# Adolescent Vulnerability to Alcohol Use Disorder: Neurophysiological Mechanisms from Preclinical Studies

Brian A. McCool and Molly M. McGinnis

## Contents



**Abstract**

Adolescent alcohol use in human populations dramatically increases the likelihood of adult alcohol use disorder. This adolescent vulnerability is recapitulated in preclinical models which provide important opportunities to understand basic neurobiological mechanisms. We provide here an overview of GABAergic and glutamatergic neurotransmission and our current understanding of the sensitivity of these systems to adolescent ethanol exposure. As a whole, the preclinical literature suggests that adolescent vulnerability may be directly related to region-specific neurobiological processes that continue to develop during adolescence. These processes include the activity of intrinsic circuits within diverse brain regions (primarily represented by GABAergic neurotransmission) and activity-dependent regulation of synaptic strength at glutamatergic synapses. Furthermore, GABAergic and glutamatergic neurotransmission within regions/circuits that regulate cognitive function, emotion, and their integration appears to be the most vulnerable to adolescent ethanol exposure. Finally, using documented

B. A. McCool (✉) · M. M. McGinnis
Department of Physiology and Pharmacology, Wake Forest School of Medicine, Winston-Salem, NC, USA
e-mail: bmccool@wakehealth.edu



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behavioral differences between adolescents and adults with respect to acute ethanol, we highlight additional circuits and regions for future study.

**Keywords**
AMPAR · $GABA_A$ · NMDAR

## 1 Background and Overview

There is a robust literature in humans on the vulnerability of adolescents for the development of alcohol use disorders (AUDs) following early drinking experiences. Over seven million individuals ages 12–20 (~19% of all adolescents) report alcohol use in the past month with approximately 77% of these exhibiting "risky" drinking, like heavy or binge-like use (five or more drinks/occasion, SAMHSA 2017). The lifetime prevalence for alcohol dependence drops tenfold as the age of first use increases from early adolescence (~14 years old) into young adulthood (>20 years old; Grant and Dawson 1997). Consistent with these findings, individuals reporting first use of alcohol between the ages of 11 and 14 are five times more likely to repeatedly use alcohol despite persistent negative consequences (abuse) over a subsequent 20-year period and eight times more likely to develop alcohol dependence (inability to quit drinking, withdrawal symptoms, increased tolerance to the acute intoxicating effects) over the next 10 years compared to individuals initiating alcohol use when they were >19 years old (DeWit et al. 2000). Longitudinal studies confirm that adolescents who drink to intoxication during this period are at greatest risk for developing AUD as adults (Warner et al. 2007). These findings all suggest that adolescents are uniquely sensitive to the long-term consequences of ethanol exposure. This age-group is characterized by dramatic development of brain structures involved with fine motor skills, habit formation, executive function, memory, and emotional regulation (Bundy et al. 2017). As a result, understanding both the developmental changes in the neural systems regulating drinking behavior and the neurophysiological consequences of adolescent ethanol exposure is particularly important for defining the neurophysiological mechanisms governing vulnerability to AUD in this population.

Identification of neurobiological mechanisms responsible for adolescent vulnerability to AUD has required the development of preclinical models. These models, primarily rodents but also including some studies in nonhuman primates, have strong face validity. In rats, for example, adolescence is generally defined as the period from postweaning (post-natal day 21–28 or P21–28) to young adulthood (~P60) (Sengupta 2013). Adolescent rats are less sensitive to the locomotor impairing and sedative effects of acute ethanol compared to adults (Pian et al. 2008; Schramm-Sapyta et al. 2010; White et al. 2002). Notably, subjective feelings of intoxication in humans are diminished in the sons of alcoholics (Schuckit 1984) who have greater risk for the development of AUD. Adolescent rats also self-administer greater amounts of ethanol compared to adults in many paradigms (Bell et al. 2011; Vetter et al. 2007; Walker et al. 2008) and are less sensitive to aversive properties of ethanol during noncontingent administration (Morales et al. 2014; Schramm-Sapyta et al. 2010, 2014), although this latter finding may be sex-specific (Morales et al. 2014).

Preclinical studies thus parallel many aspects of human adolescent ethanol abuse and have produced a number of important insights into the adult behavioral consequences resulting from adolescent ethanol dependence-like exposures that produce both heighten negative affective behaviors and acute withdrawal symptoms. There have been a number of exceptional reviews highlighting these advances (Crews and Boettiger 2009; Crews et al. 2016; Doremus-Fitzwater and Spear 2016; Maldonado-Devincci et al. 2010; Spear 2016; Spear and Swartzwelder 2014; White and Swartzwelder 2005). Most relevant for this chapter, adolescent dependence-like exposure in rodents dramatically increases adult ethanol consumption/preference (Alaux-Cantin et al. 2013; Amodeo et al. 2017; Criado and Ehlers 2013; Gass et al. 2014; Pascual et al. 2009), ethanol-seeking behavior (Amodeo et al. 2017), motivation to consume ethanol (Serlin and Torregrossa 2015), and decreases sensitivity to ethanol impairment/aversion (Graham and Diaz-Granados 2006; Jury et al. 2017; Mejia-Toiber et al. 2014). Preclinical models therefore provide opportunities both to understand basic neurophysiological mechanisms conferring adolescent vulnerability and may help identify potential therapeutic targets. This chapter will summarize our understanding of these neurophysiological mechanisms with a specific focus on glutamate and GABA neurotransmission and their alteration by adolescent ethanol exposures.

