Ethanol Effects on *N*-Methyl-D-Aspartate Receptors in the Bed Nucleus of the Stria Terminalis

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The extended amygdala is a series of interconnected, embryologically similar series of nuclei in the brain that are thought to play key roles in aspects of alcohol dependence, specifically in stress-induced increases in alcohol-seeking behaviors. Plasticity of excitatory transmission in these and other brain regions is currently an intense area of scrutiny as a mechanism underlying aspects of addiction. *N*-methyl-D-aspartate (NMDA) receptors (NMDARs) play a critical role in plasticity at excitatory synapses and have been identified as major molecular targets of ethanol. Thus, this article will explore alcohol and NMDAR interactions first at a general level and then focusing within the extended amygdala, in particular on the bed nucleus of the stria terminalis (BNST).

During the transition to ethanol dependence, a shift is thought to occur in the motivational drive for ethanol use. The beginning stages of ethanol use are thought to be driven primarily by the positive reinforcing effects of ethanol (pleasurable effects from ethanol). However, as addiction progresses, it is thought that the negative reinforcing properties of the ethanol (withdrawal/negative affect) are a major motivational force behind compulsive ethanol intake (Koob and Le Moal 1997). This increased negative affect (depression and anxiety) that accompanies ethanol withdrawal (De Soto et al. 1985; Roelofs 1985) is

cited as an important contributor to relapse in alcoholics and is known to persist long after the outwardly physical symptoms of withdrawal have dissipated (Hershon 1977; Begleiter and Porjesz 1979; Roelofs 1985; Grant et al. 1987). One factor that is likely to contribute to the manifestation of negative affect during withdrawal is stress. Both clinical and animal studies have illustrated that stress during withdrawal increases the risk of relapse in dependent subjects (Le et al. 2000; Sinha 2001). The extended amygdala is an area of the brain thought to be a key player in these withdrawal-related behaviors.

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The extended amygdala is a series of interconnected, embryologically similar nuclei in the brain that are thought to play key roles in aspects of alcohol dependence, specifically in stress-induced increases in alcohol-seeking behaviors (reviewed in McCool 2011 and Wills et al. 2012). Plasticity of excitatory transmission in these and other brain regions is currently an intense area of scrutiny as a mechanism underlying aspects of addiction. NMDA receptors (NMDARs) play a critical role in plasticity at excitatory synapses (de Marchena et al. 2008), and have been identified as major molecular targets of ethanol. Thus, this article will explore alcohol and NMDAR interactions first at a general level, and then focusing within the extended amygdala, in particular on the bed nucleus of the stria terminalis (BNST).

ACUTE ETHANOL EFFECTS ON NMDARs

Seminal studies by several laboratories have shown that physiologically relevant ethanol concentrations (25–100 mM) potently inhibit NMDARs (Dildy and Leslie 1989; Hoffman et al. 1989; Lovinger et al. 1989) and modulate NMDAR plasticity in several brain regions (Blitzer et al. 1990; Morrisett and Swartzwelder 1993; Givens and McMahon 1995; Grover and Frye 1996; Schummers and Browning 2001; Hendricson et al. 2002; Weitlauf et al. 2004; Izumi et al. 2005). The NMDAR is a heterotetrameric complex composed of two obligatory GluN1 subunits and two GluN2 or GluN3 subunits. Numerous subunit combinations are possible with eight different variations of GluN1 subunit and four distinct GluN2 subunit isoforms (A, B, C, and D). However, the NMDARs primarily expressed in the adult forebrain are composed of GluN2A and/or GluN2B subunits. A large body of work has been conducted to determine ethanol's actions on specific subunits of the NMDAR complex.

