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Developmental Changes in the Acute Ethanol Sensitivity of Glutamatergic and GABAergic Transmission in the BNST

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Abstract

Glutamatergic and GABAergic transmission undergo significant changes during adolescence. Receptors for both of these transmitters (N-methyl-D-aspartate, NMDAR, and GABA$_A$) are known to be key targets for the acute effects of ethanol in adults. The current study set out to investigate the acute effects of ethanol on both NMDAR-mediated excitatory transmission and GABAergic inhibitory transmission within the bed nucleus of the stria terminalis (BNST) across age. The BNST is an area of the brain implicated in the negative reinforcing properties associated with alcohol dependence, and the BNST plays a critical role in stress-induced relapse. Therefore, assessing the developmental regulation of ethanol sensitivity in this key brain region is important to understanding the progression of ethanol dependence. To do this, whole-cell recordings of isolated NMDAR-evoked excitatory postsynaptic currents (eEPSCs) or evoked GABAergic inhibitory postsynaptic currents (eIPSCs) were performed on BNST neurons in slices from 4- or 8-week-old male C57BL/6J mice. Ethanol (50 m$_M$) produced greater inhibition of NMDAR-eEPSCs in adolescent mice than in adult mice. This enhanced sensitivity in adolescence was not a result of shifts in function of the B subunit of NMDARs (GluN2B), measured by Ro25-6981 inhibition and decay kinetics measured across age. Adolescent mice also exhibited greater ethanol sensitivity of GABAergic transmission, as ethanol (50 m$_M$) enhanced eIPSCs in the BNST of adolescent but not adult mice. Collectively, this work illustrates that a moderate dose of ethanol produces greater inhibition of transmission in the BNST (through greater excitatory inhibition and enhancement of inhibitory transmission) in adolescents compared to adults. Given the role of the BNST in alcohol dependence, these developmental changes in acute ethanol sensitivity could accelerate neuroadaptations that result from chronic ethanol use during the critical period of adolescence.

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Keywords

adolescent; NMDA receptor; bed nucleus of the stria terminalis (BNST); GABA; GluN2B

Introduction

Adolescence is known to be a time of increased risk-taking behaviors, including alcohol use. This alcohol use is not only experimental but also consists of high levels of binge drinking (5 or more drinks in one occasion; Rohde et al., 1996). This adolescent alcohol exposure is one of the strongest predictors for future alcohol dependence (Grant, 1998) and produces a more rapid dependency course (time between initial use and dependence; Clark et al., 1998). Therefore, identifying the neurobiological underpinnings of these age-related differences is important to understanding the development of alcohol dependence and understanding how treatments might be able to intercede in this process.

Numerous studies using rodent models have shown that adolescent and adult behavioral responses to acute ethanol are distinct. For example, adolescents have lower sensitivity to the sedative (Little et al., 1996; Silveri & Spear, 1998), hypothermic (Silveri & Spear, 2000), conditioned taste aversion (Anderson et al., 2010; Holstein et al., 2011; Schramm-Sapyta et al., 2010), and motor impairing (White et al., 2002) effects of alcohol, but greater sensitivity to ethanol-induced memory impairment and social facilitation (Markwiese et al., 1998; Varlinskaya & Spear, 2002, 2006).

The key to these age-related effects likely arises from maturational changes in the brain that occur during adolescence. The transition from adolescence to adulthood is accompanied by a peak in grey matter volume that declines into adulthood (Giedd, 2004; Giedd et al., 1999). This reduction is in part a result of synaptic pruning of glutamatergic synapses, particularly in the cortex and hippocampus (Andersen & Teicher, 2004; Andersen et al., 2000; Giedd et al., 1999; Huttenlocher, 1984; Insel et al., 1990; Zecevic et al., 1989). Specifically in these regions, there is a substantial decline in glutamatergic NMDA receptors (NMDARs; Insel et al., 1990) and a shift in NMDAR subunit composition, with enhancement of GluN2A over GluN2B (Quinlan et al., 1999; Sheng et al., 1994; Sobczyk et al., 2005; Williams et al., 1993; Yashiro & Philpot, 2008). While this subunit shift is prevalent in the cortex, hippocampus, and lateral amygdala, the central nucleus of the amygdala (CeA) seems resistant and retains significant GluN2B levels into adulthood (Lopez de Armentia & Sah, 2003). These data illustrate regional differences in the developmental maturation of glutamatergic synapses and highlight the need for refined characterization of these changes in ethanol-sensitive brain regions (e.g. the BNST). These changes in excitatory transmission occur in conjunction with an overall increase in GABAergic tone resulting from a developmental increase in GABA$_A$ receptors in numerous brain regions (Behringer et al., 1996; Fleming et al., 2007; Moy et al., 1998; Xia and Haddad, 1992). These developmental changes in both GABAergic and glutamatergic transmission are thus candidates to underlie age-related differences in the behavioral effects of alcohol.

One of the primary effects of acute ethanol exposure is inhibition of NMDAR-mediated transmission (Dildy & Leslie, 1989; Hoffman et al., 1989; Lovinger et al., 1989). In the hippocampus and posterior cingulate cortex of adolescents, this inhibition of NMDAR transmission by acute ethanol was more robust than in their adult counterparts (Li et al., 2002; Swartzwelder et al., 1995b). Further, long-term potentiation (LTP), a form of NMDAR-dependent hippocampal plasticity, showed enhanced disruption by ethanol exposure in adolescents (Pyapali et al., 1999; Swartzwelder et al., 1995a). This enhanced

effect of acute ethanol on NMDAR transmission and LTP in adolescents is likely a result of developmental changes in NMDAR levels and subunit composition.