## 2 Adolescence and Glutamate/GABA Neurotransmitter Systems

After the perinatal period, glutamate and GABA act as the major excitatory and inhibitory neurotransmitter systems in the central nervous system, respectively. Both systems regulate neuronal activity through ion-conducting (ionotropic) and G protein-coupled (metabotropic) neurotransmitter receptors. Glutamate ionotropic receptors, all cation-conducting channels, consist of at least three pharmacologically and biophysically identifiable subtypes – α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate, and *N*-methyl-D-aspartate (NMDA) receptors. AMPA receptors are homomeric or heteromeric assemblies of four subunits arising from four different gene produces (GluA1–GluA4). GluA1–GluA3 are widely expressed throughout the central nervous system at all developmental stages, with GluA4 showing more restricted expression during early development and restricted localization to thalamic subnuclei postweaning period. Kainate receptors, pharmacologically, structurally, and functionally similar to AMPA receptors, are composed of multi-subunit assemblies of tetramers arising from five distinct genes (GluK1–GluK5). Although the neurophysiology of kainate receptors is generally less well-characterized than AMPA receptors, they are highly permeable to calcium and, in many instances, appear to be localized to presynaptic glutamate terminals where they act as feedback facilitators of glutamate release (Huettner 2003; Zhuo 2017). NMDA receptors are also tetrameric assemblies but are believed to consist of two obligatory GluN1 subunits (eight alternatively spliced isoforms) and, at most synapses, two subunits encoded by at least one of four

different GluN2 subunits (GluN2A-D). Like kainate receptors, NMDA receptors are also highly permeable to extracellular calcium but are more commonly localized at postsynaptic sites (but see Bouvier et al. 2015; Dore et al. 2017). Postsynaptic NMDA receptors are typically blocked by intracellular magnesium bound to the channel pore which is displaced by membrane depolarizations, usually mediated by AMPA receptors. This type of "coincidence" detection by NMDA receptors, requiring both synaptic glutamate and membrane depolarization, likely underlies their role in the activity-dependent changes in synaptic efficacy (plasticity) that is believed to represent the synaptic correlate of learning and memory. $GABA_A$ receptors, members of the Cys-loop family of ligand-gated ion channels, are all anion-selective channels that mediated much of the "fast" inhibitory neurotransmission in the adult central nervous system. Like other members of the Cys-loop family, these receptors are pentameric assemblies that, for $GABA_A$, contain at least alpha and beta subunits. Synaptic $GABA_A$ receptors are believed to require gamma subunits as part of the pore-forming complex since these subunits contain binding sites for gephyrin which localizes $GABA_A$ receptors to postsynaptic sites. Delta subunits, which can replace gamma subunits in the assembly, dramatically alter complex pharmacology, function, and localization. Delta-containing $GABA_A$ receptors are frequently found in extrasynaptic $GABA_A$ receptors providing "tonic" inhibition mediated by GABA spillover from synaptic site. In addition to ionotropic receptors, glutamate and GABA also bind to heterotrimeric G protein-coupled receptors (mGluR and $GABA_B$, respectively). These receptors couple to a variety of signaling cascades and can regulate the production of second messengers like intracellular calcium, cyclic AMP, and inositol phosphates and can directly regulate the activity of ion channels like voltage-gated calcium channels and inwardly rectifying potassium channels. Compared to the ionotropic receptors, these metabotropic signaling events occur somewhat slowly owing to their localization (generally peripheral to the active zone) and their reliance on multistep signaling processes.

The late prenatal/early postnatal period is defined by rapid development of brain structures and neurotransmitter systems. For example, the expression and synaptic function of $GABA_A$ and ionotropic glutamate receptors generally mature during this period, prior to adolescence. These receptors, as well as their associated postsynaptic anchoring proteins which are involved with receptor trafficking and localization, reach adult levels/distributions prior to weaning in rodents (Dong et al. 1999; Korpi et al. 1993; Martin et al. 1998; Pandey et al. 2015; Virtanen et al. 2018; Yu et al. 2006; Zhong et al. 1995). Similar observations have been reported for mGluRs (Defagot et al. 2002) and $GABA_B$ receptors (Fritschy et al. 1999; Gaiarsa et al. 1995). The developmental trajectories of these various neurotransmitter receptor systems in nonhuman primates appear to be very similar (Gonzalez-Burgos et al. 2008; Shaw et al. 1991). These findings suggest that the functional aspects related to "fast" neurotransmitter systems like glutamate and GABA are largely in place prior to adolescence. However, activity-related "plastic" changes in synaptic function of these neurotransmitter systems appear to develop throughout the adolescent period in many brain regions. For example, long-term potentiation (LTP) at glutamate synapses, most typically characterized as activity-dependent upregulation of synaptic

