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Ethanol Dual Modulatory Actions on Spontaneous Postsynaptic Currents in Spinal Motoneurons

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Ziskind-Conhaim, Lea, Bao-Xi Gao, and Christopher Hinckley. Ethanol dual modulatory actions on spontaneous postsynaptic currents in spinal motoneurons. *J Neurophysiol* 89: 806–813, 2003. First published October 30, 2002; 10.1152/jn.00614.2002. Recently we have shown that acute ethanol (EtOH) exposure suppresses dorsal root-evoked synaptic potentials in spinal motoneurons. To examine the synaptic mechanisms underlying the reduced excitatory activity, EtOH actions on properties of action potential-independent miniature excitatory and inhibitory postsynaptic currents (mEPSCs and mIPSCs) were studied in spinal motoneurons of newborn rats. Properties of mEPSCs generated by activation of *N*-methyl-D-aspartate receptors (NMDARs) and non-NMDA receptors and of mIPSCs mediated by glycine and γ-aminobutyric acid-A receptors (GlyR and $GABA_A$R) were examined during acute exposure to 70 and 200 mM EtOH. In the presence of 70 mM EtOH, the frequency of NMDAR- and non-NMDAR-mediated mEPSCs decreased to 53 ± 5 and 45 ± 7% (means ± SE) of control values, respectively. In contrast, the frequency of GlyR- and $GABA_A$R-mediated mIPSCs increased to 138 ± 15 and 167 ± 23% of control, respectively. Based on the quantal theory of transmitter release, changes in the frequency of miniature currents are correlated with changes in transmitter release, suggesting that EtOH decreased presynaptic glutamate release and increased the release of both glycine and GABA. EtOH did not change the amplitude or rise and decay times of either mEPSCs or mIPSCs, indicating that the presynaptic changes were not associated with changes in the properties of postsynaptic receptors/channels. Acute exposure to 200 mM EtOH increased mIPSC frequency two- to threefold, significantly higher than the increase induced by 70 mM EtOH. However, the decrease in mEPSC frequency was similar to that observed in 70 mM EtOH. Those findings implied that the regulatory effect of EtOH on glycine and GABA release was dose-dependent. Exposure to the higher EtOH concentration had opposite actions on mEPSC and mIPSC amplitudes: it attenuated the amplitude of NMDAR- and non-NMDAR-mediated mEPSCs to ~80% of control and increased GlyR- and $GABA_A$R-mediated mIPSC amplitude by ~20%. EtOH-induced changes in the amplitude of postsynaptic currents were not associated with changes in their basic kinetic properties. Our data suggested that in spinal networks of newborn rats, EtOH was more effective in modulating the release of excitatory and inhibitory neurotransmitters than changing the properties of their receptors/channels.

INTRODUCTION

EtOH, a potent modulator of synaptic transmission, affects neural network function by interacting with a spectrum of specific membrane proteins that initiate cellular signal transduction processes (reviewed by Faingold et al. 1998; Weight 1992). In a variety of neuronal preparations, it modulates both excitatory and inhibitory synaptic transmission, reducing glutamate-mediated excitation and increasing GABA-, glycine-, and adenosine-mediated inhibition (reviewed by Crews et al. 1996; Narahashi et al. 2001; Peoples et al. 1996). Its dual modulatory action on nicotinic acetylcholine receptors resulted in depressing or facilitating acetylcholine-mediated currents depending on the composition of the receptor subunits (Aistrup et al. 1999).

Glutamate receptors, the major excitatory receptors in the CNS, consist of three ionotropic receptor subtypes: *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate receptors. Until recently the general consensus was that NMDA receptors (NMDAR) are the primary targets of EtOH because at relatively low concentrations, it antagonizes NMDAR-mediated responses in a diverse populations of neurons (Lima-Lindman and Albuquerque 1989; Lovinger et al. 1990; Morrisett and Swartzwelder 1993; reviewed by Faingold et al. 1998; Li et al. 2002; Tabakoff and Hoffman 1996; Woodward 2000). NMDA receptor sensitivity to EtOH is regulated by its subunit composition, but factors such as phosphorylation also play important role in modulating EtOH sensitivity (reviewed by Woodward 2000). The effect of EtOH on non-NMDA receptors is somewhat controversial (Crews et al. 1996; Lovinger 1997), but in spinal and cortical neurons, NMDAR- and non-NMDAR-mediated synaptic events are similarly affected by EtOH inhibitory actions (Wang et al. 1999, Wirkner et al. 2000).

Numerous studies have demonstrated that EtOH-induced intoxication is correlated with its interaction with γ-aminobutyric acid-A ($GABA_A$) receptors (reviewed by Crews et al. 1996). In isolated brain preparations and cultured neurons, EtOH increases $GABA_A$ receptor-mediated Cl^- conductance (Aguayo 1990; Celentano et al. 1988; Nestores 1980; Reynolds and Prasad 1991), and similarly it potentiates glycine-mediated Cl^- flux in synaptoneurosomes (Engblom and Åkerman 1991) and enhances glycine currents generated in hippocampal neurons (Aguayo and Pancetti 1994). A comparison between EtOH actions on glycine- and GABA-mediated Cl^- currents in dissociated spinal neurons indicated that glycine receptors are more sensitive to EtOH than GABA receptors (Celentano et al.1988). It has been proposed that $GABA_A$ and glycine recep-

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tor sensitivity to EtOH depends primarily on the expression of the $\alpha 1$ receptor subunit (Eggers et al. 2000; Mascia et al. 1996).

In addition to EtOH opposite actions on excitatory and inhibitory neurotransmitter receptors, its dual effects on presynaptic release of excitatory and inhibitory neurotransmitters have also been documented. EtOH inhibits NMDAR-mediated glutamate release in the rat striatum (Carboni et al. 1993), and in the hippocampus, it suppresses high-K^+-evoked release of endogenous glutamate, aspartate, and GABA (Martin and Swartzwelder 1992). In contrast, EtOH increases the frequency of action potential-independent glycinergic currents in hypoglossal motoneurons, implying that it increases presynaptic glycine release (Eggers et al. 2000).

We have demonstrated that EtOH suppresses motoneuron electrical activity by reducing motoneuron excitability, decreasing the amplitude of dorsal root-evoked excitatory postsynaptic potentials and decreasing the frequency of spontaneous excitatory postsynaptic currents, while increasing the frequency of inhibitory postsynaptic currents (Cheng et al. 1999). In that study, spontaneous excitatory and inhibitory currents were recorded concurrently; therefore it is conceivable that EtOH action partly resulted from modifying the synaptic interaction between those currents rather than directly influencing excitatory and inhibitory synaptic transmission (Marszalec et al. 1998). In this study, properties of pharmacologically isolated action potential-independent postsynaptic currents were examined to determine EtOH effects on action potential-independent presynaptic release of glutamate, glycine, and GABA and its modulatory actions on postsynaptic NMDA, non-NMDA, glycine, and $GABA_A$ receptors/channels.

A preliminary report of this study was published in an abstract form (Gao and Ziskind-Conhaim 2000).