Another system vulnerable to ethanol in adults is GABAergic transmission. Similar to the ethanol sensitivity of NMDARs, much of the research on the sensitivity of GABAergic transmission to alcohol has been performed in the hippocampus. In this region, $GABA_A$-mediated evoked inhibitory post-synaptic currents (eIPSCs) had greater ethanol-induced enhancement in adults compared to adolescents (Li et al., 2003). This age-related difference was produced by greater ethanol-induced augmentation of I_h current in adults, which results in an enhancement of interneuron excitability (Li et al., 2006; Yan et al., 2010). Conversely, other interneurons in the hippocampus were shown to have greater enhancement of inhibitory tonic current by ethanol in adolescents compared to adults (Yan et al., 2010). This age-related difference in response of tonic GABAergic current to ethanol was later found to be at least partially mediated through developmental changes in GABA transporter (Fleming et al., 2011). These disparate findings in hippocampal neurons, with adults being more ethanol sensitive in one instance and less in another, were found to be restricted to different subclasses of GABAergic interneurons, thus highlighting the complexity of ethanol effects.

In the current study, we set out to investigate the age-dependent effects of ethanol on both NMDAR-mediated excitatory transmission and GABAergic inhibitory transmission within the bed nucleus of the stria terminalis (BNST). The BNST is an area of the brain associated with the negative reinforcing properties of drug/alcohol dependence, and has been shown in numerous studies to be critical for expression of stress-induced reinstatement of drug-seeking behavior (Briand et al., 2010; Buffalari & See, 2011; Erb et al., 2001; Erb & Stewart, 1999; Leri et al., 2002; Wang et al., 2006). In the adult BNST, the acute effects of ethanol are dependent on the GluN2B subunit of the NMDAR (Wills et al., 2012). However, the developmental regulation of this subunit and the effects of ethanol in this region have not been evaluated. Therefore, determining the developmental regulation of ethanol sensitivity in this brain region is important to understanding the progression of ethanol dependence.

Materials and methods

Subjects

Male C57BL/6J (4 weeks old, postnatal days 28–34; or 8 weeks old, postnatal days 56–62; Jackson Laboratories, Bar Harbor, ME) were housed in groups of five. Food and water were available *ad libitum*. All procedures were approved by the Animal Care and Use Committee at Vanderbilt University.

Procedures

Slice preparation—Mice were transported from the animal colony to the laboratory and placed in sound-attenuated cubicles for 1 hour. They were then decapitated under isoflurane anesthesia. The brains were quickly removed and placed in ice-cold sucrose artificial cerebrospinal fluid (ACSF) (in m$_M$: 194 sucrose, 20 NaCl, 4.4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 1.2 NaH_2PO_4, 10.0 glucose, and 26.0 $NaHCO_3$), saturated with 95% O_2/5% CO_2. Slices 300 μm thick were prepared using a Tissue Slicer (Leica). Slices containing ventral BNST (vBNST; bregma, 0.26–0.02 mm) were selected using the internal capsule, anterior commissure, and stria terminalis as landmarks.

Whole-cell recordings—After dissection, slices were transferred to a holding chamber containing heated (~29 °C), oxygenated (95% O_2/5% CO_2) ACSF (in m$_M$: 124 NaCl, 4.4 KCl, 2 $CaCl_2$, 1.2 $MgSO_4$, 1 NaH_2PO_4, 10.0 glucose, and 26.0 $NaHCO_3$; pH 7.2–7.4; 290–310 mOsm). Recording electrodes (3–6 M) were pulled on a Flaming-Brown Micropipette

Puller (Sutter Instruments) using thin-walled borosilicate glass capillaries. Excitatory postsynaptic currents (eEPSCs) were evoked by local fiber stimulation with bipolar nichrome electrodes. Electrical stimulation (5–25 V with a 100–150 µsec duration) was applied at 0.0167 Hz. An electrical stimulation intensity that evoked a 100–300 pA NMDAR-EPSC was used in these experiments. Recording electrodes were filled with a solution containing in m$_M$): 117 Cs^+-gluconate, 20 HEPES, 0.4 EGTA, 5 TEA, 2 $MgCl_2$, 4 ATP, 0.3 GTP, pH= 7; 285–290 mOsm). NMDAR EPSCs were isolated by adding 25 µM picrotoxin and 10 µM NBQX, and recording at a holding potential of +40 mV in normal ACSF at a fluid exchange rate of 2 mL/min. Inhibitory post-synaptic current (eIPSC) recordings were isolated by applying kynurenic acid (3 m$_M$) and recording at a holding potential of −70 mV. Signals were acquired via a Multiclamp 700B amplifier (Axon Instruments), and digitized and analyzed via pClamp 10.2 software (Axon Instruments). Input resistance, holding current, and series resistance were all monitored continuously throughout the duration of experiments. Experiments in which changes in series resistance were greater than 20% were not included in the data analysis. Experiments were analyzed by measuring peak amplitude of the synaptic response, which was then normalized to the baseline period.