GluN1

The GluN1 subunit is an obligate subunit of the NMDAR and is, therefore, a likely target for the inhibitory effects of ethanol. There are eight

possible variations of the GluN1 subunit produced through alternative splicing (four sites on the carboxyl terminus and two sites on the amino terminus). This carboxyl terminus of the GluN1 subunit is composed of four cassettes (C0, C1, C2, and C2) but the C0 cassette is conserved within all splice variants. Removal of this C0 cassette decreases ethanol inhibition of NMDARs (Anders et al. 2000). The C0 cassette was also found to be critical for the Ca^{2+} -sensitive modulation of ethanol inhibition on NMDARs (Mirshahi and Woodward 1995; Mirshahi et al. 1998). Interestingly, the ethanol sensitivity of this C0 cassette was only found in conjunction with the GluN2A subunit (Mirshahi et al. 1998; Anders et al. 2000). Evidence also suggests that several phosphorylation sites in the C1 cassette region can modulate ethanol sensitivity as well, but again these effects are selective for GluN2A-containing NMDARs (Xu et al. 2011). Further, sites within the transmembrane regions (TM) of the GluN1 and GluN2A subunits have been investigated for the effects of ethanol. Several mutations in the GluN1 subunit (TM3 F637, F639; TM4 M813; TM4 L819) and GluN2A subunit (TM4 M832) have been found to alter ethanol sensitivity (Ronald et al. 2001; Ren et al. 2003; Honse et al. 2004; Smothers and Woodward 2006; Ren et al. 2007).

GluN2

As shown above, ethanol sensitivity is partially regulated by GluN1, but this sensitivity was only seen in conjunction with the GluN2A subunit suggesting subunit combinations are critical (Mirshahi et al. 1998; Anders et al. 2000; Xu et al. 2011). The GluN2 subunit has four isoforms (A, B, C, and D) but the NMDARs primarily expressed in the adult forebrain are composed of GluN2A and/or GluN2B subunits. These GluN2 subunits dictate many channel properties such as decay time, localization, intracellular signaling, and conductance (Cull-Candy et al. 2001; Traynelis et al. 2010).

Investigations on GluN2 subunit selectivity of ethanol have been performed in mammalian heterologous expression systems such as HEK cells and *Xenopus* oocytes expressing recombi-

nant NMDARs. In HEK cells, some studies find equal ethanol inhibition in GluN1/GluN2A, GluN1/GluN2B, GluN1/GluN2A/GluN2B subunit combinations (Lovinger 1995; Blevins et al. 1997; Popp et al. 1998; Anders et al. 1999a,b), whereas GluN1/GluN2B-containing receptors seem to be slightly more sensitive in other studies (especially at low ethanol doses) (Lovinger 1995; Blevins et al. 1997; Anders et al. 1999b). This greater sensitivity of GluN1/GluN2B-containing receptors was not seen when *Xenopus* oocytes were used (Kuner et al. 1993; Masood et al. 1994; Chu et al. 1995; Mirshahi and Woodward 1995). Later work confirmed this finding, showing that GluN2B-containing receptors were more ethanol sensitive but that the differences in sensitivity between GluN2A and B were greatly dependent on the expression system used (Smothers et al. 2001). These GluN2A- and GluN2B-containing receptors, however, were much more sensitive than their GluN2C- or GluN2D-containing counterparts (Chu et al. 1995; Mirshahi and Woodward 1995). In recent work, an extensive evaluation of the acute effects of ethanol in HEK cells with all combinations of the eight GluN1 isoforms and four GluN2 subunits was performed. Ethanol inhibition was greater in GluN2B subunits than in GluN2A and when coupled with GluN1-1a, 3a, 4a. However, there was no difference in ethanol inhibition when GluN2B and GluN2A were coupled with GluN1-2a, 1-4b (Jin and Woodward 2006).

Another commonly used technique to evaluate the GluN2B subunit specificity of ethanol inhibition has been with the use of ifenprodil and its derivatives (GluN2B-selective antagonists). In a number of brain regions (hippocampus, cerebellum, vBNST, CeA, lateral septum, cortex), these drugs are able to attenuate the inhibitory effects of acute ethanol (Lovinger 1995; Fink and Gothert 1996; Engblom et al. 1997; Criswell et al. 2003; Roberto et al. 2004; Izumi et al. 2005; Kash et al. 2008). However, these effects might be regionally and/or developmentally selective because other studies fail to see modulation of ethanol inhibition with these drugs (Popp et al. 1999; Criswell et al. 2003). One of the major disadvantages to these phar-

macological approaches is that parallel studies for other subunits are not feasible owing to lack of sufficient pharmacological tools (GluN2A vs GluN2B) (Weitlauf et al. 2005). Additionally, the efficacy of these GluN2B-selective compounds can be modulated by zinc occupancy of the receptor, pH, and whether the receptor contains di- versus triheteromeric subunit composition (Kash and Winder 2007; Paoletti and Neyton 2007). As will be discussed below, recent genetic deletion studies now provide additional evidence for a key role of GluN2B in NMDAR ethanol sensitivity (Wills et al. 2012).