efficacy, is more robust in adults in brain regions like the prefrontal cortex (Konstantoudaki et al. 2018), hippocampal dentate gyrus (Zitman and Richter-Levin 2013), and interpeduncular nucleus (Koppensteiner et al. 2017). In contrast, LTP in the barrel cortex (Konstantoudaki et al. 2018) and nucleus accumbens (Schramm et al. 2002) either develops prior to adolescence (cortex) or is greater in adolescents compared to adults (n. accumbens). These findings suggest that LTP related to sensory processing and reward circuitry develop relatively early while plasticity related to executive control, spatial memory/emotion regulation, and negative control of reward circuitry (interpeduncular nucleus, Nishikawa et al. 1986) occurs postadolescence. On the other hand, long-term depression (LTD) at glutamate synapses is typical in many adolescent brain regions (Bergerot et al. 2013; Zhang et al. 2015) and may reflect processes related to the robust pruning of synapses during this developmental period (Selemon 2013). Recent work also suggests that circuits integrating emotional control and executive function are also established during adolescence. In adults for example, ventral hippocampal (vHC) and basolateral amygdala (BLA) inputs to the prefrontal cortex (PFC) converge to dynamically regulate synaptic plasticity in the latter region. High-frequency stimulation of BLA inputs in vivo produces LTP of PFC synaptic responses, while coincidental stimulation of vHC inputs either de-potentiates (normalizes) or prevents, depending on the temporal sequence of BLA and vHC input activation, BLA-mediated PFC plasticity. vHC de-potentiation/block of BLA-mediated plasticity is notably absent in adolescent rats (Thomases et al. 2014). Similarly, high-frequency stimulation of vHC inputs to the PFC alone produces LTD of local field potentials in the PFC; picrotoxin, a $GABA_A$ receptor noncompetitive antagonist, converts this depression to potentiation. Both this picrotoxin-sensitive LTD and the resulting LTP are expressed in adult animals but not adolescents (Caballero et al. 2014). This suggests a robust developmental regulation of PFC GABAergic control of plasticity in this region. Further, the development of GABAergic control of glutamatergic plasticity in the PFC appears directly related to the maturation of local GABA circuits (Kang et al. 2018; Konstantoudaki et al. 2018; Morishita et al. 2015).

Compared to glutamate synapses, less is known about the adolescent development of GABAergic synaptic plasticity. However, the distribution and localization of synaptic specializations associated with GABA neurotransmission may continue to develop during adolescence as well. For example, gephyrin, the $GABA_A$ receptor anchoring protein which stabilizes these receptors in postsynaptic compartments, declines markedly in axonal initial segments of nonhuman primate medial prefrontal cortical pyramidal neurons during adolescence (Cruz et al. 2009), while gephyrin clusters on the dendritic shafts of these neurons appears to be stable prior to weaning in rodents (Virtanen et al. 2018). These observations suggest a subtle shift in GABAergic control over neuronal excitability during the adolescent period that may be reflected by GABAergic adaptations to adolescent ethanol exposure (below). Thus, while the basal function of many glutamate and GABA synapses may be "adult-like" prior to adolescence, the processes involved with their dynamic, activity-dependent regulation as well as the circuits themselves may continue to develop throughout this period. In particular, adolescent development of

glutamatergic and GABAergic synaptic function appears in regions like the prefrontal cortex, hippocampus, and basolateral amygdala. This suggests that integration of emotional information, memory, and executive control continues developing during adolescence and may suggest why these processes are particularly vulnerable to disruption by external influences including ethanol exposure.

## 3 Adolescent Ethanol Exposure

Longitudinal studies in humans show that adolescents who drink to intoxication are at greatest risk of developing AUD as adults (Warner et al. 2007). Preclinical rodent models have therefore relied primarily upon noncontingent ethanol exposure given the limited self-administration in this species. Although there is limited data currently, adolescent self-administration in nonhuman primates appears to cause disruptions in neurotransmitter function which parallel those using noncontingent exposure in rodents suggesting that the exposure itself is a major factor in adolescent vulnerability. Most rodent preclinical studies utilize repeating cycles of brief, robust intoxication (ethanol delivered intraperitoneally, intragastrically, or through vapor inhalation) followed by short-term withdrawal to mimic the binge-like drinking patterns that are common in human adolescents. These adolescent exposures dysregulate adult behaviors and suggest an overall increase in an "addiction-prone" phenotype. For example, adult rats with a history of adolescent ethanol exposure exhibit greater ethanol-seeking behavior (Amodeo et al. 2017; Gass et al. 2014), consumption (Amodeo et al. 2017; Criado and Ehlers 2013; Pascual et al. 2009), and preference (Pascual et al. 2009). Exceptional reviews highlighting an array of adult behavioral consequences related to adolescent ethanol exposure are in the literature (Crews and Boettiger 2009; Doremus-Fitzwater and Spear 2016; Spear and Swartzwelder 2014; Varlinskaya et al. 2016; White and Swartzwelder 2005). In general, these reviews suggest that adult outcomes can be characterized as a persistent, adolescent-like behavioral phenotype in adults exposed to adolescent intermittent ethanol. These phenotypes include reduced executive function, increased reward sensitivity, and reduced sensitivity to ethanol sedation and motor impairment. While there has been a few reviews integrating these rodent behavioral outcomes in the context of dopamine neurochemistry/neurotransmission (Doremus-Fitzwater and Spear 2016; Maldonado-Devincci et al. 2010; Spear 2016) and neuro-immune function (Crews et al. 2016; Pascual et al. 2014; Ward et al. 2014), the current review will focus on the central role of GABA and glutamate in the central nervous system and their vulnerability to adolescent ethanol exposure.

### 3.1 Adolescent Ethanol Exposure and Glutamate Neurotransmission

**Dendritic Spine Morphology** The morphological correlates of glutamatergic neurotransmission are dendritic spines. These postsynaptic specializations oppose

presynaptic release sites and contain glutamate receptors and signaling pathways responsible for moment-by-moment synaptic activity as well as activity-dependent changes in synaptic efficacy. During spine morphogenesis, immature spines appear as thin filopodial-like projections that mature into mushroom-shaped specializations. In general, adolescent ethanol exposure appears to influence adult spine density and morphology (hence maturation) in a brain region-dependent manner. There is a dramatic increase in both hippocampal principal neuron dendritic branching and the number of mature spines during adolescence (Aoki et al. 2017); in the dentate gyrus (Mulholland et al. 2018), adolescent ethanol exposure modestly reduces the number of "immature" spines. In contrast, in the CA1 (Risher et al. 2015), adolescent exposure increases the density of immature spines while decreasing the relative number of mature spines. These ethanol-related alterations in adult spine morphology may be an anatomical correlate of memory dysfunction in adults exposed to adolescent ethanol (Swartzwelder et al. 2015).