Weighted tau ( ) was calculated by fitting NMDA-eEPSC decays using Clampfit 10.2 (Axon Instruments) from averaged traces obtained 5 min prior to drug (50 m$_M$ ethanol) application and 5 min after washout (minimum 10–15 minutes of washout). The decay phase of currents was fitted using a simplex algorithm for least-squares exponential-fitting routine with a double exponential equation, where Ix is the peak current amplitude and x is the corresponding decay time constant. To allow for easier comparison of decay times between experimental conditions, the 2 decay time components were combined into a weighted time constant.

Drug Treatments

Stable baselines of at least 5 min were recorded before drug application: 10 min for ethanol (10, 25, or 50 m$_M$) and 10 min for Ro25-9681 hydrochloride (Ascent Scientific; 2 µM). NBQX hydrochloride (Ascent Scientific), picrotoxin (Tocris Bioscience), and kynurenic acid (Sigma-Aldrich), when used, were applied for the duration of the experiment.

Statistical Analysis

Analyses of the effects of Ro25-6981 and 50 m$_M$ ethanol on NMDAR-EPSCs were performed with unpaired *t* tests. Analyses of the effects of 50 m$_M$ ethanol on IPSCs were performed with an unpaired *t* test using a Welch correction due to unequal variance between groups. A 1-way ANOVA was performed on the ethanol dose response on NMDAR-EPSCs in 4-week-old pups. All analyses were made by calculating the percent change from baseline (averaged 5 min before drug application) to peak drug effect (first 5 min of washout). This peak drug effect occurs during the washout phase because it takes 6–8 minutes for solutions to equilibrate to a steady state concentration in the slice chamber.

The *N* for these data analyses is a reflection of the number of slices used per group. These slices were collected from at least 4 mice per group in all cases. The specific *n* for each of the treatment groups were as follows. Four-week-old mice, NMDA EPSCs: 10 m$_M$ ethanol (*n* = 4); 25 m$_M$ ethanol (*n* = 4); 50 m$_M$ ethanol (*n* = 7); Ro25-6981 (*n* = 6). Four-week-old mice, IPSCs: 50 m$_M$ ethanol (*n* = 7). Eight-week-old mice, NMDA EPSCs: 50 m$_M$ ethanol (*n* = 7); Ro25-6981 (*n* = 6). Eight-week-old mice, IPSCs: 50 m$_M$ ethanol (*n* = 5).

Results

Effects of acute ethanol on NMDAR transmission in the BNST

Acute ethanol application produces a dose-dependent inhibition of NMDAR-EPSC amplitude in vBNST neurons of adult C57BL/6J male mice (Kash et al., 2008). To determine potential age-related differences in acute ethanol sensitivity within the vBNST, an intermediate ethanol dose (50 m$_M$) was chosen from these previous findings in adult mice (Kash et al., 2008). Whole-cell recordings were made from neurons in the vBNST in coronal brain slices from 4- or 8-week-old male C57BL/6J mice. We selected smaller cell somas with large input resistance, as these characteristics have been previously ascribed to projection neurons (Dumont & Williams, 2004; Kash et al., 2008). NMDAR-EPSCs were generated by local afferent stimulation at a holding potential of +40 mV in the presence of picrotoxin and NBQX. Basal peak amplitude of NMDAR-EPSCs was not significantly different between 4- and 8-week-old mice (t [13] = 0.6443; p = N.S.; 8-week-old mice = 164.5 pA ± 35.57; 4-week-old mice = 133.1 pA ± 26). Ethanol (50 m$_M$) produced an inhibition of NMDAR-EPSC peak amplitude in 8-week-old mice, as was previously shown (Kash et al., 2008). This same inhibition of peak amplitude, however, was larger in 4-week-old mice (t[17] = 3.849; p < 0.005; Figs. 1A & C). This age-related difference was also found in the inhibition of NMDAR-EPSC area (t[17] = 2.152; p < 0.05; Figs. 1D & E). These age-related differences in NMDAR-EPSCs were also apparent in representative traces from 4- and 8-week-old mice before and after ethanol application (Fig. 1B). Dose-response experiments in 4-week-old mice revealed a significant effect of ethanol dose (10, 25, or 50 m$_M$) on NMDAR-EPSC peak ($F_{(2,21)}$ = 4.757; p = 0.021; Fig. 2A,B) but not on NMDAR-EPSC area ($F_{(2,21)}$ = 1.637; p = N.S.; Fig. 2A,C). In NMDAR-EPSC peaks, the percent of baseline values for 10 m$_M$ ethanol and 50 m$_M$ ethanol were significantly different, with 10 m$_M$ ethanol producing no appreciable effect. Collectively, these measurements demonstrate that ethanol inhibition at NMDARs is more robust in 4-week-old compared to 8-week-old mice.