Posttranslational Effectors

Several kinases are known to regulate the phosphorylation/dephosphorylation of NMDARs (Ron 2004). Some of these kinases show subunit-specific phosphorylation so it is likely that they can also play a role in modulating the effects of acute ethanol. Initial studies in HEK cells, however, produced either no or minimal effect of kinase activity (Src, Fyn, CaMKII) on acute ethanol sensitivity (Anders et al. 1999a,b; Xu et al. 2008). In contrast, later work in hippocampal neurons showed that H-Ras activation and Src inhibition were responsible for the specific internalization of GluN2A over GluN2B-containing receptors via the endocytic pathway from acute ethanol (Suvarna et al. 2005). Further, acute ethanol effects on NMDAR activation could be correlated with dephosphorylation of Tyr1472 on the GluN2B subunit (Alvestad et al. 2003). This dephosphorylation of Tyr1472 on the GluN2B subunit can occur through activity of striatal-enriched protein tyrosine phosphatase (STEP). A recent study (Hicklin et al. 2011) showed that ethanol is unable to inhibit NMDAR excitatory postsynaptic currents (EPSCs) in STEP knockout (KO) mice but could be rescued with STEP TAT-fusion protein. Lack of STEP in KO mice prevented the ethanol-induced dephosphorylation of Tyr1472 on the NR2B subunit suggesting the critical nature of this site in ethanol's acute inhibitory effects. Finally, the enhancement of phosphoprotein DARPP-32 activity leads to enhanced phosphorylation of Ser-897 of the GluN1

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subunit, which in turn reduces ethanol sensitivity (Hardy et al. 1999; Maldve et al. 2002). Collectively this work indicates that the acute effects of ethanol on NMDARs has some subunit dependence; however, the particular subunit implicated varies depending on the methodological approach.

CHRONIC ETHANOL EFFECTS ON NMDARs

Although acute ethanol application has been shown to inhibit NMDAR transmission, the converse can be said for the effects of chronic ethanol applications and withdrawal. There is a general hyperexcitability of glutamate synapses and NMDARs during withdrawal from chronic ethanol (Thomas et al. 1998; Hendricson et al. 2007), which is thought to arise mainly from increased expression of NMDAR subunits after chronic ethanol administration (Follesa and Ticku 1996; Kalluri et al. 1998; Kumari 2001; Nagy et al. 2003; Pawlak et al. 2005; Sheela Rani and Ticku 2006). Further in contrast to the uncertain subunit specificity of acute ethanol effects, there are more consistent findings linking chronic ethanol administration and changes in GluN2B. Numerous studies have illustrated enhanced messenger RNA (mRNA) or expression of the GluN2B subunit either during chronic ethanol administration (Hardy et al. 1999; Henniger et al. 2003) or within the first 24 h of withdrawal (Follesa and Ticku 1995; Hu et al. 1996; Kalluri et al. 1998; Narita et al. 2000; Nagy et al. 2003; Hendricson et al. 2007; Qiang et al. 2007) in the hippocampus and cortex. This enhancement of GluN2B from chronic ethanol could also be mimicked by a chronic GluN2B antagonist suggesting that acute ethanol acts at GluN2B-containing receptors leading to these changes in expression (Follesa and Ticku 1996). In line with these changes in the GluN2B subunit, multiple studies find that the amount of ifenprodil-induced inhibition is also enhanced by chronic ethanol (Blevins et al. 1995; Blevins et al. 1997; Nagy et al. 2003). Therefore, this enhanced GluN2B subunit expression translates to greater functional GluN2B-containing receptors. This enhanced expression is likely related to the persistent up-regulation of GluN2B gene

expression via DNA demethylation (Sheela Rani and Ticku 2006; Qiang et al. 2010). In conjunction with these changes in the GluN2B subunit, chronic ethanol also increases expression of the GluN1 subunit (Follesa and Ticku 1996; Kalluri et al. 1998; Floyd et al. 2003; Hendricson et al. 2007; Qiang et al. 2007) and in a few cases GluN2A (Follesa and Ticku 1996; Kalluri et al. 1998). The parallel changes in GluN1 and GluN2B are not surprising because the GluN1 subunit is the obligatory subunit for functional NMDARs.

