HHS Public Access

Author manuscript

Prog Neurobiol. Author manuscript; available in PMC 2019 December 01.

Published in final edited form as:

Prog Neurobiol. 2018 December ; 171: 32–49. doi:10.1016/j.pneurobio.2018.10.001.

Role of glutamatergic system and mesocorticolimbic circuits in alcohol dependence

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Abstract

Emerging evidence demonstrates that alcohol dependence is associated with dysregulation of several neurotransmitters. Alterations in dopamine, glutamate and gamma-aminobutyric acid release are linked to chronic alcohol exposure. The effects of alcohol on the glutamatergic system in the mesocorticolimbic areas have been investigated extensively. Several studies have demonstrated dysregulation in the glutamatergic systems in animal models exposed to alcohol. Alcohol exposure can lead to an increase in extracellular glutamate levels in mesocorticolimbic brain regions. In addition, alcohol exposure affects the expression and functions of several glutamate receptors and glutamate transporters in these brain regions. In this review, we discussed the effects of alcohol exposure on glutamate receptors, glutamate transporters and glutamate homeostasis in each area of the mesocorticolimbic system. In addition, we discussed the genetic aspect of alcohol associated with glutamate and reward circuitry. We also discussed the potential therapeutic role of glutamate receptors and glutamate transporters in each brain region for the treatment of alcohol dependence. Finally, we provided some limitations on targeting the glutamatergic system for potential therapeutic options for the treatment alcohol use disorders.

Keywords

Alcohol Dependence; Glutamate; Glutamate Receptors; Glutamate Transporters; NAc; PFC; Striatum; Amygdala; Hippocampus; VTA.

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1. Introduction

Alcohol use disorders (AUD) are chronic relapsing disorders with profound implications on the socioeconomic status as well as morbidity and mortality of the addict (Grant et al., 2015). The 2015 National Survey on Drug Use and Health (NSDUH) reported that about 86% of adults ages 18 and older drank alcohol during their lifetime in US (SAMHSA, 2015a). The NSDUH reported also that about 26.9% of adults ages 18 and older have been involved in binge alcohol drinking and about 7% were engaged in heavy alcohol use in US (SAMHSA, 2015d). In addition, the 2015 NSDUH reported that 15.1 million of people ages 18 and older had AUD, including 9.8 million men and 5.3 million women in US (SAMHSA, 2015b; SAMHSA, 2015c). The World Health Organization reported in 2014 that alcohol use led to more than 200 diseases and injuries associated with health conditions such as alcohol dependence, liver cirrhosis, cancers and others (WHO, 2014). Increased tendency to consume high amounts of alcohol with limited control on the consumption, despite the detrimental health outcomes associated with it, is a characteristic of the progression from an acute social drinking pattern to the state of alcohol dependence. The clinical signature of the person occasionally consuming alcohol is distinct from that of escalated alcohol intake leading to emergence of chronic compulsive alcohol seeking behavior (Koob and Volkow, 2010).

Corticostriatal circuitry involving both dopaminergic and glutamatergic projections play a critical role in the initiation and progression of dependence to most drugs of abuse (Figure 1) (Kalivas et al., 2009; Wise, 1987). The limbic subcircuit involves the prefrontal cortex (PFC), amygdala, nucleus accumbens (NAc) and ventral tegmental area (VTA); and the motor subcircuit encompasses the motor cortex, dorsal striatum and substantia nigra. The NAc acts as a gateway between these interconnected subcircuits, which collectively form the larger corticostriatal circuitry (Figure 1). Interestingly, both subcircuits play a critical role in drug dependence processes, with the limbic system being the key center for processing new information based on the motivational cues and developing new behavioral responses to those cues, while the motor subcircuit acts when the behavioral pattern to that motivational cue is already established (Kalivas et al., 2009).