Adolescent ethanol exposure produces similar outcomes in rat prelimbic cortex. There ethanol exposure increases the density of immature spines (Trantham-Davidson et al. 2017). In contrast, studies with Thy-1 transgenic mice (Jury et al. 2017) found that adolescent exposure had no effect on spine density in prelimbic cortex but instead increased the width of mature spines. Both of these studies utilized intermittent ethanol vapor exposure; it is therefore not clear if the differences between the rat and mouse studies represent distinct, model-dependent outcomes or other procedural differences like the use of pyrazole in mice to stabilize blood-ethanol concentrations or higher blood-ethanol concentrations and longer exposures in the rat study. Regardless, in the same study, Jury et al. also reported adolescent ethanol exposure produced (1) similar effects in the basolateral amygdala (no effect on spine density, increase in the width of mature spines) and (2) a completely novel reduction in spine density and increase in mature spine width in the infralimbic cortex. While changes in spine density and shape are difficult to interpret in the context of synaptic function, these data nicely illustrate that adolescent ethanol exposure alters the synaptic architecture associated with glutamate neurotransmission in a brain region-dependent manner.

**Adolescent Ethanol and Glutamate Receptors** Similar to the regionally-dependent alterations in dendritic spine density and morphology, adolescent ethanol exposure appears to regulate the expression/function of glutamate receptors in a region- and age-specific manner. In a study comparing the short-term consequences of ethanol exposure during adolescence (P23) and adulthood in rats (P60), Pian et al. (2010) showed that adolescent exposure decreased cortical NR1 subunit protein levels during the exposure which normalized within 24 h post-ethanol. There was no effect on NR2A or NR2B subunit protein expression. In adults, cortical NR1, 2A, and 2B subunit proteins were also decreased immediately after the exposure. While NR1 levels normalized 2 weeks after the exposure (more slowly than adolescents), NR2A and NR2B subunit levels were dramatically elevated at this later time point albeit with distinct time courses. While adolescent exposure likewise decreases hippocampal NR1 and NR2A subunit protein levels, the expression of both proteins

is elevated following a 2 week withdrawal. There was no effect on adolescent hippocampal NR2B subunits; exposure-dependent effects on these subunits in adults rapidly normalize within 24 h. A more recent proteomic analysis of adult hippocampal proteins following adolescent ethanol exposure focused on synaptic and extrasynaptic proteins directly associated with the NMDA NR2B subunit (Swartzwelder et al. 2016). This study again did not find significant effects of the adolescent exposure on adult levels of NR2B in either the synaptic or non-synaptic/extrasynaptic subcellular compartments. However, among the dozens of proteins associated with NR2B that were altered by the adolescent ethanol exposure, the treatment up-regulated pathways associated with the actin cytoskeleton in the synaptic compartment providing some indication of the molecular mechanisms controlling changes in spine density/morphology discussed in a previous paragraph.

In the non-synaptic fraction, adult NR1 subunit proteins associated with NR2B were also upregulated by the adolescent ethanol exposure suggesting increased function of NMDA receptors at these extra-synaptic sites. Importantly, extrasynaptic, NR2B-containing NMDA receptors in the hippocampus appear to help mediate long-term, activity-dependent regulation of glutamate neurotransmission (Lu et al. 2001; Yang et al. 2017), excitotoxic insult (Lai et al. 2011; Liu et al. 2007), and neuron excitability/network synchrony (Papouin and Oliet 2014). In contrast to these dynamic effects of ethanol exposure on adolescent NMDA subunit proteins in the cortex and hippocampus, neither adolescent nor adult ethanol exposure alter expression of NMDA receptor NR2 subunit mRNAs in lateral/basolateral amygdala (BLA) tissue (Falco et al. 2009; Floyd et al. 2003) or in individual BLA principal-like neurons (Floyd et al. 2003). However, NR1 subunit mRNA levels in this region are increased by adolescent ethanol; this is associated with increased NMDA receptor-mediated whole-cell currents (Floyd et al. 2003). Notably, the Floyd et al. study also showed that adolescent ethanol exposure increases NMDA current inhibition by the NR2B-selective antagonist, ifenprodil. These data, along with changes in the biophysical properties and calcium permeability of NMDA-mediated currents (Floyd et al. 2003), suggest increased functional contributions by NR2B subunits in BLA principal neurons following adolescent ethanol despite minimal impact of the exposure on subunit mRNAs or protein levels. These data together suggest that adolescent ethanol exposure regulates NMDA receptor expression/function in a regionally-specific manner and can involve transcription, translation, receptor function, and potentially localization.