In conjunction with subunit expression changes, localization of NMDARs within the synapse can also play an important role in their function. NMDARs are known to exist in both synaptic and extrasynaptic populations. The location of NMDARs with these distinct populations can differentially regulate neuronal physiology and plasticity. Extrasynaptic NMDAR populations are thought to be heavily populated by GluN2B-containing NMDARs. Chronic ethanol exposure has been reported to enhance synaptic clustering of NMDARs into dendritic spines (Carpenter-Hyland et al. 2004; Hendricson et al. 2007; Qiang et al. 2007). However, recent work has also suggested that during withdrawal there is lateral movement of GluN2B-containing receptors from synaptic to extrasynaptic populations in hippocampal neurons (Clapp et al. 2010). These data show there is likely a time dependence (during ethanol exposure vs. withdrawal) to the localization of GluN2B that likely has important functional consequences. Overall the effects of chronic ethanol and withdrawal lead to a general increase in NMDAR function through increases in the GluN2B subunit. It is likely that these changes in NMDAR function contribute to aberrant forms of plasticity that occur in addiction and persist during abstinence.

ETHANOL EFFECTS ON PLASTICITY

One of the primary ways that stimuli (like ethanol) can impart long-lasting changes on neural systems is through alterations in synaptic plasticity (reviewed in McCool 2011). A wide range of studies performed primarily in the

hippocampus have shown that acute application of ethanol inhibits the induction of long-term potentiation (LTP) (Blitzer et al. 1990; Morrisett and Swartzwelder 1993; Givens 1995; Grover and Frye 1996; Schummers et al. 1997; Pyapali et al. 1999; Schummers and Browning 2001; Izumi et al. 2005; Fujii et al. 2008) and long-term depression (LTD) (Hendricson et al. 2002; Izumi et al. 2005; Jeanes et al. 2011). Meanwhile, chronic ethanol administration reduced LTP induction threshold (Fujii et al. 2008) and reduced the magnitude of LTD during withdrawal (Thinschmidt et al. 2003) in the hippocampus. Nucleus accumbens LTD was not only absent during withdrawal from chronic ethanol but instead developed into LTP (Jeanes et al. 2011). These disruptions in plasticity were attributed either fully or partially to ethanol's actions at NMDARs.

The work above illustrates a key role of ethanol in modulating the transmission of NMDARs and plasticity in a number of brain regions. More recent work has begun to evaluate the effects of ethanol in other brain areas thought to be critical for the modulation of negative affect during withdrawal (i.e., the extended amygdala). The following sections will primarily focus on a key portion of the extended amygdala, the BNST, and its importance in regulating the negative affect associated with ethanol dependence. Further, evidence will be provided to suggest that the modulation of NMDARs by ethanol is a likely mechanism for negative affect that accompanies ethanol dependence in this region.

BNST CONNECTIVITY

Evidence suggests that the BNST is a key “midline management” region, serving as an information hub upstream of key nuclei such as the VTA and the paraventricular nucleus of the hypothalamus (PVN) (Georges and Aston-Jones 2001, 2002; Jalabert et al. 2009; Ulrich-Lai and Herman 2009). The BNST is considered an integral regulator of the hypothalamic-pituitary-adrenal (HPA) stress axis (Saper and Loewy 1980; Weller and Smith 1982; Moga et al. 1989; Cullinan et al. 1993; Herman et al. 1994; McDonald et al. 1999; Dong et al. 2001a,b; Herman et al.