METHODS

Spinal cord preparation

Lumbar spinal cords were isolated from 1- to 4-day old postnatal Sprague-Dawley rats (P1–4). The procedure for spinal cord dissection was similar to that described previously (Gao and Ziskind-Conhaim 1998; Ziskind-Conhaim 1990). Postnatal rats were anesthetized by hypothermia. The lumbar region of the spinal cord was removed and placed in oxygenated cold dissection solution. The dissection solution contained (in mM): 113 NaCl, 3 KCl, 1 $CaCl_2$, 6 $MgCl_2$, 25 $NaHCO_3$, 1 NaH_2PO_4, and 11 glucose (pH 7.2–7.4). The isolated spinal cord was embedded in agar (2% in extracellular solution). Transverse slices, 350 μm thick, were cut using a Vibratome (Technical Products International). Prior to whole cell recordings, slices were incubated in extracellular solution at room temperature for 30–60 min. The extracellular solution contained (in mM): 113 NaCl, 3 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 25 $NaHCO_3$, 1 NaH_2PO_4, and 11 glucose. The solution was equilibrated with 95% O_2-5% CO_2 (pH 7.2 at 20–22°C).

Whole cell recording

Slices were transferred into a recording chamber, which was mounted on the stage of an upright microscope and were superfused with aerated extracellular solution at room temperature (20–22°C). Large neurons in the medial and lateral ventral horn were visualized using infrared DIC optics, and those were assumed to be motoneurons. Whole cell patch-clamp recordings in motoneurons were performed using patch electrodes pulled to tip resistances of 3–5 MΩ using a multi-stage puller (Sutter Instruments). Electrodes were filled with solution containing (in mM): 149 CsCl, 10 HEPES, 0.2 EGTA, 1 Mg-ATP, and 0.1 GTP. The solution was adjusted to pH 7.2 using CsOH, and the osmolarity was 290 mosM. Typically, the series resistance was two- to threefold higher than the pipette resistance. Experiments were rejected if the series resistance changed >15%.

Action potential-independent miniature excitatory and inhibitory postsynaptic currents (mEPSCs and mIPSCs) were recorded in the presence of TTX (1 μM). At P1–4, mEPSC and mIPSC frequencies were 0.5 and 0.3 Hz, respectively (Gao et al. 1998), and the frequencies of pharmacologically isolated NMDAR- and non-NMDAR-mediated mEPSCs and GlyR- and $GABA_A$R-mediated mIPSCs were ~50% lower. Therefore to acquire a large sample of mEPSCs and mIPSCs for statistical analysis, experiments were performed in high extracellular K^+ (18 mM, Gao et al. 1998, 2001). Synaptic currents were recorded using an Axopatch 200A amplifier (Axon Instruments). Currents were filtered at 1 kHz, digitized at 5 kHz, and stored on a disk for later analysis. In most motoneurons, continuous recordings were carried out until >100 events were recorded.

Data analysis

The threshold for detection of miniature currents was set at 2 pA above the background noise. The average noise SD was 1.7 pA at −60 mV (see also Gao et al. 2001), not significantly different from the 1.9 pA measured at +40 mV. mEPSCs and mIPSCs were measured using Mini Analysis software (Synaptosoft). Kinetic analysis was performed on averaged miniature currents, which were obtained by lining up the rising phase of single currents. In most motoneurons, >100 events were averaged. Kinetic analysis included: peak amplitude, rise time from 10 to 90% peak amplitude and decay time constant (decay τ). Typically, the time course of decay of mEPSCs and mIPSCs was best fitted with the sum of two exponentials. Data are presented as means ± SE. Student's *t*-test was used to determine the statistical significance ($P < 0.05$).

RESULTS

EtOH modulation of mEPSC and mIPSC properties was examined in spinal motoneurons of P1–4 rats. Acute EtOH exposure does not significantly change the properties of dorsal root-evoked potentials at concentrations <70 mM (Cheng et al. 1999). To determine whether lower EtOH concentration altered the properties of action potential-independent miniature synaptic currents, mEPSC and mIPSC frequencies and amplitudes were examined during a 10- to 15-min exposure to 30 mM EtOH ($n = 4$). At that concentration, EtOH did not alter mEPSC and mIPSC frequencies (not shown), confirming our previous findings that higher EtOH concentrations were required to significantly reduce excitatory synaptic transmission and motoneuron excitability in newly formed spinal networks (Cheng et al. 1999). Therefore in this study, EtOH actions on pharmacologically isolated miniature synaptic currents were examined only at higher concentrations of 70 and 200 mM.

EtOH decreased mEPSC frequency and increased mIPSC frequency

Glutamate-mediated mEPSCs were recorded in the presence of strychnine (0.5 μM) and bicuculline (5 μM), glycine, and $GABA_A$ receptor antagonists, respectively. mEPSCs were recorded at a holding potential (HP) of +40 mV to remove the voltage-dependent Mg^{2+} block of NMDA channels. Some experiments were initially carried out at a HP of −60 mV, and Mg^{2+} was omitted from the extracellular solution (see following text). However, because of the possibility that Mg^{2+} mod-

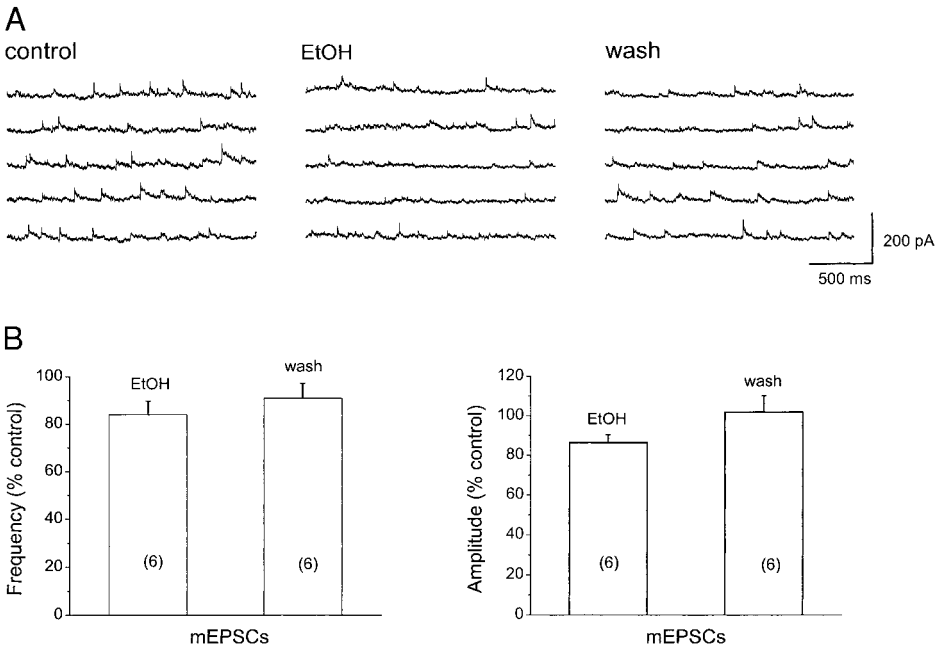

FIG. 1. Ethanol, EtOH reversibly suppressed the frequency of miniature excitatory postsynaptic currents (mEPSCs) in a P4 motoneuron. *A*: traces of continuously recorded mEPSCs before (control), during EtOH exposure (EtOH) and after the removal of EtOH from the extracellular solution (wash). mEPSC frequency decreased from 3.7 Hz before EtOH application to 3.0 Hz in its presence. mEPSC frequency of 3.2 Hz was recorded 20 min after EtOH removal. *B*: mEPSC frequency and amplitude during EtOH exposure (% of control) and after its removal. EtOH significantly suppressed mEPSC frequency to 84 ± 6% of control ($P < 0.04$), and the frequency increased to 91 ± 6% of control 20 min after EtOH removal. The averaged amplitude did not significantly change, decreasing to 86 ± 4% of the control value ($P < 0.07$) in the presence of EtOH and increasing to 101 ± 8% after its removal. Values are means ± SE of 6 motoneurons.

ulated presynaptic release, mEPSC properties were analyzed only when recorded at +40 mV.