The sensitivity of adolescent AMPA-type glutamate receptors in general, and particularly in the context of adult outcomes, is less well documented. In a study examining differences between adolescent and adult mouse AMPA receptors in the amygdala immediately following intermittent-access ethanol drinking, Agoglia et al. (2015) found no effects on total protein levels of GluA1 subunit in either the amygdala or striatum. In the amygdala however, adolescent drinking decreased phosphorylation of Serine 831 (Ser831) on the GluA1 subunit, in contrast to adult drinking which increased phosphorylation of this same site. The decreased phosphorylation in adolescents was associated with decreased phosphorylation of the auto-regulatory Threonine 286 site on CamKII suggesting a mechanistic link

between decreased CamKII activity and GluA1 phosphorylation at Ser831. In contrast to these findings in mice, a dependence-like ethanol exposure in adolescent rats increased phosphorylation of lateral/basolateral amygdala AMPA subunits GluA1 at Ser831 as well as GluA2 at Ser880. This exposure also increased phosphorylation of the autoregulatory sites, Thr286 and Thr305, on CamKII and the phosphorylation of the PKC substrate, neurogranin (Christian et al. 2012). Like the mouse study, this rat study also found that an adolescent ethanol exposure had little impact on total protein levels of AMPA receptor subunits. Notably, GluA1 phosphorylation at S831 and GluA2 at Ser880 are both associated with increased receptor trafficking to the plasma membrane that is typically observed during activity-dependent synaptic plasticity; increased trafficking of AMPA receptors to the plasma membrane was directly demonstrated in the rat study (Christian et al. 2012). There are numerous procedural differences between the Agoglia and Christian study including exposure paradigm (hence level of intoxication), model system (mouse versus rat), and a specific focus on the cortical-like lateral and basolateral subdivisions in the rat study.

**Adolescent Ethanol and Glutamate Synaptic Function** In light of the regionally-dependent effects of adolescent ethanol exposure on glutamate receptor expression, it is perhaps no surprise that studies focused on glutamatergic neurotransmission likewise appear to highlight alterations in synaptic function that are again dependent upon the brain region. In the CA1 hippocampus for example, adolescent ethanol exposure increases NMDA-mediated synaptic currents (Swartzwelder et al. 2017) and increases the expression of long-term potentiation measured (LTP) with field recordings (Risher et al. 2015; Sabeti and Gruol 2008). Similar ethanol exposures during late adolescence/young adulthood actually decrease LTP expression (Sabeti and Gruol 2008) suggesting the effects of ethanol on NMDA-mediated synaptic currents and synaptic plasticity are age-dependent.

In the lateral/basolateral amygdala, chronic ethanol and withdrawal differentially modulate pre- and post-synaptic properties of glutamatergic synapses in adolescent rats. The BLA receives qualitatively distinct information from excitatory inputs arising from both cortical and subcortical/thalamic brain regions, which project to the BLA via the lateral external capsule or medial stria terminalis, respectively (Sah et al. 2003). In line with these afferents arising from different brain regions and entering the BLA through different anatomical pathways, the effects of adolescent ethanol exposure on these glutamatergic synapses also differ. For example, the subcortical/thalamic afferents entering the BLA through the medial stria terminalis arise from regions like the medial prefrontal cortex, anterior cingulate cortex, hippocampus, thalamus, and somatosensory cortex. In contrast, afferents entering the BLA through the lateral external capsule originate from lateral cortical areas such as the temporal, occipital, piriform, entorhinal, and insular cortices. Adolescent ethanol exposure increases in 'basal' glutamate synaptic transmission in the BLA, evidenced by increased frequency of spontaneous excitatory postsynaptic currents (sEPSC) as well as an increase in the frequency and amplitude of action potential-independent miniature EPSCs recorded in the presence of the sodium channel

blocker, tetrodotoxin (Lack et al. 2007). Notably, this pre- and postsynaptic facilitation of BLA glutamate neurotransmission occurs in an input-specific fashion. Several studies have found increased presynaptic glutamate release following adolescent ethanol exposure when stimulating the medial stria terminalis inputs, with no presynaptic alterations at the lateral external capsule inputs (Christian et al. 2012, 2013; Lack et al. 2009; Morales et al. 2018). Christian et al. (2013) further revealed that this increased presynaptic function was characterized by increased synaptic glutamate concentrations, decreased 'failure-rates' ('no response' following minimal electrical stimulation), and enhanced contributions by the readily releasable pool of synaptic vesicles. These presynaptic physiological responses to adolescent ethanol were also associated with increased levels of vesicle-associated proteins like VAMP2 (part of the SNARE complex) and the vesicular glutamate proteins, VGLUT1, and VGLUT2. Additionally, BLA CB1 cannabinoid receptors located on medial stria terminalis terminals normally inhibit excitatory transmission. Robinson et al. (2016) found that adolescent ethanol exposure impairs CB1 function at these inputs and decreases CB1 protein expression.

Adolescent ethanol increases postsynaptic function, but not presynaptic function, at external capsule afferents onto BLA principal neurons (Christian et al. 2012, 2013; Floyd et al. 2003; Lack et al. 2007, 2009; Morales et al. 2018). Using a strontium ($Sr^{2+}$) substitution method to specifically separate pre- and postsynaptic function (Dodge et al. 1969) at these external capsule inputs, we found a significant increase in the $Sr^{2+}$-dependent EPSC (asynchronous EPSCs or aEPSCs) amplitude but not effect on frequency (Christian et al. 2012; Morales et al. 2018). In addition to postsynaptic AMPA receptor function, adolescent ethanol exposure also increases synaptic function of postsynaptic NMDA (Floyd et al. 2003; Lack et al. 2007) and kainate-type glutamate receptors (Lack et al. 2009). Notably, the input-specific alterations in BLA glutamatergic synaptic transmission induced by adolescent ethanol described above are also exposure duration- and sex-dependent. Morales et al. (2018) recently found that increased presynaptic function at medial stria terminalis inputs required shorter exposure durations relative to postsynaptic alterations at lateral external capsule inputs; and this was true for both sexes. However, synaptic alterations in females required longer ethanol exposures than males. These data all suggest that adolescent ethanol up-regulates the synaptic function of all three major subtypes of ionotropic glutamate receptors expressed by BLA principal neurons and increases presynaptic function stria terminalis inputs onto BLA principal neurons.