Ample evidence suggests that the positive reinforcing effects of acute alcohol exposure are associated with increased dopaminergic transmission in mesocorticolimbic brain regions (Gonzales et al., 2004; Weiss and Porrino, 2002). An increase in dopaminergic neuronal firing in the VTA has been reported following exposure to alcohol (Diana et al., 1993; Gessa et al., 1985; Morzorati et al., 2010). Concomitantly, alcohol also increases dopamine release in the pathway projecting to the NAc (Clarke et al., 2014). In accordance, several studies have reported that an increase in dopamine release in the NAc mediates the initial positive reinforcing effects of alcohol [For review see (Koob and Le Moal, 2001; Wise and Rompre, 1989)],(Bainton et al., 2000; Yoshimoto et al., 1992a). Similarly, the increase in dopamine release in the NAc was observed in humans consuming alcohol, as compared to those consuming non-alcoholic beverage, using a PET scanning technique (Boileau et al., 2003). It is noteworthy that the NAc, PFC and amygdala receive dopaminergic inputs from the VTA (Kalivas and O'Brien, 2008). In addition, repeated alcohol intake also increases dopamine release into PFC and amygdala, which facilitates learning behavior associated with drug

dependence (Trantham-Davidson and Chandler, 2015). It has been suggested that the glutamatergic projections from PFC to NAc play a crucial role in retrieving and integrating drug-associated memories (Kalivas and Volkow, 2005; Kalivas and O'Brien, 2008). These glutamatergic projections from the PFC to NAc are implicated in the initiation and learning of drug seeking behaviors and are essential for reinstituting biological reward behavior (Kalivas and Volkow, 2005; Moussawi and Kalivas, 2010). Glutamatergic projections from the amygdala and hippocampus to the NAc and PFC are involved in the behavioral response to the stimuli (craving) from previously established associations with motivational and neutral cues (Kalivas and Volkow, 2005). Supporting this hypothesis, several studies have reported an elevation of extracellular glutamate levels in the NAc during and after -alcohol exposure (Das et al., 2015; Ding et al., 2013; Hinton et al., 2012; Melendez et al., 2005; Pati et al., 2016; Saellstroem Baum et al., 2006; Szumlinski et al., 2007).

Importantly, during alcohol withdrawal, rats showed a marked increase in extracellular glutamate concentration in the NAc (Saellstroem Baum et al., 2006). These studies indicate that repeated exposure to alcohol and withdrawal from chronic alcohol exposure increases extracellular glutamate level in the NAc (Figures 2–3). The increase in glutamate levels in mesocorticolimbic brain regions during abstinence is considered critical for relapsing to alcohol after long-term exposure to this drug. It is suggested that the PFC sends projections, which can trigger relapse (Goldstein and Volkow, 2002), to the NAc, a brain region implicated in goal-directed behavior, including drug seeking (Childress et al., 1999). Interestingly, glutamate, a key excitatory neurotransmitter in the brain, is suggested to be the principal driver of PFC neurons involved in relapse to drug abuse by releasing this glutamate into the NAc (Capriles et al., 2003; Childress et al., 1999; Goldstein and Volkow, 2002; McFarland et al., 2003; Shalev et al., 2002). The amygdala, another brain region involved in drug seeking and relapse (Di Ciano and Everitt, 2004), sends glutamatergic projections to several other brain regions, including PFC as well as receives glutamatergic projections from these brain region (Kalivas et al., 2009; Marek et al., 2013). Drug seeking behaviors and relapse require glutamate release into the NAc and the source of this glutamate is the amygdala and PFC (Kalivas et al., 2009). Another important brain region involved in relapse to drug abuse is the hippocampus (Fuchs et al., 2005; Vorel et al., 2001), the memory center of the brain; which also sends glutamatergic projections into the NAc and PFC (Britt et al., 2012; Gigg et al., 1994).

The effects of acute and chronic exposure to alcohol on the glutamatergic system have been investigated in several studies (Basavarajappa et al., 2008; Das et al., 2015; Ding et al., 2012; Griffin et al., 2015; Reynolds and Brien, 1994). While acute exposure to alcohol reduced the glutamatergic activity and stimulated GABAergic activity, chronic alcohol exposure resulted in the opposite effects [For review see (Rao et al., 2015a)]. Acute alcohol exposure inhibited the glutamate transmission in part by activating the endocannabinoid receptor-1 in the hippocampal neurons (Basavarajappa et al., 2008). In accordance, in guinea pigs, acute exposure to alcohol induced depressing effects on glutamate release in the hippocampus (Reynolds and Brien, 1994). Moreover, acute exposure to high dose of alcohol (2.0 g/kg) reduced the extracellular glutamate concentration, while lower dose of alcohol (0.5 g/kg) increased extracellular glutamate concentration in the posterior VTA (Ding et al., 2012). This latter study also found that repeated exposure to alcohol for seven days

increased extracellular glutamate concentration and reduced glutamate clearance in the posterior VTA in rats. This is in agreement with a recent study showing that continuous long-term exposure to alcohol reduced GLT-1 expression and increased extracellular glutamate in the NAc in rats (Das et al., 2015), which indicates that chronic alcohol exposure increased extracellular glutamate concentration through reducing glutamate uptake. However, chronic limited access to alcohol elevated extracellular glutamate concentrations (Griffin et al., 2015) without altering the expression of glutamate transporters (Alasmari et al., 2018; Griffin et al., 2015; Stennett et al., 2017).