2002a,b, 2003; Choi et al. 2007, 2008; Ulrich-Lai and Herman 2009; Radley and Sawchenko 2011). The BNST receives “proceptive” or “psychogenic” stressor input from the basolateral amygdala (BLA), insular cortex, prefrontal cortex, and the hippocampal formation. The BNST also receives “systemic” stressor information (i.e., hypotension or hemorrhage) directly from ascending visceral efferent pathways. The BNST projects heavily to the PVN, the key initiator of stress responses. Control of corticotropin-releasing factor (CRF) release from the PVN (and thus ultimately levels of circulating corticosterones) is a major site of regulation of the stress response. Parvocellular CRF-containing PVN neurons receive synaptic input from a discrete set of brain regions and are indirectly regulated by several cortical regions and the hippocampal formation (Herman et al. 2003; Radley et al. 2009; Radley and Sawchenko 2011). Direct inputs include other hypothalamic subnuclei, brain stem nuclei such as the raphe and the nucleus of the solitary tract (NTS), and the BNST.

BNST IN STRESS AND ANXIETY

Consistent with its interconnections with the BLA and the PVN, the BNST plays a critical role in anxiety (Walker et al. 2003). Intra-BNST stimulation in the awake behaving rats resulted in a behavioral phenotype very similar to the one seen during restraint stress (Casada and Dafny 1991). Restraint stress produces an increase in norepinephrine (NE) levels in the anterolateral BNST (alBNST), and microinjection of either β - or α_1 -AR antagonists into the alBNST attenuates restraint stress-induced reactivity on the elevated plus maze (Cecchi et al. 2002). Curiously, α_1 - but not β -AR antagonists in the BNST also disrupted stress-induced HPA axis recruitment. In addition to actions within the hypothalamus, it is now clear that CRF exerts important extrahypothalamic actions as well (Muller et al. 2003). Intracerebroventricular (ICV) CRF administration enhances fear-potentiated startle responses in rats, and this enhancement is ablated by BNST lesions (but not CeA lesions), or by microinjections of CRF antagonists into the BNST (Lee and Davis

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1997). Further, light enhancement of fear-potentiated startle is also disrupted by BNST lesions (Walker and Davis 1997). Finally, inactivation of the BNST, as well as inhibition of NE signaling in the BNST, blocks the freezing response induced by unconditioned stressors such as predator scent (Fendt et al. 2003, 2005).

THE BNST PLAYS A KEY ROLE IN MEDIATING ACTIONS OF ALCOHOL

Much evidence suggests that the BNST plays a key role in mediating aspects of alcohol abuse. First, administration of alcohol activates a relatively small number of nuclei, one of which is the alBNST (Chang et al. 1995; Demarest et al. 1998; Knapp et al. 2001; Crankshaw et al. 2003; Leriche et al. 2008; Ibba et al. 2009). Although neurons within the BNST are among a small subset of neurons to be activated by acute intraperitoneal (i.p.) ethanol administration, 45 min later this region is also one of the few to show decreased metabolic activity (Lyons et al. 1998). Withdrawal from chronic alcohol exposure is also associated with strong Fos activation in the region (Kozell et al. 2005), as is exposure to conditioned stimuli associated with ethanol (Hill et al. 2007). Evidence also suggests that rodents bred for differences in alcohol preference and intake show significant variance in expression of BDNF in restricted regions of the brain, including the BNST (Prakash et al. 2008). Moreover, manipulation of the BNST in the preferring (P) rat strain disrupts alcohol-seeking behaviors (Eiler and June 2007), and stimulation in the P but not nonpreferring (NP) rat's BNST can support reward-related behavior (Eiler et al. 2007). Intraperitoneal administration of ethanol leads to increases in extracellular dopamine levels in BNST (Carboni et al. 2000), and blockade of D1 dopamine receptors (Eiler et al. 2003) or activation of GABA_ARs (Hyytia and Koob 1995) within the BNST reduces ethanol self-administration. Removal from a chronic ethanol diet results in an increase in CRF levels in BNST, which can be normalized by reexposure to ethanol (Olive et al. 2002). Finally, the BNST is one of several structures in which NMDA recruitment of c-fos activation is reduced by eth-

anol (Knapp et al. 2001). Thus multiple lines of evidence suggest that alcohol regulates the activity of BNST neurons, and that this regulation is relevant to alcohol-mediated behaviors.