An age-related difference in ethanol sensitivity could reflect developmental regulation of NMDAR subunits. Previous work has illustrated that ethanol sensitivity in the BNST is dependent on the GluN2B subunit (Wills et al., 2012), and expression of this subunit is known to be developmentally regulated in other brain regions. Since subunit composition is a determinant of the decay kinetics of NMDAR currents, weighted Tau ( ) measurements were taken from NMDAR-isolated eEPSCs before and after ethanol inhibition in 4- and 8-week-old mice. There was no age-related difference in before (t[17] = 1.002; p = N.S.; 8-week-old mice = 130.6 ± 14.23; 4-week-old mice = 106.4 ± 14.18) or after ethanol application (t[17] = 0.194; p = N.S.; 8-week-old mice = 102.7 ± 14.25; 4-week-old mice = 108.6 ± 25.59). A secondary assessment of subunit composition was performed evaluating the amount of inhibition by Ro25-6981 (2 μM; selective GluN2B antagonist) on NMDAR-EPSCs in 4- and 8-week-old mice. There were no differences in the magnitude of Ro25-6981 inhibition on NMDAR-EPSC peak amplitude (t[14] = 1.477; p = N.S.; Fig. 3A) or NMDAR-EPSC area between ages (t[14] = 0.1597; p = N.S.; Fig. 3B). These findings suggest that GluN2B composition may be similar between 4- and 8-week-old mice in the vBNST, despite age-related differences in ethanol sensitivity.

Effects of acute ethanol on GABA transmission in the BNST

In conjunction with this excitatory transmission, there is also substantial inhibitory transmission in the vBNST. Previous work in adult mice found that 100 m$_M$ acute ethanol had no effect on IPSCs in the BNST, but this concentration was more than sufficient to inhibit NMDAR-EPSCs (Weitlauf et al., 2004). In the current experiments, the effect of 50 m$_M$ ethanol (shown to produce age-related differences on NMDAR transmission) on IPSCs was evaluated in neurons within the vBNST. Ethanol (50 m$_M$) produced no significant age-

related difference in eIPSC peak amplitude (t[11] = 1.343; p = N.S.; Figs. 4A & C), although there was a trend for ethanol-induced enhancement of eIPSCs in 4-week-old mice. However, when eIPSC area was evaluated, there was an age-related difference in ethanol sensitivity (t[12] = 2.213; $p < 0.05$; Figs. 4D & E). This difference was a consequence of ethanol-induced enhancement of eIPSCs in 4-week-old mice with no ethanol-induced change in eIPSCs in 8-week-old mice (see representative traces before and after 50 m$_M$ ethanol in Fig. 4B).

Discussion

These studies demonstrate clear developmental differences in the effects of ethanol on NMDAR-mediated excitatory transmission and GABAergic inhibitory transmission. Adolescent mice were more sensitive to ethanol-mediated inhibition of NMDAR transmission and more sensitive to ethanol-mediated enhancement of inhibitory transmission. The culmination of these effects illustrates that ethanol produces greater inhibition of neural transmission in the BNST in 4-week-old mice than the same ethanol concentration in 8-week-old mice. Further, it is important to note that, while the ethanol concentrations used in these studies are somewhat high (translates into approximately 230 mg%), they are well within the range of blood ethanol concentrations (BECs) achieved by drinking adolescents. One of the potential reasons for the greater preponderance of these higher BECs among adolescents is that they are less susceptible to sedative properties of ethanol than adults are (Little et al., 1996; Silveri & Spear, 1998). Additionally, lower concentrations (25 m$_M$) were also effective in inhibiting NMDAR-EPCSs in adolescents, as has been previously demonstrated in adults (Kash et al., 2008).

Age-related differences in NMDAR ethanol sensitivity have been found in other brain regions (Li et al., 2002; Swartzwelder et al., 1995b), with adolescents displaying enhanced ethanol-induced inhibition on NMDARs. A number of factors could contribute to these age-related differences in ethanol sensitivity: subunit composition, co-agonist activation, Mg blockade, phosphorylation states, intracellular signaling cascades, etc. Any of these factors could account for the disparate actions of ethanol on NMDARs across age.

Developmental changes in NMDAR subunit composition are one possible explanation for age-dependent differences in ethanol sensitivity. As was discussed above, there is an overall decline in NMDARs and a shift in subunit composition from predominantly GluN2B to GluN2A in many regions (e.g. hippocampus and cortex). GluN2A and GluN2B (with the obligate GluN1 subunit) are the primary NMDAR subunits expressed in the forebrain and confer many of the receptor properties. NMDAR composition (especially GluN2B vs. GluN2A) confers many of the receptor properties, such as decay time, localization, signaling, and conductance. Further, previous work in our laboratory has shown that the acute inhibitory effects of acute ethanol on the NMDAR are GluN2B-dependent (Wills et al., 2012). We assessed the developmental shift in subunit composition by evaluating the amount of Ro25-6981 (GluN2B antagonist) inhibition and basal age-related differences in decay kinetics (with T). Our results found no age-related difference in inhibition by Ro25-6981 or basal decay kinetics. These results suggest that the BNST does not undergo the same developmental shift to GluN2A-containing NMDARs found in many brain regions (hippocampus, cortex, striatum, lateral amygdala, and cerebellum). This lack of a developmental shift has also been found in the CeA (Lopez de Armentia & Sah, 2003). While our data suggest that a large-scale shift from GluN2B to GluN2A does not occur in the BNST, it is possible that changes in subunit composition between NMDARs containing 2 subunits types (e.g., two GluN2B-two GluN1) and those containing 3 subunit types (e.g., GluN2A-GluN2B-two GluN1) NMDARs might occur developmentally. Ro inhibition may be unable to detect such developmental shifts since the efficacy of this antagonist on