In this review, we discussed the available evidence documenting the effects of alcohol on glutamatergic system. We also discussed the genetic aspect of alcohol associated with changes in glutamate and reward circuity. More specifically, we discussed the effects of alcohol exposure on the expression and function of glutamate receptors and transporters in mesocorticolimbic brain regions. We also discussed the potential therapeutic effects of pharmacological modulators of glutamate receptors (Table 1) and transporters, microinjected directly into specific mesocorticolimbic brain regions, on the attenuation of alcohol dependence.

2. Alcohol and Glutamate Receptors

Glutamate receptors have been categorized into two different categories based on the structure and function: metabotropic glutamate receptors (mGluRs) and ionotropic glutamate receptors (iGluRs). mGluRs are G-protein coupled receptors (GPCR) that mediate the slow excitatory and inhibitory glutamatergic transmission through intracellular second messenger systems. In contrast, iGluRs are ligand-gated ion channels, which regulate rapid glutamatergic neurotransmission in the brain (Meldrum, 2000). Acute and chronic alcohol exposure has been shown to be associated with dysregulated glutamatergic neurotransmission. Not surprisingly, glutamate receptors (both presynaptic and postsynaptic) have been extensively implicated in the development and progression of alcohol dependence [For review see (Goodwani et al., 2017)].

2.1 Alcohol and iGluRs

There have been inconsistent reports of the effect of alcohol exposure on N-methyl-D-aspartate receptor (NMDAR) expression. For example, a few studies reported a reversible upregulation of NMDARs in preclinical models exposed to alcohol (Kroener et al., 2012; Ron, 2004) while others have reported a decrease in NMDAR expression after alcohol exposure (Abrahao et al., 2013). Specifically, chronic exposure to alcohol increases the expression of GluN2B mRNA as well as protein expression in both *in-vitro* and *in-vivo* models (Kash et al., 2008; Kash et al., 2009; Obara et al., 2009; Roberto et al., 2006;Snelletal.,1996).In addition, increased expression of GluN1 subunit of NMDAR was found following chronic alcohol exposure (Roberto et al., 2006; Trevisan et al., 1994).

Despite the inconsistency in reports examining the effects of alcohol on NMDAR expression, there is some consensus regarding its effect on NMDAR functions such as synaptic plasticity and mediating calcium influx. Generally, acute alcohol exposure has an inhibitory effect while chronic exposure leads to an increase in NMDAR-mediated synaptic

plasticity as well as NMDAR-mediated glutamatergic synaptic transmission [For review see (Goodwani et al., 2017)]. Interestingly, another study reported that exposure and withdrawal from alcohol induced a long-lasting increase in GluN2B NMDAR activity which facilitate alcohol consumption (Chen et al., 2011). Conversely, inhibiting the GluN2B-NMDAR using an antagonist, ifendropil, resulted in a significant decrease in alcohol consumption in rats (Wang et al., 2010). These findings suggest that GluN2B-NMDAR plays a critical role in excessive alcohol intake.

Supporting the hypothesis that alcohol regulates expression of iGluRs, alcohol also increases α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) expression as well as its synaptic localization in several in vitro and in vivo models (Brückner et al., 1997; Chandler et al., 1999; Christian et al., 2012; Wang et al., 2012). The increase in AMPAR expression is accompanied by an increase in neuronal activity dependent pentraxin (NARP) levels, which further facilitates synaptic distribution of AMPAR, a critical element for regulating neuroplasticity (Ary et al., 2012). Long-term exposure to chronic alcohol upregulates GluA1 subunit of AMPAR (Ortiz et al., 1995). In addition, chronic alcohol exposure also led to upregulation of GluA2/3 subunit (Brückner et al., 1997; Chandler et al., 1999). Acute alcohol exposure also inhibits AMPAR functionally, but this inhibition is attained at much higher alcohol concentrations than that required for NMDAR inhibition (Akinshola et al., 2003; Dildy-Mayfield and Harris, 1992; Lovinger et al., 1989; Möykkynen et al., 2003).