In contrast to the dynamic regulation of glutamate synapses in hippocampus and lateral/basolateral amygdala, recent work (Cuzon-Carlson et al. 2018) compared striatal miniature EPSC frequency (presynaptic), amplitude (postsynaptic), and biophysical properties in ethanol drinking monkeys across age-at-first-access that included adolescents (4–5 years old, equivalent to 15–18 years old humans), young adults (5–6 years old, 20–24 years old humans), and mature adults (7–11 years old, equivalent to 25–40 years old humans). After 14 months of drinking, the study found no significant age-by-exposure interactions for mEPSC frequency or amplitude in either the caudate or putamen. Similar studies in rodents showed no effect of

adolescent exposure on extracellular glutamate concentrations in the caudate (Boutros et al. 2014). These studies together show that the region-specific effects of adolescent exposure on glutamate receptor expression function are likewise reflected at the level of the synapse. Importantly, glutamatergic transmission in reward- and habit-related regions appear to achieve adult-like resilience to ethanol exposure during adolescence while synaptic function in regions involved with executive function and emotional control remain vulnerable.

## 3.2 Adolescent Ethanol Exposure and GABA Neurotransmission

**Adolescent Ethanol and GABA Receptors** Like glutamatergic receptors, adolescent ethanol exposure appears to produce region-dependent changes in the expression and localization of $GABA_A$ receptors. In the prelimbic cortex for example, adolescent ethanol exposure does not appear to modulate total protein levels of the α1, α4, α5, δ, or γ2 subunits and does not appear to alter the plasma membrane levels of delta-containing receptors in adults (Centanni et al. 2017). But this contrasts with substantive changes in $GABA_A$-mediated extrasynaptic currents mediated by delta-containing $GABA_A$ receptors that is produced by a similar exposure (below). In contrast to the prelimbic cortex, $GABA_A$ protein expression in adult hippocampus is dramatically altered by adolescent ethanol exposure. In a separate study, Centanni et al. (2014) used total hippocampus and separated lysates into synaptic and non-synaptic fractions. Adolescent exposure decreased α4 subunit protein in the detergent-resistant, synaptic fraction and decreased δ subunit levels in the detergent-soluble, extrasynaptic fraction. Thus, adolescent ethanol appears to shift the subunit composition of adult hippocampal $GABA_A$ receptors. Surprisingly, α4 subunit mRNA was increased by the adolescent exposure – a potential compensation to changes in subunit protein levels. Adult ethanol exposure had no effect on either subunit in hippocampus. Similarly, long-term adolescent ethanol drinking did not alter levels of the $GABA_A$ α1 subunit mRNA in the lateral/basolateral amygdala; although adult drinking experience increased levels of the subunit mRNA (Falco et al. 2009). This contrasts with studies of $GABA_A$ subunit proteins in this brain region which found that an adolescent dependence-like exposure decreased both α1 subunit proteins levels and diminished α1-containing receptors found on the plasma membrane (Diaz et al. 2011). Although adult outcomes were not measured in the Diaz et al. study, this work also found that adolescent ethanol increased the levels of α4-containing receptors at the cell surface without altering total levels of α4 subunit protein. $GABA_A$ gamma2 subunit and gephyrin protein levels were also increased by the adolescent ethanol exposure; these proteins localize $GABA_A$ receptors to postsynaptic specializations (Schweizer et al. 2003). While these findings highlight the region-specific effects of the exposure, they indicate that adolescent ethanol appears to also alter the proteins involved with receptor trafficking/localization. Importantly, trafficking/localization can occur independently from or in conjunction with alterations in protein or mRNA expression.

**Adolescent Ethanol and GABAergic Synaptic Function** Like the effects on subunit expression, adolescent ethanol exposure exerts region-specific effects on adult GABAergic neurotransmission. Generally, those regions in which GABAergic synaptic function are developing during adolescence remain sensitive to ethanol exposure during this period. In prelimbic cortex for example, adult 'basal' GABAergic synaptic function, reflected by tetrodotoxin-resistant or 'miniature' inhibitory postsynaptic currents (mIPSCs), remains unaltered by adolescent ethanol exposure. However, the amplitude of spontaneous IPSCs – which reflect both basal transmission and the activity of intrinsic cortical GABAergic connections – is decreased by adolescent ethanol (Centanni et al. 2017) highlighting the vulnerability of developing adolescent GABAergic circuits in this brain region. Importantly, adolescent ethanol exposure also decreases electrically-evoked, repetitive firing of prelimbic cortical fast-spiking interneurons (Trantham-Davidson et al. 2017). Together these findings suggest that ethanol-dependent modulation of GABAergic circuitry may reflect direct effects on intrinsic interneurons or their synapses. Importantly, extrasynaptic $GABA_A$ receptors, which mediate the tonic currents expressed by prelimbic principal neurons, are also vulnerable to adolescent ethanol exposure. During the transition from adolescence to adulthood, the number of prelimbic layer 5/6 pyramidal neurons expressing tonic currents increases from roughly 20% of these cells at P45 to 100% of neurons at P90; adolescent ethanol exposure 'freezes' neurons in the adolescent phenotype such that number of adult neurons expressing tonic currents is greatly reduced (Centanni et al. 2017). Thus, both the intrinsic GABAergic circuitry and extrasynaptic GABAergic function in the prelimbic cortex are shaped by adolescent ethanol exposure.