heterotrimeric NMDARs is not well understood. Another possibility is that subunit composition is conserved across these developmental stages and that ethanol-related differences are a response of divergent signaling, total NMDAR number, or changes in receptor phosphorylation. Several studies have found phosphorylation sites on GluN1 (expressed with GluN2A) that alter acute ethanol sensitivity (Honse et al., 2004; Ren et al., 2003; Ren et al., 2007; Ronald et al., 2001; Smothers & Woodward, 2006). Further, various kinases (e.g. H-Ras, Syc, Fyn, STEP, DARPP-32) are known to alter the phosphorylation/ dephosphorylation of the NMDAR subunit and in turn influence NMDAR sensitivity to ethanol (Alvestad et al., 2003; Hardy et al., 1999; Hicklin et al., 2011; Maldve et al., 2002; Suvarna et al., 2005; Yaka et al., 2003a, 2003b). Therefore, developmental changes in NMDAR phosphorylation states and kinase activity could also account for these age-related effects in the BNST and will need to be investigated in future studies.

In conjunction with these age-related changes in NMDAR-mediated transmission, ethanol sensitivity differences were also found for inhibitory transmission in the BNST. In these studies, there was an enhancement of IPSC by acute ethanol (50 m$_M$) in slices from adolescent mice, whereas no effect of ethanol was seen in their adult counterparts. These changes in ethanol sensitivity are potentially a consequence of developmental changes in GABA receptor expression and/or subunit composition. GABA$_A$ has been shown to steadily increase in expression into adulthood (Behringer et al., 1996; Xia & Haddad, 1992). However, certain brain regions show distinct rates of GABA$_A$ receptor expression (measured by zolpidem binding) during adolescence (Moy et al., 1998). These overall GABA$_A$ changes are also accompanied with developmental shifts in subunit composition with increases in the subunit, and general decline with age in 1, 3, and 5 subunits (Laurie et al., 1992; Yu et al., 2006). Developmental ethanol sensitivity has been most extensively characterized in the hippocampus where work has shown adolescents to be either more or less sensitive to the effects of acute ethanol depending on the type of GABAergic transmission being evaluated (discussed above). Our current work did not isolate specific GABA subunits so it is unclear if the acute ethanol mechanisms at work in the BNST are similar to those seen in the hippocampus. Another potential mechanism for enhanced GABA transmission by acute ethanol comes from work in the adult CeA, another subregion of the extended amygdala. In this region, enhancement of GABAergic transmission by acute ethanol occurs via enhancement of presynaptic GABA release via CRF receptor signaling through PKC (Bajo et al., 2008; Roberto et al., 2004). Further studies will be necessary to determine the precise mechanisms underlying these developmental changes in ethanol sensitivity in the BNST.

In sum, these developmental changes in ethanol sensitivity demonstrate that ethanol exposure during adolescence produces a larger inhibition of transmission (through greater excitatory inhibition and enhancement of inhibitory transmission) in the BNST than the same amount of ethanol in adults. This suggests that lower amounts of ethanol would be required to affect BNST-related behaviors and neuroadaptions that occur during chronic ethanol use. Given the role of the BNST in negative effect and relapse, it is likely that this increased sensitivity to ethanol in the BNST during adolescence could accelerate the progression of ethanol dependence. While these studies provide intriguing initial findings about developmental differences in the BNST, future studies are needed to evaluate later adolescent periods, more chronic ethanol treatments, and the mechanisms for these changes in ethanol sensitivity.

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Zecevic N, Bourgeois JP, Rakic P. Changes in synaptic density in motor cortex of rhesus monkey during fetal and postnatal life. Brain Research. Developmental Brain Research. 1989; 50:11–32. [PubMed: 2582602]