At +40 mV, mEPSCs were recorded as outward currents (Fig. 1), and consisted of a mixed population of fast-rising, fast-decaying non-NMDAR-mediated mEPSCs and slow-rising, slow-decaying NMDAR-mediated mEPSCs. A third subpopulation of dual-component mixed fast- and slow-decaying mEPSCs was also apparent, and it was assumed that those were generated by co-activation of NMDA and non-NMDA receptors. Pharmacologically isolated non-NMDAR-mediated mEPSCs were recorded in the presence of D-2-amino-5-phosphonovaleric acid (D-APV, 20 μM), a NMDA receptor antagonist (Fig. 2). NMDAR-mediated mEPSCs were recorded in the presence of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 5–10 μM), an AMPA/kainate receptors antagonist (Fig. 3).

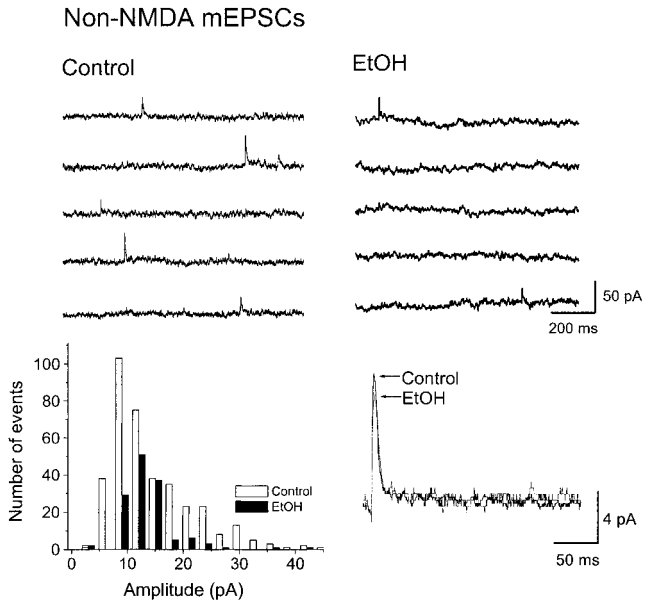

FIG. 2. EtOH significantly reduced the frequency of fast-decaying non-*N*-methyl-D-aspartate receptor (non-NMDAR)-mediated mEPSCs, without changing mEPSC amplitudes and basic kinetic properties. *Top*: traces of continuously recorded mEPSCs in a P4 motoneuron before and during a brief exposure to 70 mM EtOH. *Bottom left*: amplitude distributions of non-NMDAR-mediated mEPSCs recorded in control and EtOH-containing solution. mEPSC amplitudes >45 pA are not shown. Mean peak amplitudes were 16.5 (control) and 15.0 pA (EtOH). mEPSC frequency decreased from 0.6 Hz before EtOH application to 0.2 Hz in its presence. *Bottom right*: averaged non-NMDAR-mediated mEPSCs before and during EtOH exposure (control mEPSCs, $n = 373$; EtOH mEPSCs, $n = 134$). Overlapping currents were not averaged.

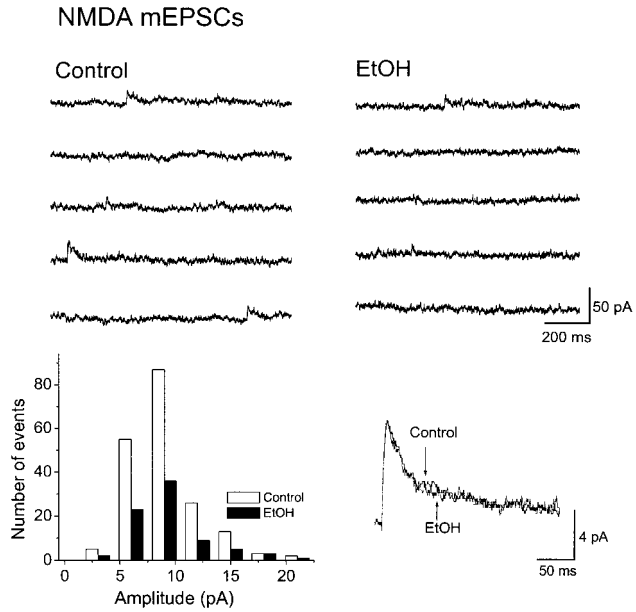

FIG. 3. EtOH significantly reduced the frequency of slow-decaying NMDAR-mediated mEPSCs but did not change mEPSC amplitudes and basic kinetic properties. *Top*: traces of continuously recorded mEPSCs in a P3 motoneuron before EtOH application and in its presence (70 mM). *Bottom left*: amplitude distributions of NMDAR-mediated mEPSCs before and during EtOH exposure. mEPSC amplitudes >25 pA are not shown. Mean peak amplitudes were 10.6 and 10.7 pA before and after EtOH exposure, respectively. mEPSC frequency was reduced from 0.4 Hz (control) to 0.2 Hz (EtOH). *Bottom right*: averaged NMDAR-mediated mEPSCs before and during EtOH exposure (control mEPSCs, $n = 192$; EtOH mEPSCs, $n = 79$).

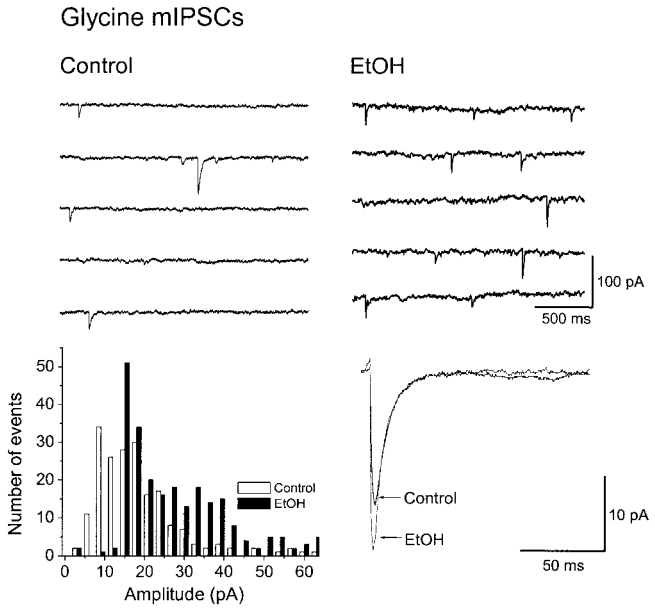

FIG. 4. EtOH significantly increased the frequency of fast-decaying glycine receptor (GlyR)-mediated miniature inhibitory postsynaptic currents (mIPSCs), without changing mIPSC basic kinetic properties. *Top*: traces of continuously recorded mIPSCs in a P4 motoneuron before and during exposure to 70 mM EtOH. *Bottom left*: amplitude distributions of GlyR-mediated mIPSCs in control and EtOH-containing solution. mIPSC frequency increased from 0.6 Hz (control) to 0.9 Hz (EtOH). mIPSC amplitudes >65 pA are not shown. Mean peak amplitudes were 18.3 (control) and 25.1 pA (EtOH), the largest amplitude increase induced by 70 mM EtOH. *Bottom right*: averaged GlyR-mediated mIPSCs before and during EtOH exposure (control mIPSCs, n = 194; EtOH mIPSCs, n = 253). The large EtOH-induced increase in the amplitude of GlyR-mediated mIPSCs was not associated with changes in their rise time and decay time constants.