In the hippocampus, acute ethanol potentiates sIPSC frequency to a greater extent in adults compared to adolescents, with minimal effects on mIPSCs (Li et al. 2003, 2006). This again suggests development of intrinsic hippocampal GABAergic circuitry during adolescence. However, in contrast to the cortex, adolescent exposure has no effect on adult sIPSC amplitude or frequency suggesting that hippocampal GABAergic circuitry is more resilient than cortex during this period. In contrast, the amplitude of tonic GABA currents in the dentate decrease from adolescence to adulthood; adolescent exposure accentuates this decline (Fleming et al. 2013). Acute ethanol facilitation of GABA tonic currents is also more pronounced in adolescent-ethanol animals compared (Fleming et al. 2012, 2013). Thus, while adolescent exposure has modest impact on adult GABAergic circuitry in the hippocampus, it produces persistent changes in both the tonic GABAergic currents and the acute effects of ethanol on these extrasynaptic currents.

In the BLA, at least two anatomically and functionally distinct populations of GABAergic interneurons, the lateral pericapsular intercalated cells (LPC) and local interneurons, synapse onto principal neurons. LPCs are GABAergic interneurons found in concentrated clusters along the external capsule while local GABAergic interneurons that are scattered throughout the BLA (Spampanato et al. 2011). Similar to the hippocampus, acute ethanol potentiates $GABA_A$ mediated inhibitory postsynaptic currents (IPSCs) recorded from both distal LPCs and local interneuron synapses in the BLA (Silberman et al. 2008). Chronic adolescent ethanol exposure robustly

decreases presynaptic function at LPC GABAergic synapses which provide robust feed-forward inhibition to principal neurons (Diaz et al. 2011). Interestingly, adolescent ethanol does not alter GABAergic release from local interneurons. In addition to these presynaptic changes, Diaz and colleagues reported an increase in the decay kinetics of miniature IPSCs, likely arising from local interneurons which synapse onto principal neuron soma and proximal dendrites, suggesting an ethanol-induced modulation of postsynaptic function in intrinsic BLA GABAergic circuitry. This paralleled changes in the $GABA_A$ receptor subunit composition (described above).

In contrast to the specific vulnerability of adolescent GABAergic synapses/circuits in the cortex, hippocampus, and lateral/basolateral amygdala, adolescent and adult ethanol drinking alter GABAergic neurotransmission in nonhuman primate caudate/putamen to a similar extent. At these synapses, there is a general trend for an age-dependent increase in mIPSC frequency in both brain regions; an ethanol drinking history suppresses mIPSC frequency regardless of age (Cuzon-Carlson et al. 2018). There was no impact of drinking on mIPSC amplitude in these studies. However, sIPSCs were not measured so the impact of ethanol drinking on intrinsic GABAergic circuitry, particularly the excitability of GABA interneurons is not yet certain.

## 4 Concluding Remarks

A critical observation for GABA and glutamate within this review is that synaptic processes developing during adolescence appear to be the most vulnerable to ethanol exposure. Fundamental aspects of GABAergic and glutamatergic neurotransmission (i.e., presynaptic release, postsynaptic receptor function) are largely intact in many brain region by adolescence with some notable exceptions. But, the literature suggests that substantial components of GABAergic circuitry continue to develop during adolescence. These components can include the localization of GABAergic synapses on principal neurons (reflected by shifts in gephyrin immunoreactivity), GABAergic neuron firing (circuit 'activity'), and extrasynaptic receptor activity. All these aspects of GABAergic neurotransmission are sensitive to adolescent ethanol exposure. For glutamate synapses, activity-dependent modulation of synaptic efficacy ('plasticity') likewise develops during adolescence and appears most vulnerable to ethanol exposure. This may be a product of developing signaling cascades or NMDA receptor function/activity/localization which can be influenced by subunit composition. Although these conclusions are specific for GABA and glutamate, similar outcomes are apparent for other neurotransmitters as well. With dopamine for example, adolescence can be characterized as a 'reward-focused period' (Doremus-Fitzwater and Spear 2016). This reward-centric focus is highlighted anatomically by a dramatic peak in dopaminergic projection development, particularly fibers from the ventral tegmental area and substantia nigra to the striatum, nucleus accumbens, and throughout the cortex (Doremus-Fitzwater and Spear 2016). Functionally, there are also peaks in dopamine cell firing rates (Marinelli and McCutcheon 2014) and receptor levels (Doremus-Fitzwater and Spear 2016) during

adolescence. Adolescent ethanol exposure modulates the development of these processes. In the prelimbic cortex for example, ethanol exposure reduces dopamine fiber density and decreases D1-mediated regulation of pyramidal cell firing (Boutros et al. 2014; Trantham-Davidson et al. 2017). Similar to dopamine, the cholinergic system continues to develop during the transition from adolescence to adulthood (Carcoba et al. 2014; Nordberg et al. 1992). Also, adolescent ethanol exposure profoundly decreases the number of choline acetyltransferase-positive neurons in the basal forebrain (Boutros et al. 2014; Coleman et al. 2011; Fernandez and Savage 2017; Swartzwelder et al. 2015; Vetreno et al. 2014; Vetreno and Crews 2018). These data all suggest that vulnerability to ethanol exposure is directly related to neural processes which continue to mature during adolescence.