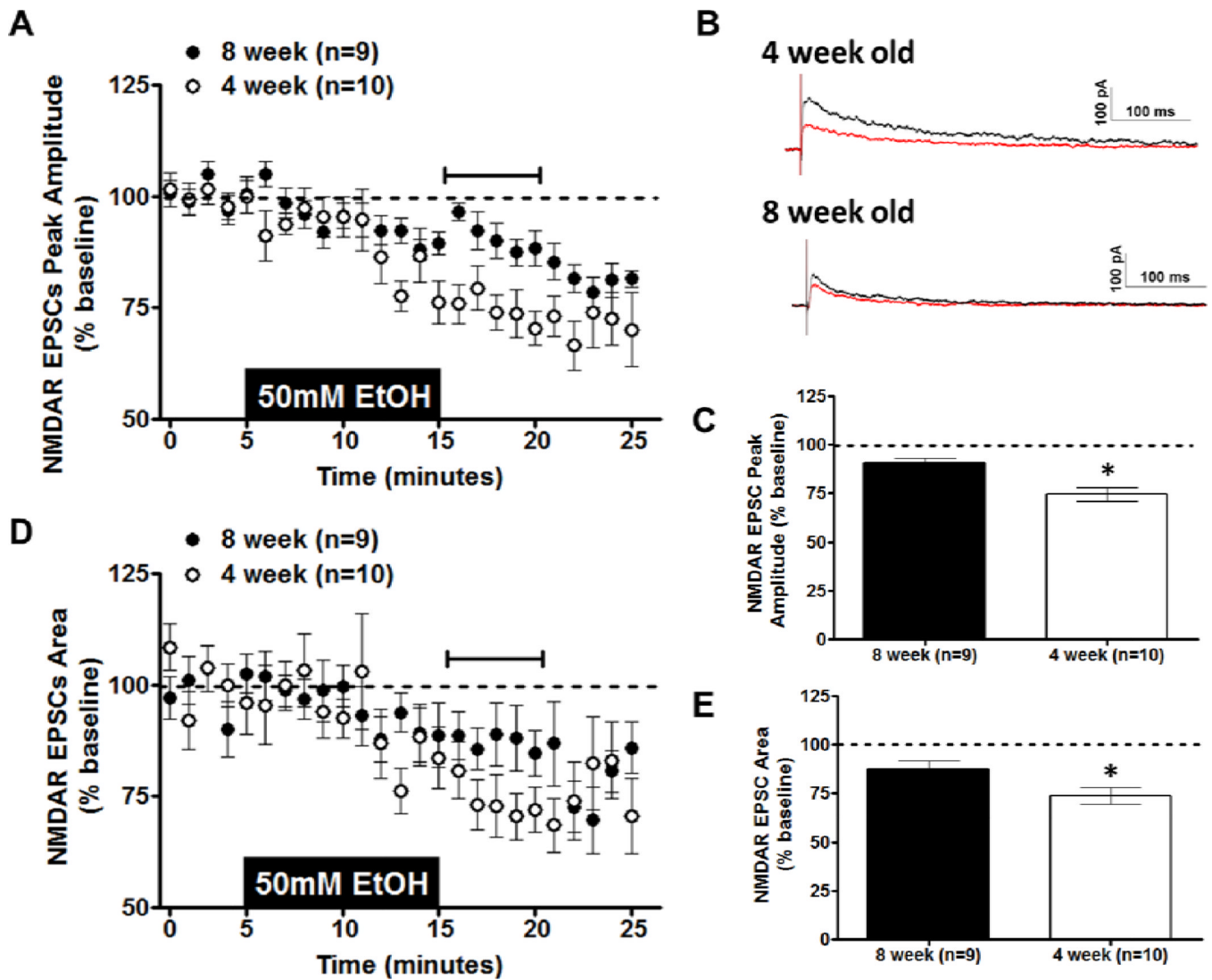**Figure 1. Effects of Acute Ethanol on NMDAR transmission in the BNST**