Chronic exposure to alcohol enhances AMPAR-mediated currents in several brain regions (Ary et al., 2012; Christian et al., 2012; Heikkinen et al., 2009; Läck et al., 2007). In addition, chronic alcohol exposure also enhanced AMPAR-evoked Ca2+ influx in developing neurons (Netzeband et al., 1999). As with AMPAR, kainate receptor (KAR) expression was increased following exposure and withdrawal from alcohol *in vitro* (Carta et al., 2002). However, another study reported that there was no change in the KAR expression following exposure to alcohol (Chandler et al., 1999). Functional inhibition of KAR following exposure to alcohol has been reported in several studies (Dildy-Mayfield and Harris, 1995; Martin et al., 1995; Weiner et al., 1999). Pharmacological inhibition of AMPAR/KAR with mixed antagonists, CNQX and NBQX, attenuated alcohol seeking behavior as well as cue-induced reinstatement to alcohol (Bäckström and Hyytiä, 2004; Czachowski et al., 2012; Stephens and Brown, 1999). However, this effect may not necessarily be specific to alcohol (Roberto and Varodayan, 2017). In contrast to CNQX, a AMPAR positive allosteric modulator, aniracetam, increased alcohol seeking-behavior determined by alcohol self-administration and cue-induced reinstatement of alcohol in animal models (Cannady et al., 2013). In summary, a general inhibition in the neuronal excitability is observed with acute alcohol exposure, while chronic alcohol exposure increased iGluRs functioning [For review see (Roberto and Varodayan, 2017)]. Therefore, counteracting these iGluRs mediated alterations in neuronal excitability and synaptic activity appears to be a promising therapeutic approach in treating acute and chronic effects of alcohol exposure.

2.2 Alcohol and mGluRs

Unlike iGluRs, alcohol exposure has only modest effect on mGluRs. Group I (mGluR1 and mGluR5) mGluRs and Group II (mGluR2/3) mGluRs are widely studied for their roles in alcohol dependence processes. Group I mGluRs are predominantly localized in the post-synaptic neurons regulating slow excitatory neurotransmission while group II mGluRs are mainly located in pre-synaptic neurons, with limited post-synaptic and glial localization, and are involved in slow inhibitory neurotransmission [For review see (Goodwani et al., 2017)]. Upregulation of group I mGluRs (mGluR1 and mGluR5) following prolonged exposure to alcohol has been extensively reported (Cozzoli et al., 2012; Cozzoli et al., 2014; Obara et al., 2009; Szumlinski et al., 2008). Increase in mGluR5 expression following alcohol exposure was also associated with activation of its intracellular pathways including phosphatidylinositol 3-kinase (Cozzoli et al., 2009; Cozzoli et al., 2012). In contrast, excessive alcohol exposure downregulated *Grm2* (mGluR2 gene) transcripts, which resulted in an mGluR2 deficit leading to an increase in reinstatement to alcohol. This effect was abolished after restoring mGluR2 expression in the infralimbic cortex (Meinhardt et al., 2013). This finding has been confirmed in several preclinical pharmacological studies as well. For example, pharmacological blockade of mGluR1 (by JNJ16259685, EMQMCM, or CPCCOEt) resulted in a decrease in alcohol self-administration, condition place preference (CPP) and withdrawal associated seizures (Besheer et al., 2008a; Kotlinska et al., 2011). Similarly, the mGluR5 antagonists 3-((2-Methyl-4-thiazolyl)ethynyl) pyridine (MTEP) and 2-Methyl-6-(phenylethynyl) pyridine (MPEP) diminished alcohol self-administration, cue-induced alcohol seeking, relapse, withdrawal and CPP (Adams et al., 2008; Backstrom et al., 2004; Backstrom and Hyytia, 2006; Besheer et al., 2008b; Besheer et al., 2010; Bird et al., 2008; Blednov and Adron Harris, 2008; Cowen et al., 2005; Cowen et al., 2007; Gupta et al., 2008; Hodge et al., 2006; Kotlinska et al., 2011; Lominac et al., 2006; Olive et al., 2005; Schroeder et al., 2005; Schroeder et al., 2008; Sidhpura et al., 2010). In contrast, mGluR2/3 plays a critical role in reducing the release of glutamate from pre-synaptic glutamatergic neurons which might attenuate drug seeking behavior (Moran et al., 2005). mGluR2/3 agonists LY379268 and LY404039 attenuated alcohol self-administration, cue-induced alcohol seeking as well as behaviors associated with relapse to alcohol (Bäckström and Hyytiä, 2005; Rodd et al., 2006; Sidhpura et al., 2010; Zhao et al., 2006). Interestingly, a study comparing alcohol-preferring (P) versus non-preferring (NP) rats reported that mGluR2/3 expression was lower in alcohol-naïve P rats compared to NP rats. In addition, there was also a decrease in group III mGluR (mGluR4, mGluR7 and mGluR8) expression in alcohol-naïve P rats as compared to NP rats (McBride et al., 2012; McBride et al., 2013). Alcohol exposure also decreased mGluR7 mRNA expression in rats without affecting mGluR4 and mGluR8 (Simonyi et al., 2004). In summary, the literature suggests that antagonism of post-synaptic group I mGluRs and/or agonism of group II mGluRs restores glutamate homeostasis, which attenuates alcohol seeking behavior in several preclinical models (Backstrom et al., 2004; Kufahl et al., 2011; Lum et al., 2014).