mIPSCs were recorded in the presence of D-APV (20 μM) and CNQX (5–10 μM), and at a HP of −60 mV they appeared as inward currents (Cl^- equilibrium potential was approximately −5 mV). Based on their kinetic properties, mIPSC population consisted of three groups: fast-decaying glycinergic mIPSCs, slow-decaying GABAergic mIPSCs, and dual-component, fast-slow-decaying mixed glycine-GABA-mediated mIPSCs (Gao et al. 2001). Pharmacologically isolated GlyR-mediated mIPSCs were recorded in the presence of bicuculline (5 μM, Fig. 4), and $GABA_A$R-mediated mIPSCs were recorded in the presence of strychnine (0.5 μM, Fig. 5).

A brief (10–15 min) exposure to 70 mM EtOH reversibly suppressed mEPSC frequency to ~50% of control (Figs. 1–3). The frequency of non-NMDAR-mediated mEPSCs was significantly reduced from 0.64 ± 0.16 Hz (SE, n = 8) before EtOH exposure to 0.29 ± 0.11 Hz in its presence and that of NMDAR-mediated mEPSCs decreased from 0.51 ± 0.13 Hz (n = 11) to 0.27 ± 0.09 Hz (Fig. 6). Opposite modulatory action was apparent on mIPSC frequency, significantly increasing the frequency of GlyR- and $GABA_A$R-mediated mIPSCs to 138 ± 15% (n = 8) and 167 ± 23% (n = 9) of control, respectively (Fig. 6). Although we cannot rule it out, it is unlikely that the apparent reduction in mEPSC frequency reflected a failure to detect currents that decreased in amplitude during EtOH exposure. EtOH did not significantly change mEPSC amplitude distributions (Figs. 2 and 3), and small mEPSCs (2.5–6 pA) constituted only a small fraction of mEPSC population. Therefore even if EtOH acted on postsynaptic glutamatergic receptors to reduce the amplitude of the small currents below the level of detection, it could not have accounted for the 50% reduction in mEPSC frequency.

At this concentration, EtOH did not significantly alter the amplitude or rise and decay times of either mEPSCs or mIPSCs. The mean amplitude of non-NMDAR-mediated mEPSCs was 18.3 ± 1.1 pA before EtOH application and 16.7 ± 1.2 pA in its presence and that of NMDAR-mediated mEPSCs changed from 11.2 ± 1.8 to 11.8 ± 1.8 pA (Fig. 6). It is conceivable that small mEPSCs were not detected at a HP of +40 mV because of a potential leak, which could have masked changes in mEPSC amplitudes during EtOH exposure. However, at a HP of −60 mV (extracellular Mg^{2+} was omitted), the mean amplitude of non-NMDAR-mediated mEPSCs was 18.9 ± 2.1 pA (n = 5) and that of NMDAR-mediated mEPSCs was 11.6 ± 1.6 (n = 4), not significantly different from those recorded at +40 mV. Therefore it is unlikely that the failure to detect changes in mEPSC amplitudes resulted from the inability to record small currents at a HP of +40 mV.

The amplitude of GlyR- and $GABA_A$R-mediated mIPSCs increased ~10% during EtOH exposure. Our recent study has demonstrated that EtOH does not significantly change motoneuron membrane resistance (Cheng et al. 1999), therefore it is unlikely the inability to detect changes in mEPSC and mIPSC amplitudes resulted from a decrease in membrane resistance during EtOH exposure.

Our findings that EtOH changed the frequency but not amplitude of mEPSCs and mIPSCs implied that acute exposure to 70 mM EtOH modulated presynaptic release of glutamate,

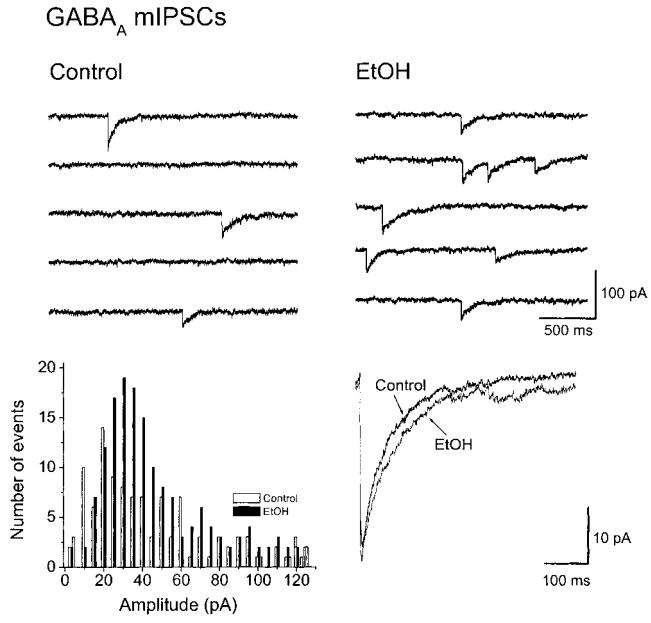

FIG. 5. EtOH significantly increased the frequency of slow-decaying $GABA_A$R-mediated mIPSCs but did not change mIPSC amplitudes and basic kinetic properties. *Top*: traces of continuously recorded mIPSCs in a P3 motoneuron before EtOH application and in its presence (70 mM). *Bottom left*: amplitude distributions of $GABA_A$R-mediated mIPSCs before and during EtOH exposure. mIPSC amplitudes larger than 130 pA are not shown. Mean peak amplitude increased from 35.5 (control) to 36.5 pA (EtOH). mIPSC frequency increased from 0.2 Hz (control) to 0.6 Hz (EtOH), with a significant number of overlapping currents. *Bottom right*: averaged $GABA_A$R-mediated mIPSCs before and during EtOH exposure (control mIPSCs, n = 111; EtOH mIPSCs, n = 161).