Acute ethanol (50 m$_M$; 10 min) was applied to vBNST slices from 4- and 8-week-old C57BL/6J mice; evoked NMDA-receptor isolated EPSCs were then recorded. A) Time course of NMDAR-EPSC peak amplitude in 4- and 8-week-old mice. B) Representative traces of NMDAR-EPSCs before (black trace) and after (red trace) removal of ethanol in 4- and 8-week-old mice. C) Averaged peak amplitude of NMDAR-EPSC for the first 5 min after ethanol exposure in 4- and 8-week-old mice. D) Time course of NMDAREPSC area in 4- and 8-week-old mice. E) Averaged area of NMDAR-EPSC for the first 5 min after ethanol exposure in 4- and 8-week-old mice. *$p < 0.05$
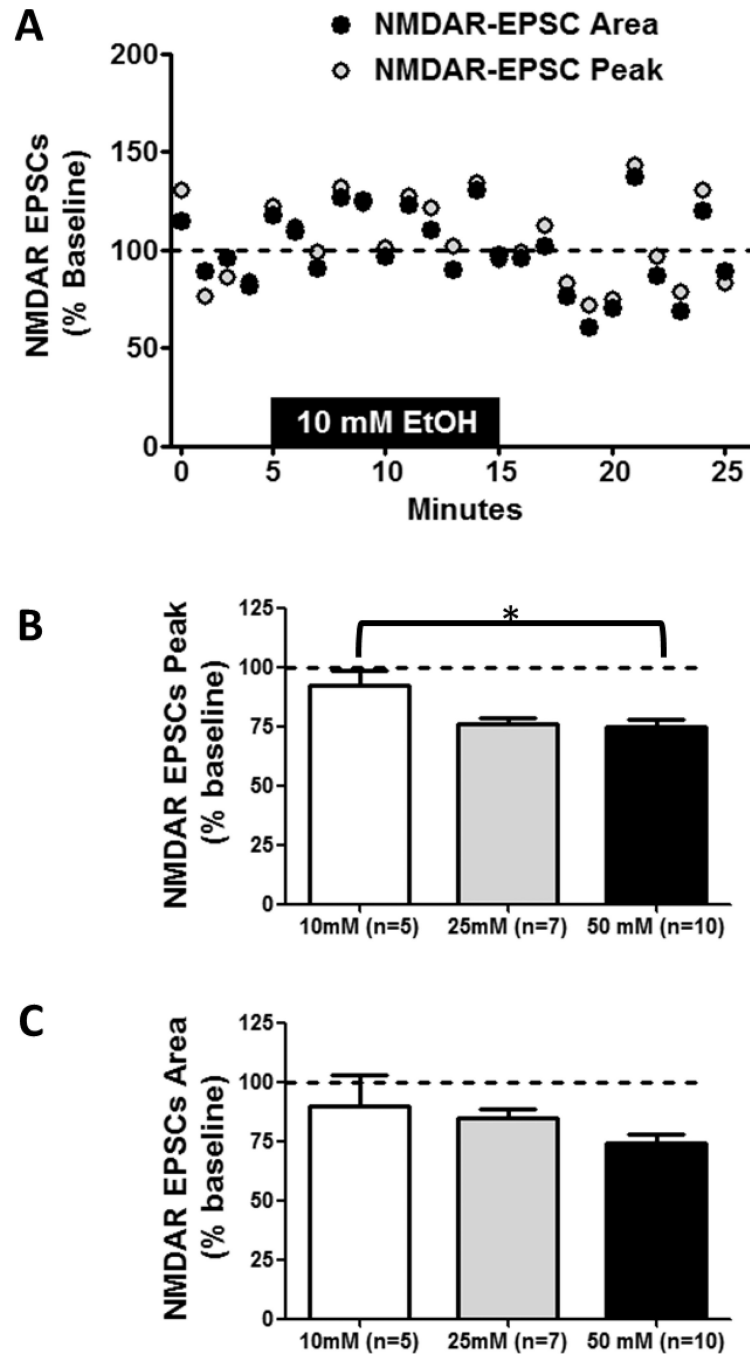
Figure 2. Dose Response of Acute Ethanol on NMDAR transmission in 4-week-old mice
Acute ethanol (10, 25, or 50 m$_M$; 10 min) was applied to vBNST slices from 4-week-old
C57BL/6J mice; evoked NMDA receptor isolated EPCSs were then recorded. A)
Representative time course of 10 m$_M$ ethanol exposure on NMDAR-EPSC peak and area. B)
Averaged peak amplitude of NMDAR-EPSC for the first 5 min after ethanol exposure in 4-
week-old mice for each concentration. C) Averaged area of NMDAR-EPSC for the first 5
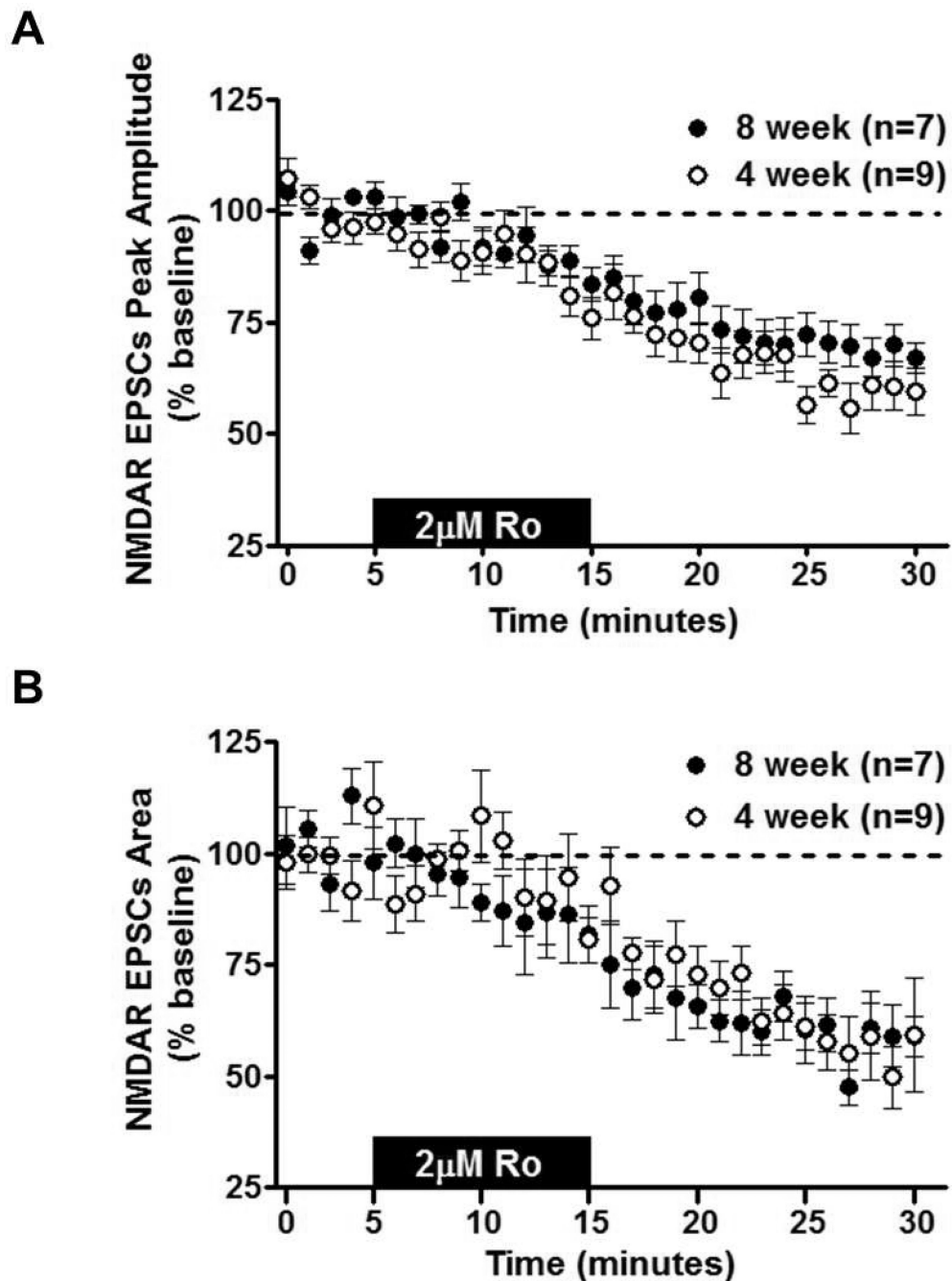min after ethanol exposure in 4-week-old mice for each concentration. *$p < 0.05$