3. Alcohol and Glutamate Transporters

3.1 Expression of glutamate transporters

Glutamate transporters are high-affinity transporters belonging to the solute carrier 1 family (Danbolt, 2001). These transporters co-transport three Na^+ and one H^+ along with counter-transport of K^+ which creates a concentration gradient to transport glutamate out of the synapse into the glial cells (mainly astrocytes) (Goodwani et al., 2017). Glutamate transporters regulate synaptic and extra synaptic glutamate levels. There are five known glutamate transporters (also known as excitatory amino acid transporters; EAATs), which have been characterized based on their expression patterns and functions. The EAAT1 (also known as glutamate/aspartate transporter, GLAST) is largely expressed in the cerebellum, while expression of EAAT2 (also known as glutamate transporter 1, GLT-1) is predominantly localized in the forebrain (Danbolt, 2001). Glutamate transporters expression is region and cell subtype-specific, facilitating excitatory neurotransmission via regulation of glutamate homeostasis (Danbolt, 2001; Lehre and Danbolt, 1998; Mitani and Tanaka, 2003).

Unlike GLAST and GLT-1, which are expressed mainly in the astrocytes, EAAT3 is primarily expressed in neurons with more homogeneous but limited expression in the astrocytes. This transporter is highly expressed during the brain development, suggesting that EAAT3 plays an important role during early neuronal development (Bar-Peled et al., 1997; Haugeto et al., 1996; Nieoullon et al., 2006; Torp et al., 1994). In addition, like EAAT2, EAAT4 is primarily expressed in cerebellum (Bar-Peled et al., 1997; Dehnes et al., 1998). Expression of EAAT5 is limited to the retinal bipolar cells and rod photoreceptor (Wersinger et al., 2006). Cystine-glutamate antiporter (xCT), another glial glutamate transporter, also plays an important role in glutamate homeostasis (Moran et al., 2005). xCT regulates extra-synaptic glutamate levels by exchanging extracellular cystine for intracellular glutamate in astrocytes (Figures 2–3) (Baker et al., 2002).

3.2 Alcohol exposure modulates glutamate transporters

Accumulating evidence demonstrates the effects of alcohol exposure on the expression and functions of the glutamate transporters. For example, acute alcohol exposure increased EAAT3 (Kim et al., 2003; Kim et al., 2005) as well as EAAT4 (Park et al., 2008) activity; however, EAAT3 activity was also reduced following incubation of Xenopus oocytes to alcohol for 24 to 96 hours (Kim et al., 2003; Kim et al., 2005). These studies suggest that the effect of alcohol exposure on these glutamate transporters might depend on the duration of exposure and type of model being employed. Furthermore, we have shown that chronic alcohol intake in male alcohol preferring (P) rats using a five-week continuous two-bottles free-choice paradigm results in a significant decrease in expression of GLT-1 and xCT, but not GLAST, in brain regions associated with reward (Aal-Aaboda et al., 2015; Alhaddad et al., 2014; Hakami et al., 2016b; Rao et al., 2015b), (Figure 2B). Moreover, studies from our laboratory also found that GLT-1 and xCT upregulators attenuated chronic and relapse-like alcohol drinking behavior. Additionally, we have shown that this upregulation of GLT-1 by β-lactam antibiotics is accompanied by activation of Akt/NF-κB pathway (Figure–3) (Aal-Aaboda et al., 2015; Alasmari et al., 2015a; Alhaddad et al., 2014; Qrunfleh et al., 2013; Rao et al., 2015b; Rao et al., 2015c; Sari and Sreemantula, 2012). However, additional

studies employing specific genetic knockdown or pharmacological inhibition of each of these signaling pathways are required to understand the mechanism of upregulation of GLT-1 by these compounds. Overall, these studies emphasize the importance of glutamate transporters in the alcohol addiction process.