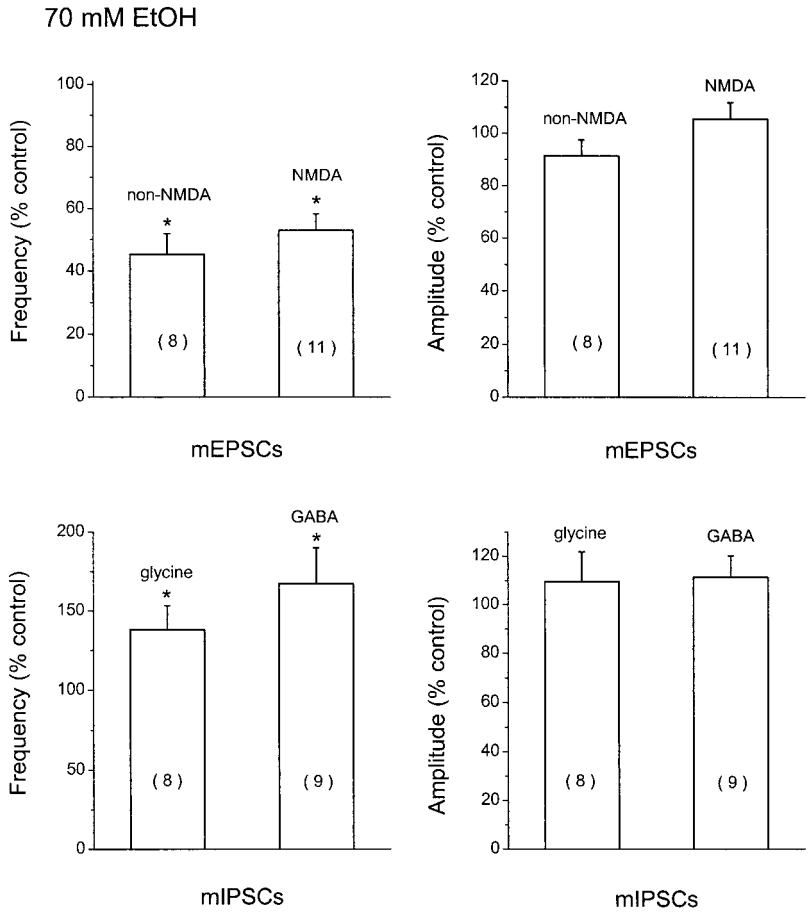

FIG. 6. The frequency of non-NMDAR- and NMDAR-mediated mEPSCs significantly decreased and the frequency of GlyR- and $GABA_A$R-mediated mIPSCs significantly increased during EtOH exposure (70 mM). At this concentration, EtOH did not significantly alter mEPSC and mIPSC amplitudes. Averages ± SE of 8 and 11 motoneurons for non-NMDAR- and NMDAR-mediated mEPSCs, respectively, and 8 and 9 motoneurons for GlyR- and $GABA_A$R-mediated mIPSCs.

glycine, and GABA without changing the properties of their postsynaptic receptors/channels.

High EtOH concentration reduced mEPSC amplitude and increased mIPSC amplitude

To determine whether the amplitude and basic kinetic properties of mEPSCs and mIPSCs were modulated by high EtOH concentration, current properties were examined in the presence of 200 mM EtOH. The ~30% decrease in mEPSC frequency (Table 1) was similar to the reduction induced by 70 mM EtOH, indicating that the lower concentration produced a maximal inhibitory effect on mEPSC frequency. At that concentration, the decrease in mEPSC frequency was associated with a significant decrease in mEPSC amplitude, with a reduction of ~20% in the amplitude of both NMDAR- and non-NMDAR-mediated mEPSCs (Table 1). EtOH-induced amplitude attenuation was not associated with changes in basic kinetic properties of glutamate-mediated mEPSCs.

mIPSC frequency increased two- to threefold during the exposure to 200 mM EtOH (Table 2), significantly higher than the increase induced during the exposure to 70 mM EtOH. This effect was reversible on removal of EtOH from the extracellular solution (Fig. 7). These data demonstrated that unlike its inhibitory action on mEPSC frequency, EtOH facilitatory action on mIPSC frequency was dose-dependent. At the higher concentration EtOH induced an ~20% increase in the amplitude of both GlyR- and $GABA_A$R-mediated mIPSCs, but it did not affect mIPSC basic kinetic properties (Table 2).

TABLE 1. *Acute exposure to 200 mM EtOH significantly decreased the frequency and amplitude of non-NMDAR- and NMDAR-mediated mEPSCs*

	Frequency, Hz	Amplitude, pA	Rise Time, ms	Decay τ1, ms	Decay τ2, ms
Non-NMDA (n = 6)					
Control	0.50 ± 0.12	16.8 ± 1.9	1.8 ± 0.4	2.3 ± 0.4	11.5 ± 1.9
EtOH	0.33 ± 0.08*	14.0 ± 2.0*	1.7 ± 0.3	2.3 ± 0.4	11.7 ± 2.1
NMDA (n = 6)					
Control	0.35 ± 0.05	15.0 ± 2.1	5.3 ± 0.1	33.9 ± 4.2	168.0 ± 20.5
EtOH	0.24 ± 0.04*	11.8 ± 1.3*	4.8 ± 0.3	24.5 ± 2.8	122.6 ± 14.1

The frequencies of non-*N*-methyl-D-aspartate receptor (NMDAR)- and NMDAR-mediated miniature excitatory postsynaptic currents (mEPSCs) decreased to 67 and 68% of control levels, respectively, and their amplitudes decreased to 83 and 78% of control. EtOH did not significantly change the rise time and decay time constants of the two populations of mEPSCs. n = number of cells. * Significantly different than control, $P < 0.05$.

TABLE 2. *Changes in mIPSC properties during acute exposure to 200 mM EtOH*

	Frequency, Hz	Amplitude, pA	Rise Time, ms	Decay τ1, ms	Decay τ2, ms
Glycine (n = 6)					
Control	2.04 ± 0.76	31.3 ± 9.7	2.3 ± 0.4	7.6 ± 0.9	37.9 ± 4.5
EtOH	4.7 ± 1.5*	37.5 ± 10.5*	2.8 ± 0.3	9.6 ± 1.8	39.3 ± 6.1
GABA (n = 7)					
Control	0.58 ± 0.09	31.4 ± 4.9	3.0 ± 0.2	27.9 ± 3.1	147.0 ± 21.5
EtOH	1.88 ± 0.40*	39.1 ± 4.8*	3.1 ± 0.2	31.6 ± 3.5	155.9 ± 17.1

The frequency and amplitude of glycine receptor (GlyR)- and $GABA_A$ receptor ($GABA_A$R)-mediated miniature inhibitory postsynaptic currents (mIPSCs) significantly increased during exposure to 200 mM EtOH, but there was no change in their rise time and decay time constants. The frequencies of GlyR- and $GABA_A$R-mediated mIPSCs increased 2- to 3-fold, but their amplitudes increased only by 19.9 and 24.5%, respectively. n = number of cells. * Significantly different than in control, $P < 0.05$.

DISCUSSION

This study demonstrated that EtOH shifted the balance between excitation and inhibition toward inhibition by modulating both pre- and postsynaptic mechanisms underlying spontaneous synaptic transmission. EtOH dominant action was to increase high-K^+-induced mIPSC frequency and depress mEPSC frequency, probably reflecting an increase in glycine and GABA release and a suppression of glutamate release. The dual presynaptic effects might contribute to EtOH-induced attenuation of dorsal root evoked potentials in spinal motoneurons (Cheng et al. 1999; Wang et al. 1999). At high concentrations, presynaptic modulation was associated with opposite actions on excitatory and inhibitory postsynaptic receptors/channels as evident by the decrease in mEPSC amplitude and the increase in mIPSC amplitude. The concept of opposite EtOH actions on excitatory and inhibitory synaptic transmission is not new, but this is the first study to show that during the period of spinal network formation in the rat (Seebach and Ziskind-Conhaim 1994), EtOH at the lower concentration (70 mM) was more effective in modulating presynaptic release of excitatory and inhibitory neurotransmitters than changing the properties of their postsynaptic receptors/channels. The finding that relatively high EtOH concentrations were required to affect miniature current properties might be related to the age-dependent sensitivity to EtOH in the rat (Fang et al. 1997). It is conceivable that lower EtOH concentrations would effectively modulate synaptic currents in more mature rats.