ETHANOL ACTIONS ON NMDAR IN THE BNST

Glutamatergic signaling in several subnuclei of the BNST is altered by acute and chronic ethanol. In the juxtacapsular (jc)BNST, protracted withdrawal from ethanol, cocaine, and heroin disrupted LTP of intrinsic excitability at glutamatergic synapses, which is characterized by reduced neuronal firing threshold and enhanced temporal firing fidelity (Francesconi et al. 2009). This effect was blocked with a CRF₁ antagonist and mimicked by CRF itself indicating the dysregulation of stress circuitry in the BNST during protracted withdrawal. In the dorsal lateral BNST (dlBNST), acute in vitro ethanol administration was able to reduce the early portion of NMDAR-dependent LTP. Additionally, acute in vitro ethanol also produced a reversible dose-dependent reduction in isolated NMDAR responses, which could be mimicked by partial NMDAR blockade (Weitlauf et al. 2004). In the ventral BNST (vBNST) using whole cell recordings, acute ethanol also inhibited NMDAR EPSCs without changing glutamate release. This inhibition by acute ethanol was still present in GluN2A knockout mouse but attenuated with a GluN2B antagonist (Kash et al. 2008). Recent work within dlBNST found that the acute inhibitory effects of ethanol were absent in a GluN2B knockout mouse and in the presence of a GluN2B antagonist. Collectively, this evidence defines the GluN2B subunit as a key determinant of acute ethanol sensitivity within the BNST (Fig. 1A,C) (Wills et al. 2012).

Similar to findings in other brain regions, chronic ethanol administration and withdrawal also enhances NMDAR transmission in the BNST. In the vBNST, chronic intermittent ethanol exposure increases NMDAR EPSC temporal summation during the early withdrawal period (Kash et al. 2009). In these chronically treated ethanol mice, there was no change in glutamate release but there were elevated GluN2B protein

Figure 1. The acute and chronic effects of ethanol in control and GluN2B KO mice in the BNST. (A) Basally, our data suggests that many BNST synapses are populated by AMPARs and NMDARs (GluN2A- and GluN2B-containing). During acute ethanol application to the slice, there is an inhibition of glutamate transmission. Our work has shown that this is specific to the NMDARs that are GluN2B-containing because no ethanol inhibition occurs in slices from GluN2B KO mice (B). (C) Following chronic intermittent ethanol, we find enhancement of long-term potentiation (LTP) that is GluN2B dependent because this effect is absent in GluN2B KO mice (D). We postulate that this LTP enhancement results from increased synaptic GluN2B-containing receptors. Further, our data illustrate that extrasynaptic GluN2B-containing receptors contribute to synaptic signaling (although the mechanism is unknown) to enhance LTP after chronic intermittent ethanol.

levels and increased GluN2B antagonism on NMDAR EPSCs and decay kinetics (Kash et al. 2009). These changes are consistent with functional up-regulation of GluN2B from chronic ethanol and it is this increase that likely contributes to enhanced temporal summation of NMDAR EPSCs. Later work in the dBNST also suggested an enhancement of GluN2B-dependent transmission. Chronic intermittent ethanol exposure enhanced LTP during early withdrawal compared to air-exposed controls. This ethanol-induced enhancement was absent in the GluN2B knockout mice lending further

support to the role of GluN2B in adaptations from chronic ethanol (Fig. 1B,D) (Wills et al. 2012). Additionally, chronic ethanol exposure blunted the inhibitory effects of acute in vitro ethanol administration in the vBNST suggesting that NMDARs likely develop tolerance to the acute ethanol effects (Kash et al. 2009). This is particularly curious given the key role of GluN2B in ethanol sensitivity of NMDARs in this region coupled with its increased expression after chronic exposure. Again, this discrepancy likely points to the importance of posttranslational modifications of NMDARs in control of

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ethanol sensitivity. In total, this work in the BNST shows that, similar to other brain regions, acute ethanol has an inhibitory role on glutamatergic signaling, whereas chronic ethanol treatment enhances its function. Subsequently, with the combined approach of both pharmacological modulation and genetic deletion, these ethanol effects have been shown to be GluN2B dependent.