4. Effects of alcohol on the glutamate homeostasis in the nucleus accumbens (NAc)

Nucleus accumbens, NAc, also known as the central reward brain region, receives glutamatergic inputs from the amygdala, hippocampus and PFC (LaLumiere and Kalivas, 2008; McFarland et al., 2003) (Figure 1). It is important to note that NAc is anatomically divided into two sub-compartments: the core and the shell, which are thought to be organized in a specific pattern to serve different functions (Zahm and Brog, 1992). The VTA dopaminergic innervation into the NAc shell is critical in establishing and maintaining learning and motivation events associated with alcohol dependence.

With distinct interconnections with anterior cingulate cortex (ACC) and orbital frontal cortex (OFC), the glutamatergic projections into the NAc core mediate motivational impulses which induce learned behaviors-associated with alcohol dependence (Kalivas and Volkow, 2005). Therefore, the effects of alcohol in NAc are not limited to an increase in glutamatergic neurotransmission, but also extend to an enhancement in other neurotransmitters such as dopamine and serotonin (Boileau et al., 2003; Melendez et al., 2005; Olive et al., 2001; Yoshimoto et al., 1992b). Interestingly, studies that investigated the effects of voluntary alcohol intake (acute and chronic) on extracellular glutamate levels reported an elevation in the extracellular glutamate levels in the NAc (Das et al., 2015; Ding et al., 2013; Melendez et al., 2005; Pati et al., 2016; Szumlinski et al., 2007). These findings were also in agreement with an increase in extracellular glutamate levels in the NAc following forced alcohol administration in rodent models of alcoholism (Carrara-Nascimento et al., 2011; Ding et al., 2012; Kapasova and Szumlinski, 2008; Melendez et al., 2005). Additionally, there is an increase in extracellular glutamate levels in the NAc during cue-induced reinstatement of alcohol-seeking behavior compared to food-seeking behavior (Gass et al., 2011). However, this alcohol-induced increase in extracellular glutamate levels may be a result of either an increase in glutamate release into the synaptic cleft (Pierce et al., 1996) or decrease in glutamate uptake from the synaptic cleft or both (Melendez et al., 2005). In summary, alcohol induces an increase in extracellular glutamate levels in the NAc thereby dysregulating the glutamate homeostasis.

4.1 Role of glutamate receptors in the NAc in alcohol dependence

High abundance of mGluRs has been reported in the NAc (Testa et al.,1994).Specifically, group I and group II mGluRs (mGluR5, mGluR2/3) in NAc are very well studied in the context of alcohol dependence. The presynaptic mGluR2/3, when activated by glutamate, provides a negative feedback to decrease the glutamate release leading to a decrease in the glutamatergic neurotransmission (Moran et al., 2005). Several studies have targeted the negative feedback loop to decrease the extracellular glutamate levels by stimulating the presynaptic mGluR2/3, which could attenuate alcohol-seeking behavior. For example,

microinjections of the mGluR2/3 agonist LY379268 directly into the NAc attenuated alcohol self-administration in rats (Besheer et al., 2010). Another study found that pre-treatment with the mGluR2/3 agonist LY404039 reduced alcohol-seeking and relapse-like behavior without affecting the maintenance of operant alcohol self-administration in rats (Rodd et al., 2006), as well as alcohol intake in both dependent and non-dependent mice (Kapasova and Szumlinski, 2008). In contrast, the mGluR2/3 agonist LY379268 attenuated alcohol self-administration and reinstatement. However, the effective doses of this compound also had motor-suppressant side effects in animals (Bäckström and Hyytiä, 2005). Treatment with mGluR2/3 antagonist, LY341495 did not affect operant responses to alcohol in P rats (Schroeder et al., 2005). This suggests that alcohol-seeking behavior is more sensitive to mGluR2/3 agonists compared to its antagonists. Although the available literature provides compelling evidence of the role of mGluR2/3 in alcohol dependence, there is less known about the effects of alcohol exposure on the expression and function of mGluR2/3 in the NAc, or the role of mGluR2/3 agonists in restoring glutamate homeostasis by decreasing glutamate release into the synaptic cleft. Therefore, further studies are warranted to investigate the extent of the specific contribution of NAc mGluR2/3 in context of AUD.