Dual EtOH actions on neurotransmitter release

Our observations that EtOH depressed mEPSC frequency supported previous reports showing that it inhibited NMDA-mediated glutamate release in the rat striatum (Carboni et al. 1993) and suppressed high-K^+-evoked release of endogenous glutamate and aspartate in the hippocampal slice (Martin and Swartzwelder 1992). The finding that EtOH increased mIPSC frequency was similar to the increased frequency of glycinergic mIPSCs in postnatal hypoglossal motoneurons (Eggers et al. 2000) but contradicted its inhibitory effect on high-K^+-evoked GABA release in the hippocampus (Martin and Swartzwelder 1992). It should be noted that the decrease in endogenous GABA release was measured in the whole tissue rather than its release at synaptic sites as reflected in our study.

The mechanisms underlying EtOH-induced opposite actions on vesicular release of excitatory and inhibitory neurotransmitters are unknown. It is conceivable that different presynaptic receptors that influence intracellular calcium concentrations are

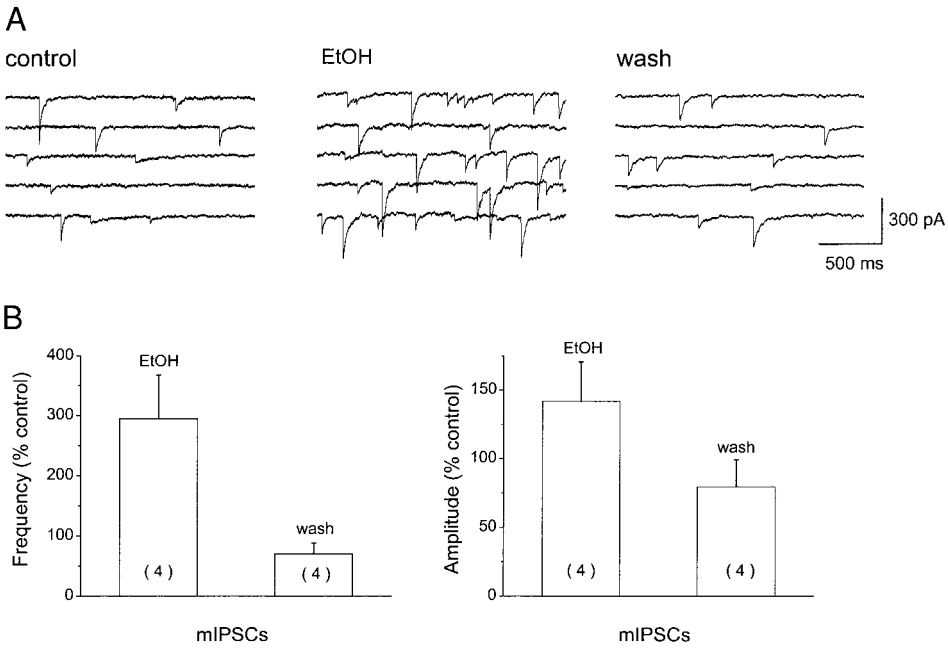

FIG. 7. mIPSC frequency and amplitude significantly increased during acute exposure to 200 mM EtOH. *A*: continuously recorded mIPSCs in a P3 motoneuron before (control), in the presence of EtOH (EtOH) and after its removal (wash). mIPSC frequency was 1.4 Hz and the mean amplitude was 94.7 pA before EtOH application and 4.6 Hz and 136 pA in its presence. EtOH effects were partially reversible and the frequency and amplitude decreased to 0.9 Hz and 45.4 pA after its removal. *B*: mIPSC frequency and amplitude during EtOH exposure (% of control) and after its removal. EtOH significantly increased mIPSC frequency to 295 ± 73% of control ($P < 0.01$), and the frequency decreased to 70 ± 18% of control 20 min after EtOH removal. The averaged amplitude increased to 142 ± 29% of control in the presence of EtOH and decreased to 80 ± 20% after its removal. Values are means ± SE of 4 motoneurons.

expressed on terminals releasing glutamate, glycine and GABA. Dual EtOH actions on muscarine-stimulated release of norepinephrine (NE) have been demonstrated in PC12 cells (Rabe and Weight 1988). At a concentration of 25 mM, EtOH inhibited both muscarine-induced NE release and the increase in intracellular free Ca^{2+}. However, at a concentration of 100 mM, it increased NE release and elevated intracellular Ca^{2+}. Those findings implied that the dose-dependent dual EtOH actions were associated with its opposite effects on intracellular Ca^{2+}. Therefore it is possible that suppression of Ca^{2+} release from internal stores in glutamate containing nerve terminals contributed to the reduced mEPSC frequency. It has been shown that spontaneous transmitter release can result from spontaneous Ca^{2+} release from internal stores in hippocampal synaptic boutons (Emptage et al. 2001), and in Purkinje cells, large mIPSCs are generated by multivesicular release mediated by Ca^{2+} release from presynaptic stores (Llano et al. 2000).

In our study, miniature postsynaptic currents were recorded in the presence of 18 mM extracellular K^+ that produced ~20 mV depolarization, sufficient to reach the threshold potential for Ca^{2+} current activation (Gao and Ziskind-Conhaim 1998). One of the most significant effects of EtOH on voltage-gated ion channels is its inhibitory action on Ca^{2+} channels, in particular the L-type channel (reviewed by Crews et al. 1996). It is feasible that the decrease in mEPSC frequency during EtOH exposure resulted from its suppression of voltage-gated Ca^{2+} current. Our recent findings have indicated that EtOH reduced the amplitude of Ca^{2+} current in spinal motoneurons of postnatal rats (Gao and Ziskind-Conhaim, unpublished data). That current was previously characterized as the N-type current (Gao and Ziskind-Conhaim 1998), which appeared to regulate synaptic transmission in spinal cords of developing rodents (e.g., Gruner and Silva 1994; Xie and Ziskind-Conhaim 1995).

It is conceivable that nerve terminals releasing glutamate have different components in the vesicular release sequence than those releasing glycine and GABA (Varoqueaux et al. 2002), resulting in different EtOH actions on the release of excitatory and inhibitory neurotransmitters. The mechanisms underlying the increase in mIPSC frequency are unknown, but it is possible that presynaptic facilitation via ligand- or voltage-gated channels is responsible for the increased mIPSC frequency.