As described above, both subunit selectivity and synaptic localization are critical factors in determining the net effect of ethanol on glutamatergic transmission and plasticity. In other brain regions chronic ethanol leads to increased synaptic clustering of NMDARs and during withdrawal GluN2B-containing NMDARs move to extrasynaptic populations (Carpenter-Hyland et al. 2004; Hendricson et al. 2007; Qiang et al. 2007; Clapp et al. 2010). Our recent work (Wills et al. 2012) in the dBNST supports this idea of enhanced extrasynaptic GluN2B populations and illuminates how their function can contribute to plasticity. This work took advantage of the unique pharmacology of ifenprodil and ifenprodil derivatives (GluN2B antagonist). These drugs have the dual role of noncompetitive inhibition of GluN2B-containing NMDARs and enhancing glutamate affinity. We found that low doses of an ifenprodil derivative, Ro25-6981, could enhance GluN2B-dependent NMDA transmission likely through its glutamate affinity enhancing effects on extrasynaptic NMDAR populations. During acute withdrawal from chronic intermittent ethanol, it was shown that this low dose of Ro25-6981 enhanced LTP only in ethanol-treated mice. This enhanced LTP could be blocked with low-dose memantine, which preferentially inhibits extrasynaptic NMDARs at low doses. Collectively, this work provides additional support for the enhancement of extrasynaptic GluN2B-containing NMDARs during withdrawal (Fig. 1B). Moreover, this population functions to enhance LTP in this region. This idea is in contrast with conventional thought regarding the role of extrasynaptic GluN2B-containing NMDARs in promoting LTD over LTP in other brain areas (Liu et al. 2004; Massey et al. 2004). Thus, these results suggest either regional differences in

extrasynaptic GluN2B signaling or ethanol-induced changes in the normal intracellular cascades of this population.

ETHANOL ACTIONS ON NMDARs IN THE BASOLATERAL AND CENTRAL AMYGDALA

NMDARs in the BLA and CeA also play important roles in negative affect and are heavily interconnected to the BNST. NMDAR LTP can be elicited at glutamate synapses in each of these brain regions (Pollandt et al. 2006). Further, acute applications of ethanol dose dependently inhibit NMDAR in the BLA and CeA (Floyd et al. 2003; Roberto et al. 2004). This acute ethanol inhibition was removed by preapplication of the GluN2B-selective antagonist in the CeA (Roberto et al. 2004), similar to the results in the BNST (Kash et al. 2008). After chronic ethanol exposure, an enhanced ethanol sensitivity of NMDAR EPSCs was observed, which was sensitive to GluN2B antagonism and associated with increased GluN2B expression (Roberto et al. 2004). In the basolateral nucleus of the amygdala, chronic ethanol treatment increased NMDAR current density, did not change acute ethanol sensitivity, and enhanced ifenprodil inhibition (Floyd et al. 2003). However, this treatment did not result in altered GluN2B mRNA levels (Floyd et al. 2003). Evidence from this work further supports that these actions of ethanol on the NMDAR are GluN2B dependent. However, regional differences in ethanol sensitivity could suggest that posttranslational modifications of NMDARs might be distinct in different brain regions. Further, it is currently unknown how synaptic and extrasynaptic populations might be affected in these regions following chronic ethanol.

CONCLUDING REMARK

In summary, a large body of data indicates that the NMDAR is a major target of ethanol in the BNST. Both acutely and chronically, the GluN2B subunit of the NMDAR plays a critical role in this sensitivity. As this receptor plays a critical role in the long-term setting of synaptic

weights, dysregulation of the receptor may reset key neural circuitries within the extended amygdala, thus increasing the drive for negative-reinforcement-based behavior. As the NMDAR plays a major role at synapses throughout the CNS, it seems unlikely that typical competitive antagonists will be an effective strategy in the treatment of addiction. However, the recognition of subunit dependence to ethanol sensitivity, coupled with an evolving variety of allosteric and use-dependent antagonists may lead to more subtle means of addressing ethanol sensitivity of these receptors.

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Ethanol Effects on N-Methyl-d-Aspartate Receptors in the Bed Nucleus of the Stria Terminalis

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