Another strategy employed to target mGluRs in context of alcohol dependence is antagonizing group I mGluRs in the NAc. Withdrawal following binge-alcohol consumption in adult mice led to an increase in mGluR1 and mGluR5 expression in the NAc shell (Lee et al., 2016). Additionally, the latter study reported that binge alcohol consumption also led to an increase in protein kinase C epsilon type (PKCε) level in the NAc core. It is noteworthy that activation (by phosphorylation) and translocation of PKCε is critical in regulating the cellular localization of mGluR5 in the NAc (Schwendt and Olive, 2017). Moreover, binge alcohol consumption was associated with an increase in the NAc levels of phospho-Ser729-PKCε (Cozzoli et al., 2016). Furthermore, inhibition of PKCε translocation decreased binge alcohol consumption in mGluR1/5 dependent manner (Cozzoli et al., 2016). Therefore, it has been concluded that chronic alcohol intake resulted in a functional increase in mGluR5-Homer2-PI3K signaling pathway in the NAc, an effect inhibited by intra-NAc infusions of mGluR5 antagonist, MPEP (Cozzoli et al., 2009). PKCε is a critical downstream of PI3K-mGluR5 signaling pathway and this cascade in NAc is required for attenuation of alcohol consumption by mGluR5 antagonism (Gass and Olive, 2009; Olive et al., 2005). Interestingly, systemic administration of mGluR5 antagonist MPEP decreased cue-induced reinstatement of alcohol-seeking behavior and ERK1/2 phosphorylation in the NAc (Schroederetal.,2008). It is important to note that this study reported an increase in ERK1/2 phosphorylation in the NAc shell, which was associated with a reinstatement of alcohol-seeking behavior in rats. In a different study, MPEP administered locally into the NAc attenuated alcohol self-administration in P rats, without affecting the response to sucrose or water (Besheer et al., 2010). Furthermore, blocking mGluR5 in the NAc also reduced cue-induced reinstatement of alcohol seeking behavior in rats (Sinclair et al., 2012). onversely, upregulation of mGluR1 in the NAc shell was also reported following stress-alcohol cross-sensitization in mice (Quadir et al., 2016). Intra-NAc infusion of JNJ-16259685, a negative-allosteric modulator of mGluR1, decreased alcohol consumption in C57BL/6J mice. The same study demonstrated the ability of mGluR1 negative allosteric modulators in decreasing alcohol consumption was associated with modulation of mGluR1-Homer2-PLC signaling in

the NAc shell (Lum et al., 2014). Therefore reported literature suggests that the activity of mGluR1/5 and its signaling pathways in the NAc are modulated by alcohol.

Similar to mGluRs, iGluRs are also expressed in the NAc (Nie et al., 1994). NMDAR-dependent long-term depression (LTD) in the NAc is associated with neuronal plasticity. Disruption of NMDAR dependent LTD in the NAc is suggested to be a compensatory neuroadaptation in response to chronic alcohol consumption (Abrahao et al., 2013; Jeanes et al., 2011; Jeanes et al., 2014). Alcohol locomotor sensitization is associated with altered NMDAR synaptic plasticity in the NAc, which may lead to an increase in alcohol consumption (Abrahao et al., 2013). Several studies have also reported an inhibition in NMDA function following acute alcohol exposure (Maldve et al., 2002; Zhang et al., 2005; Zhang et al., 2006). Another study found that alterations in NMDAR functions in the NAc mediated excessive alcohol intake in mice exposed to chronic intermittent alcohol exposure paradigm (Renteria et al., 2017). Moreover, microinjection of a competitive NMDAR antagonist directly into the NAc reduced alcohol self-administration in rats (Rassnick et al., 1992) as well as alcohol conditional place preference (CPP) (Gremel and Cunningham, 2009). Withdrawal following 2-week period of binge alcohol drinking in mice resulted in alcohol-seeking behaviors and this was associated with increase in the expression of GluN2b subunit of NMDAR in the NAc shell (Lee et al., 2016). Additionally, the same study also reported an increase in expression of Ca^{2+}/calmodulin-dependent protein kinase II (CAMKII). Upon phosphorylation and activation, CAMKII mediates NMDAR-dependent long term potentiation (LTP), which leads to forward trafficking of AMPAR to the synaptic membrane [For review see (Morisot and Ron, 2017)]. Moreover, CAMKII has been directly linked to establishment of alcohol-drinking behaviors (Easton et al., 2013). Importantly, stress-alcohol cross-sensitization displays an increase in GluN2b expression in the NAc shell, however GluN2b expression was decreased in the NAc core in mice (Quadir et al., 2016).