Opposite EtOH actions on postsynaptic receptors

EtOH opposite actions on mEPSC and mIPSC amplitudes supported the general consensus that its interaction with glutamate receptors/channels depressed glutamatergic responses, while it increased glycine- and GABA-mediated Cl^- currents (reviewed by Faingold et al. 1998; Weight et al. 1992). It is thought that NMDAR-mediated synaptic transmission is especially sensitive to acute EtOH exposure (Dildy-Mayfield et al. 1996; Lovinger et al. 1989; Morrisett and Swartzwelder 1993). However, our findings indicated that there was no differential sensitivity to EtOH of NMDA and non-NMDA receptors (see also Wang et al. 1999; Wrikner et al. 2000). NMDA receptor sensitivity to EtOH depends, at least in part, on the receptor subunit compositions (Lovinger 1995; Masood et al. 1994; Yang et al. 1996; reviewed by Crews et al. 1996), and studies have suggested that EtOH has a more prominent action on NMDAR-mediated currents in cells expressing the NR1/NR2B combination than any other receptor subunit composition (Masood et al. 1994; Woodward 2000). Although we cannot rule it out, it is unlikely that the low sensitivity of the NMDA receptor to EtOH is related to its receptor subunit composition in developing spinal motoneurons, because NR1 and NR2B subunits are expressed in postnatal motoneurons (Stegenga and Kalb 2001). Moreover, EtOH (100 mM) has similar inhibitory action on both AMPAR- and NMDAR-mediated synaptic currents in motoneurons of 14- to 23-day-old rats (Wang et al. 1999), close to the time when adult-like NMDA receptor subunit composition is expressed (Stegenga and Kalb 2001). It is conceivable that other postsynaptic mechanisms, such as EtOH actions on pathways that regulate receptor phosphorylation (reviewed by Woodward 2000), mediate at least some of its effects on glutamate-mediated synaptic transmission.

Our observation that EtOH increased the amplitudes of GlyR- and $GABA_A$R-mediated mIPSCs supported previous reports showing an increase in GABA- and glycine-induced Cl^- flux during EtOH exposure (Aguayo 1990; Aguayo and Pancetti 1994; Celentano et al. 1988; Eggers et al. 2000; Nestores 1980; Reynolds and Prasad 1991). EtOH had no differential actions on GlyR- and $GABA_A$R-mediated mIPSCs in postnatal motoneurons as reported in dissociated spinal neurons in which EtOH induced larger increase in glycine- than GABA-induced current (Celentano et al. 1988). It is possible that EtOH significantly increased mIPSC amplitude only at a concentration of 200 mM because of the immature composition of glycine and GABA receptor subunits. Expression of α1, β2, and γ2 $GABA_A$ receptor subunits (Criswell et al. 1993; Duncan et al. 1995; Wafford et al. 1991) and α1 glycine receptor domain (Eggers et al. 2000; Mascia et al. 1996) are correlated with high sensitivity to EtOH. In spinal sensory neurons, the switch in glycine receptor subunits from the embryonic α2 to the adult α1 occurs between P8 to P16 (Takahashi et al. 1992), implying that motoneurons expressed primarily the α2 subunit during the period examined in our study.

Our data demonstrated that EtOH dual modulatory actions on excitatory and inhibitory synaptic currents resulted from its interactions with both pre- and postsynaptic mechanisms that regulate synaptic transmission in the developing rat spinal cord. EtOH opposite effects on presynaptic release of excitatory and inhibitory neurotransmitters were predominant in newly established spinal networks.

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REFERENCES

Aguayo LG. Ethanol potentiates the $GABA_A$-activated Cl^- current in mouse hippocampal and cortical neurons. *Eur J Pharmacol* 187: 127–130, 1990.

Aguayo LG and Pancetti FC. Ethanol modulation of the gamma-aminobutyric acid-A- and glycine-activated Cl^- current in cultured mouse neurons. *J Pharmacol Exp Ther* 270: 61–69, 1994.

Aistrup GL, Marszalec W, and Narahashi T. Ethanol modulation of nicotinic acetylcholine receptor currents in cultured cortical neurons. *Mol Pharmacol* 55: 39–49, 1999.

Carboni S, Isola R, Gessa GL, and Rossetti ZL. Ethanol prevents the glutamate release induced by *N*-methyl-D-aspartate in the rat striatum. *Neurosci Lett* 152: 133–136, 1993.

Celentano JJ, Gibbs TT, and Farb DH. Ethanol potentiates GABA- and glycine-induced chloride currents in chick spinal cord neurons. *Brain Res* 455: 377–380, 1988.

Cheng G, Gao B-X, Verbny Y, and Ziskind-Conhaim L. Ethanol reduces neuronal excitability and excitatory synaptic transmission in the developing rat spinal cord. *Brain Res* 845: 224–231, 1999.

Crews FT, Morrow AL, Criswell H, and Breese G. Effects of ethanol on ion channels. *Int Rev Neurobiol* 39: 283–367, 1996.

Criswell HE, Simson PE, Duncan GE, McCown TJ, Herbert JS, Morrow AL, and Breese GR. Molecular basis for regionally specific action of ethanol on γ-aminobutyric acid-A receptors: generation of other ligand-gated ion channels. *J Pharmacol Exp Ther* 267: 522–537, 1993.

Dildy-Mayfield JE and Harris RA. Comparison of ethanol sensitivity in rat brain kainate, DL-a-amino-3-hydroxy-5-methyl-4-isoxalone proprionic acid and *N*-methyl-D-aspartate receptors expressed in *Xenopus* ocytes. *J Pharmacol Exp Ther* 252: 487–494, 1992.

Duncan GE, Breese GR, Criswell HE, McCown TJ, Herbert JS, Devaud LL, and Morrow AL. Distribution of [^{3}H]zolpidem binding sites in relation to mRNA encoding the α1, β2 and γ2 subunits of $GABA_A$ receptors in rat brain. *Neuroscience* 64: 1113–1128, 1995.

Eggers ED, O'Brien JA, and Berger AJ. Developmental changes in the modulation of synaptic glycine receptors by ethanol. *J Neurophysiol* 84: 2409–2416, 2000.

Emptage NJ, Reid CA, and Fine A. Calcium stores in hippocampal synaptic boutons mediate short-term plasticity, store-operated Ca^{2+} entry, and spontaneous transmitter release. *Neuron* 29: 197–208, 2001.

Engblom AC and Åkerman KEO. Effects of ethanol on gamma-aminobutyric acid and glycine receptor-coupled $C1^-$ fluxes in rat brain synaptoneurosomes. *J Neurochem* 57: 384–390, 1991.

Engblom AC, Holopainen I, and Åkerman KEO. Ethanol-induced Cl^- flux in rat cerebellar granule cells as measured by a fluorescent probe. *Brain Res* 568: 55–60, 1991.

Faingold CL, N′Gouemo P, and Riaz A. Ethanol and neurotransmitter interactions—from molecular to integrative effects. *Prog Neurobiol* 55: 509–535, 1998.

Fang Z, Gong D, Ionescu P, Laster MJ, Eger EI, and Kendig J. Maturation decreases ethanol minimum alveolar anesthetic concentration (MAC) more than desflurane MAC in rats. *Anesth Analg* 84: 852–858, 1997.

Gao B-X, Cheng G, and Ziskind-Conhaim L. Development of spontaneous synaptic transmission in the rat spinal cord. *J Neurophysiol* 79: 2277–2287, 1998.

Gao B-X, Stricker C, and Ziskind-Conhaim L. Developmental transition from GABAergic to glycinergic synaptic transmission in the rat spinal cord. *J Neurophysiol* 86: 429–502, 2001.

Gao B-X and Ziskind-Conhaim L Development of glycine- and GABA-gated currents in rat spinal motoneurons. *J Neurophysiol* 74: 113–121, 1995.

Gao B-X and Ziskind-Conhaim L. Development of ionic currents underlying changes in action potential waveform in rat spinal motoneurons. *J Neurophysiol* 80: 3047–3061, 1998.

