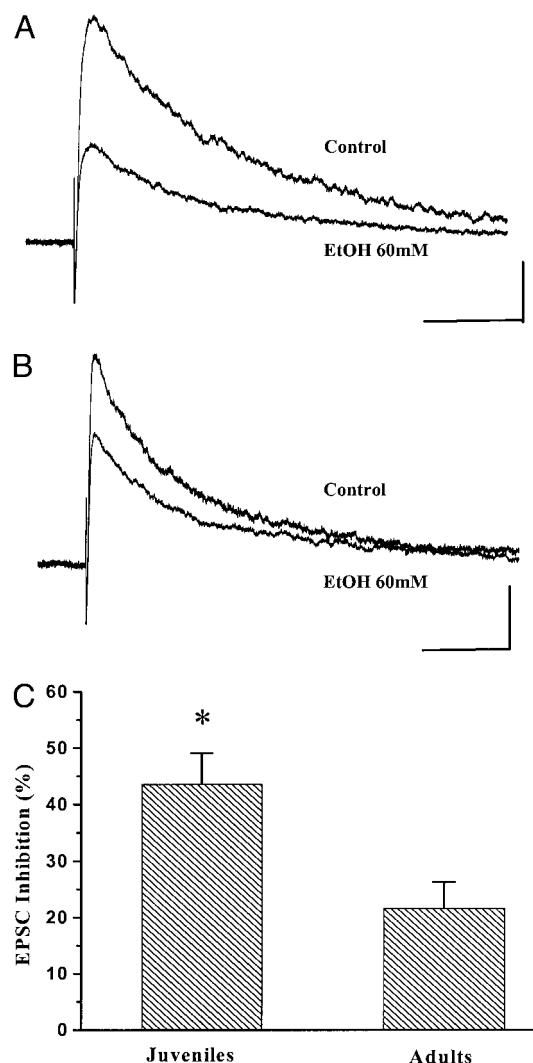


FIG. 5. Inhibitory effect of 60 mM EtOH on NMDA receptor-mediated EPSCs in neocortical cells from juvenile (A and left bar of C) and adult (B and right bar of C) rats at a holding potential of $+30$ mV. Calibration bars in A and B, 200 ms and 100 pA. * The mean percentage of inhibition was significantly different between the 2 groups of cells (unpaired t -test, $P < 0.05$).

receptor-mediated EPSCs in neocortical neurons. Previous studies using extracellular recordings have shown that ethanol more potently inhibits NMDA receptor-mediated pEPSPs (Swartzwelder et al. 1995a) and the induction of LTP (Pyapali et al. 1999; Swartzwelder et al. 1995b) in hippocampal area CA1 in brain slices from juvenile animals than in those from adults. Because of the well-known effects of acute ethanol exposure on learning, the hippocampal formation has been a region of consistent study within the ethanol literature. The inhibitory effect of ethanol against NMDA-mediated activity may be related to the learning impairments observed after acute ethanol exposure. That the effect is greater in hippocampi from juvenile and adolescent animals is consistent with the observed developmental differences in learning impairment during ethanol exposure in both humans (Acheson et al. 1998) and animal models (Markweise et al. 1998).

The present results indicate that enhanced sensitivity to ethanol in juveniles may extend to cognitive domains beyond learning and memory. The posterior cingulate cortex has been implicated in a number of neuropsychiatric disorders and is thought to mediate some subtle behaviors in humans related to spatial orientation, memory, and monitoring shifting cognitive orientation (Vogt et al. 1992). It also appears to be sensitive to the effects of chronic ethanol exposure. Such exposure in mice decreased the number and changed the morphology of PCC neurons (Marrero-Gordillo et al. 1998). As in other cortical areas, most excitatory synapses in the cingulate are thought to be glutamatergic, and include both AMPA and NMDA receptor subtypes (Hestrin 1996). The NMDA receptor/channel complex allows calcium entry; this initiates a cascade of cellular signals, some of which mediate synaptic plasticity, but also may mediate neurotoxicity (Rothman and Olney 1995). Thus the enhanced sensitivity of the pyramidal cells in this region to ethanol during juvenile development could indicate heightened vulnerability of these neurons to ethanol-mediated excitotoxicity. This could have broad implications for cognitive development in young individuals who drink ethanol in substantial amounts.

In addition to its focus on neocortical neurons, another unique feature of the present study is the cellular level of analysis. Previous direct studies of developmental sensitivity to ethanol used extracellular recordings from hippocampal slices (Pyapali et al. 1999; Swartzwelder et al. 1995a,b). Although these were valuable demonstrations of the developmental sensitivity to ethanol, it remained unclear whether the differences were due to sensitivity at the neuronal level or whether circuit interactions were the salient point difference. The present results clearly indicate that neocortical neuronal responsiveness to ethanol varies across the juvenile to adult period in rats, and these results are consistent with those from the hippocampal formation. The cellular level of analysis also provided the opportunity to compare the input resistance of NMDA EPSCs in slices from juvenile and adult animals. The fact that there was no significant difference rules out that aspect of membrane function as a mechanism underlying the increased sensitivity of juvenile neurons to ethanol.

The whole cell technique also afforded us the opportunity to test the inhibitory effects of ethanol on NMDA receptor-mediated EPSCs at multiple holding potentials. While the

developmental difference in the inhibitory potency of a single high concentration of EtOH (60 mM) remained intact when the holding potential was increased from -30 to $+30$ mV, the voltage change diminished EtOH-induced inhibition in neurons from adult animals while those from juveniles were not affected. The decrease of EtOH-induced inhibition in adult tissue is consistent with previous reports using hippocampal slices in which reductions of Mg^{2+} diminished the inhibitory effect of EtOH on NMDA receptor-mediated pEPSPs (Morrissett et al. 1991). However, a direct comparison of this effect between juvenile and adult neurons has never been made. The lack of such an effect on neurons from juvenile animals could indicate that EtOH-induced inhibition of NMDA currents is not regulated by Mg^{2+} in juvenile neurons. Among hippocampal pyramidal cells, Mg^{2+} is a less potent regulator of NMDA receptor-mediated EPSPs in juvenile rats compared to adults (Morrissett et al. 1990). Thus Mg^{2+} regulation of the channel is different in juveniles compared with adults and could be of mechanistic significance for the developmental sensitivity of NMDA receptor-mediated activity to ethanol.

The present findings add to a rapidly expanding literature on the unique potencies of ethanol against various neurophysiological and behavioral outcomes during postnatal development. We have shown that the enhanced sensitivity of NMDA receptor-mediated electrophysiological activity in the developing CNS extends to the neocortex and is observable at the cellular level. These results may figure productively in a very important and current dialogue in the U.S. related to alcohol consumption by young people. The use of ethanol by juveniles and adolescents is a consistent and growing problem. As we learn more about the parameters and mechanisms underlying the differences in developmental sensitivity to EtOH, this line of research may inform the public discussion on alcohol education and policy.

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