Figure 3. Effects of GluN2B Antagonist on NMDAR transmission in the BNST
Ro25-6981 (2 µM; 10 min) was applied to vBNST slices from 4- and 8-week-old C57BL/6J mice; evoked NMDA-receptor isolated EPSCs were then recorded. A) Time course of NMDAR-EPSC peak amplitude in 4- and 8-week-old mice. B) Time course of NMDAR-EPSC area in 4- and 8-week-old mice.
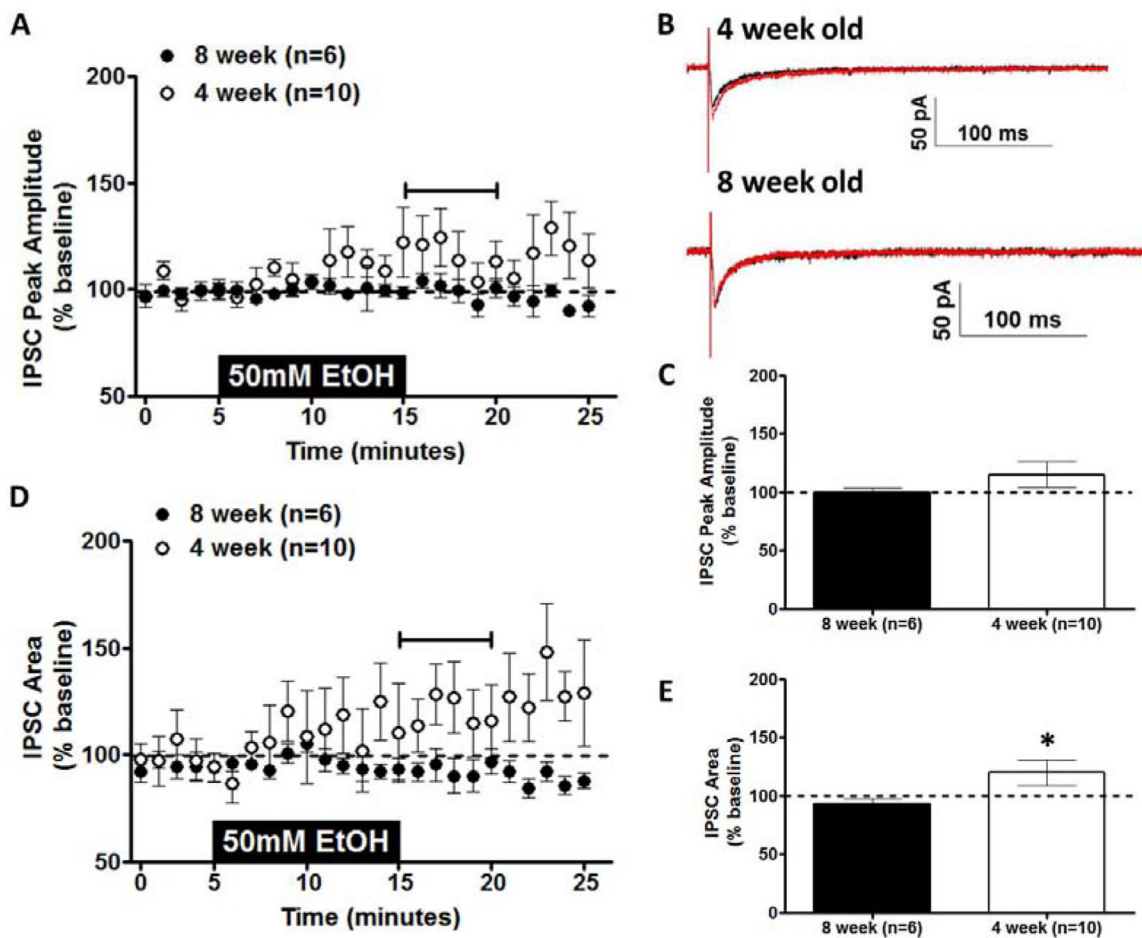
Figure 4. Effects of Acute Ethanol on GABAergic transmission in the BNST
Acute ethanol (50 mM; 10 min) was applied to vBNST slices from 4- and 8-week-old
C57BL/6J mice; evoked IPSCs were then recorded. A) Time course of IPSCs peak
amplitude in 4- and 8-week-old mice. B) Representative traces of IPSCs before (black trace)
and after (red trace) removal of ethanol in 4- and 8-week-old mice. C) Averaged peak
amplitude of IPSCs for the first 5 min after ethanol exposure in 4- and 8-week-old mice. D)
Time course of IPSCs area in 4- and 8-week-old mice. E) Averaged area of IPSCs for the
first 5 min after ethanol exposure in 4- and 8-week-old mice. $*p < 0.05$. Horizontal bars in A
and D indicate time over which the ethanol effect was calculated.