Gao B-X and Ziskind-Conhaim L Ethanol-induced opposite effects on the frequency of spontaneous excitatory and inhibitory synaptic currents in the rat spinal cord. *Soc Neurosci Abstr* 26: 692, 2000.

Gruner W and Silvia LR. Omega-conotoxin sensitivity and presynaptic inhibition of glutamatergic sensory neurotransmission in vitro. *J Neurosci* 14: 2800–2808, 1994.

Gruol DL. Ethanol alters synaptic activity in cultured spinal cord neurons. *Brain Res* 243: 25–33, 1982.

Li Q, Wilson WA, and Swartzwelder HS. 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.

Lima-Landman MTR and Albuquerque EX. Ethanol potentiates and blocks NMDA-activated single-channel currents in rat hippocampal pyramidal cells. *FEBS Lett* 247: 61–67, 1989.

Llano I, Gonzalez J, Caputo C, Lai FA, Blayney LM, Tan YP, and Marty A. Presynaptic calcium stores underlie large-amplitude miniature IPSCs and spontaneous calcium transients. *Nat Neurosci* 3: 1256–1265, 2000.

Lovinger DM. Developmental decrease in ethanol inhibition of *N*-methyl-D-aspartate receptors in rat neocortical neurons: relation to the actions of ifenprodil. *J Pharmacol Exp Ther* 274: 164–172, 1995.

Lovinger DM. Alcohols and neurotransmitter gated ion channels: past, present and future. *Naunyn-Schmiedeberg's Arch Pharmacol* 356: 267–282, 1997.

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

Lovinger DM, White G, and Weight FF. NMDA receptor-mediated synaptic excitation selectively inhibited by ethanol in hippocampal slice from adult rat. *J Neurosci* 10: 1372–1379, 1990.

Marszalec W, Aistrup GL, and Narahashi T. Ethanol modulation of excitatory and inhibitory synaptic interactions in cultured cortical neurons. *Alcohol Clin Exp Res* 22:1524, 1998.

Martin D and Swartzwelder HS. Ethanol inhibits release of excitatory amino acids from slices of hippocampal area CA1. *Eur J Pharmacol* 219: 469–472, 1992.

Mascia MP, Mihic SJ, Valenzuela CF, Schofield PR, and Harris RA. A single amino acid detemines differences in ethanol actions on strychnine-sensitive glycine receptors. *Mol Pharmacol* 50: 402–406, 1996.

Masood K, Wu C, Brauneis U, and Weight FF. Differential ethanol sensitivity of recombinant *N*-methyl-D-aspartate receptor subunits. *Mol Pahrmacol* 45: 324–329, 1994.

Morrisett RA and Swartzwelder HS. Attenuation of hippocampal long-term potentiation by ethanol: a patch-clamp analysis of glutamatergic and GABAergic mechanisms. *J Neurosci* 13: 2264–2272, 1993.

Narahashi T, Kuriyama K, Illes P, Wirkner K, Fischer W, Muhlberg K, Scheibler P, Allgaier C, Minami K, Lovinger D, Lalelmand F, Ward RJ, DeWitte P, Itatsu T, Takei Y, Oide H, Hirose M, Wang XE, Watanabe S, Tateyama M, Ochi R, and Sato N. Neuroreceptors and ion channels as targets of alcohol. *Clin Exp Res* 25: 182S–188S, 2001.

Nestores JN. Ethanol specifically potentiates GABA-mediated neurotransmission in the feline cerebral cortex. *Science* 209: 708–710, 1980.

Peoples RW, Li C, and Weight FF. Lipid VS protein theories of alcohol action in the nervous system. *Annu Rev Pharmacol Toxicol* 36: 185–201, 1996.

Rabe CS and Weight FF. Effects of ethanol on neurotransmitter release and intracellular free calcium in PC12 cells. *J Pharmacol Exp Therap* 244: 417–422, 1988.

Reynolds JN and Prasad A. Ethanol enhances $GABA_A$ receptor-activated chloride currents in chick cerebral cortical neurons. *Brain Res* 564: 138–142, 1991.

Seebach BS and Ziskind-Conhaim L. Formation of transient inappropriate sensorimotor synapses in developing rat spinal cords. *J Neurosci* 14: 4520–4528, 1994.

Stegenga SL and Kalb RG. Developmental regulation of *N*-methyl-D-aspartate and kainate-type glutamate receptor expression in the rat spinal cord. *Neuroscience* 105: 499–507, 2001.

Tabakoff B and Hoffman PL. Ethanol and glutamate receptors. In: Pharmacological effects of ethanol on the nervous system, edited by Deitrich RA and Erwin VG. New York: CRC, 1996, p. 73–94.

Takahashi T, Momiyama A, Hirai K, Hishinuma F, and Akagi H. Functional correlation of fetal and adult forms of glycine receptors with developmental changes in inhibitory synaptic receptor channels. *Neuorn* 9: 1155–1161, 1992.

Varoqueaux F, Sigler A, Rhee J-S, Brose N, Enk C, Reim K, and Rosenmund C. Total arrest of spontaneous and evoked synaptic transmission but normal synaptogenesis in the absence of Munc13-mediated vesicle priming. *Proc Natl Acad Sci USA* 99: 9037–9042, 2002.

Wafford KA, Burnett DM, Leidenheimer NJ, Burt DR, Wang JB, Kofuji P, Dunwiddie TV, Harris RA, and Sikela JM. Ethanol sensitivity of the $GABA_A$ receptor expressed in *Xenopus* oocytes requires 8 amino acids contained in the γ2L subunit. *Neuron* 7: 27–33, 1991.

Wang M-Y, Rampil IJ, and Kendig JJ. Ethanol directly depresses AMPA and NMDA glutamtae currents in spinal cord motor neurons independent of actions on $GABA_A$ or glycine receptors. *J Pharmacol Exp Ther* 290: 362–367, 1999.

Weight FF. Cellualr and molecular physiology of alcohol actions in the nervous system. *Int Rev Neurobiol* 33: 289–348, 1992.

Wirkner K, Eberts C, Poelchen W, Allgaier C, and Illes P. Mechanism of inhibition by ethanol of NMDA and AMPA receptor channel functions in cultured rat cortical neurons. *Naunyn-Schmiedeberg's Arch Pharmacol* 362: 568–576, 2000.

Woodward JJ. Ethanol and NMDA receptor signaling. *Crit Rev Neurobiol* 14: 69–89, 2000.

Wu W-L, Ziskind-Conhaim L, and Sweet M. Early development of glycine- and GABA-mediated synapses in rat spinal cord. *J Neurosci* 12: 3935–3945, 1992.

Xie HW and Ziskind-Conhaim L. Blocking Ca^{2+}-dependent synaptic release delays motoneuron differentiation in the rat spinal cord. *J Neurosci* 15: 5900–5911, 1995.

Yang X, Criswell HE, Simson PMSS, and Breese GR. Evidence for a selective effect of ethanol on NMDA responses: ethanol affects a subtype of the ifenprodil-sensitive NMDA receptor. *J Pharmacol Exp Ther* 278: 114–124, 1996.

Ziskind-Conhaim L. NMDA receptors mediate poly- and monosynaptic potentials in motoneurons of rat embryos. *J Neurosci* 10: 125–135, 1990.