A second, equally important observation from the literature is that adolescent ethanol exposure alters GABA and glutamatergic neurotransmission in a brain region-dependent manner. Exposure-dependent alterations in receptor expression (mRNA or protein), phosphorylation, or localization vary across the regions are highlighted here. However, region-specific disruption in receptor expression is not specific to GABA or glutamate. For example, adolescent ethanol exposure significantly decreasing dopamine D1 and D2 protein levels in the frontal cortex but only D2 protein in the hippocampus and striatum (Pascual et al. 2009). Even subdivisions within the same region can express unique alterations. For example, spine morphology – an anatomical marker for glutamatergic synapses – is differentially impacted by adolescent exposure in hippocampal subregions like dentate gyrus and CA1 (Mulholland et al. 2018; Risher et al. 2015) or in medial prefrontal cortical areas like prelimbic and infralimbic cortex (Jury et al. 2017). It is perhaps no surprise then that synaptic function and circuits are likewise altered by adolescent ethanol in a regionally-specific manner.

A limitation associated with the current preclinical literature is that processes maturing during adolescence remain poorly defined in many instances. The focus of this review has thus been primarily on adolescent ethanol modulation of GABA and glutamate neurotransmission in the context of executive function, memory, and emotion – processes well recognized as exhibiting profound development during adolescence. As highlighted in the Introduction, adolescents and adults also differ in self-administration behavior and are differentially sensitive to ethanol sedation/intoxication and aversion. Circuits and synaptic processes related to these behaviors are therefore important targets for future preclinical studies. For example, outside of the well described dopamine circuits influencing activity of nucleus accumbens neurons (Doremus-Fitzwater and Spear 2016), glutamate and/or GABA signaling in the lateral hypothalamus, dorsal striatum, central amygdala all regulate ethanol self-administration (Hwa et al. 2017). The circuits/processes controlling ethanol sedation/intoxication are less well-defined, but acute ethanol inhibits nicotinic receptors in brainstem nuclei involved with motor performance, attention, and sleep (McDaid et al. 2016). In a recent study with fMRI in humans, ethanol impairment of simulated driving behavior had its greatest effect on hemodynamics in cingulate/orbitofrontal circuits involved with attention and cerebellar/motor cortical circuits involved with gross and fine motor control (Meda et al. 2009). Finally, recent work

focused on aversion-like behavior suggests that projections from the lateral habenula (LHb) to the ventral tegmental area (VTA) are promising targets for study as well. The lateral habenula provides glutamatergic input to GABAergic neurons in the rostromedial tegmental nucleus (RMTg) which negatively regulate VTA dopamine neurons projecting to the nucleus accumbens (Lammel et al. 2012). Optogenetic and lesion studies suggest this pathway is intimately involved with conditioned taste aversion (Haack et al. 2014; Lammel et al. 2012). Importantly, electrical stimulation of LHb reduces voluntary ethanol drinking (Li et al. 2016); neuron activity within the LHb-RMTg pathway is highly correlated with ethanol conditioned taste aversion (Glover et al. 2016). While additional circuit mapping studies are needed to understand the brain regions controlling ethanol sedation/intoxication and aversion, a focus on the adolescent development of the systems/circuits will help define synaptic mechanisms impacted by ethanol exposure during this vulnerable period.

Finally, it is worth noting that a detailed neurophysiological understanding of how sex regulates adolescent vulnerability to ethanol is largely missing in preclinical studies. Clinical data suggest the effects of sex are likely to be subtle. For example, sex does not predict lifetime drinking trajectory (i.e., those that go on to develop drinking problems as adults) in adolescent drinkers (Warner et al. 2007). However, lifetime prevalence for alcohol abuse and dependence following adolescent drinking tends to be lower for females compared to males across the entire adolescent period (Grant and Dawson 1997). Factors that influence sex-dependent drinking trajectories are likely to be subtle and potentially species-specific. For example, parental relationships appear to differentially regulate adolescent drinking in males and females, with more 'protective' or 'controlling' relationships reducing alcohol consumption in adolescent females and increasing it in males (Leung et al. 2014). Female humans tend to consume more alcohol during early adolescence with these relationships reversing to more 'adult-like' drinking (males>females) by late adolescence/early adulthood (Patrick and Schulenberg 2013). Importantly, these studies suggest that diagnostic criteria related to clinical interventions may need to be refined to address subtle differences between sexes across the adolescent period. Unfortunately, in a study examining adolescent alcohol and drug use in pediatric care settings, Sterling et al. (2012) found that adolescent males were significantly more likely than females to receive screening for alcohol use. Similar to these human studies, there is a paucity of neurophysiological data in females from preclinical studies. Behavioral studies may give some clue to potential circuits and neurotransmitter systems. For example, there are marginal sex differences with respect to cognitive function (Pavlovian conditioned approach; Madayag et al. 2017) and anxiety-like behavior (Amodeo et al. 2018). Sex differences related to ethanol locomotor impairment are also only evident in adult animals following long-term ethanol drinking that begins during adolescence (Westbrook et al. 2018). Despite this, adolescent male and female rats do differ with respect to the impact of stress (Wille-Bille et al. 2017) and social context (Varlinskaya et al. 2015) on ethanol drinking; sex interacts with social context to influence conditioned aversion to ethanol (Morales et al. 2014; Vetter-O'Hagen et al. 2009), but this may be influenced

by both the conditioning paradigm (Pautassi et al. 2011) and rat strain (Schramm-Sapyta et al. 2014). Together, this literature suggests that subtle sex differences, particularly related to affiliative and social relationships, may distinguish the vulnerability within unique adolescent populations.

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