A prior study reported that chronic alcohol exposure increased the expression of GluN2A and GluN1 subunits of NMDAR in the NAc of low-sensitized mice compared to high-sensitized mice. The study classified these mice into low and high sensitization based on locomotor activity indicating that GluN2A and GluN1 subunits of NMDAR are implicated in mediating locomotion following repeated-alcohol exposure (Nona et al., 2014). Alcohol exposure also increased phosphorylation (activation) of GluA1 S831 subtype of AMPAR (pGluA1 S831) in the NAc, however the contribution of activated NAc AMPAR in escalating alcohol consumption appeared to be limited in this study since intra NAc core infusion of AMPAR positive modulator did not affect alcohol reinforcement (Cannady et al., 2017). Following chronic intermittent alcohol exposure, there is a marked decrease in GluA2-containing AMPAR, an effect associated with an increase in GluA2-lacking AMPAR. This alteration in AMPAR subunit composition is implicated in the loss of NMDAR-induced LTD in the NAc, which increased alcohol intake (Renteria et al., 2018). These data suggest that there are functional interactions between NMDA and AMPA receptors in the NAc affecting alcohol seeking behavior.

4.2 Role of glutamate transporters in the NAc in alcohol dependence

Several studies have reported an increase in synaptic glutamate in the NAc following exposure to alcohol (Ding et al., 2013; Kapasova and Szumlinski, 2008; Szumlinski et al., 2005; Szumlinski et al., 2007). This alcohol-induced increase in extracellular glutamate is associated with a decrease in glutamate uptake in the NAc (Das et al., 2015; Melendez et al., 2005). More importantly, increased glutamate levels in the NAc enhanced alcohol drinking in mice (Griffin et al., 2014). Moreover, we have shown that GLT-1 and xCT but not GLAST are downregulated in the NAc in rats exposed to alcohol in two bottle five weeks free-choice paradigm (Alhaddad et al., 2014; Hakami et al., 2016b). This effect was associated with an increase in extracellular glutamate levels in the NAc (Das et al., 2015) (see Figure 2 and 2B). Consistent with these findings, pharmacological upregulation of GLT-1, GLT-1 isoforms and/or xCT in the NAc attenuated both alcohol consumption and relapse to alcohol (Alasmari et al., 2015b; Alasmari et al., 2016; Alhaddad et al., 2014; Qrunfleh et al., 2013; Rao et al., 2015b; Rao et al., 2015c; Sari and Sreemantula, 2012). We also reported that this alcohol-induced downregulation of GLT-1 and xCT in NAc maybe regulated, at least in part, by phosphorylated-Akt (pAkt) and nuclear factor-kappa B (NF-κB) (Alhaddad et al., 2014; Rao et al., 2015b) (Figure 4). These findings indicate that these signaling pathways are critical in downregulatory effects of alcohol on the glial glutamate transporters.

In order to confirm the involvement of glutamate transporters in alcohol dependence, pharmacological strategies have been employed to reverse deficits in transporters expression. For example, β-lactam antibiotics and (R)-(−)-5-methyl-1-nicotinoyl-2-pyrazoline (MS-153) induced reversal of GLT-1 and xCT downregulation following alcohol exposure also increased Akt phosphorylation and NF-κB levels in the NAc (Alhaddad et al., 2014; Rao et al., 2015b; Rao et al., 2015c) (Figure 4). In summary, these findings provide critical evidence of the therapeutic effects of pharmacological modulation of glutamate transporters to restore the glutamate homeostasis in the NAc on the attenuation of alcohol seeking behaviors, including chronic and relapse-like alcohol drinking.

5. Effects of alcohol on the glutamate homeostasis in the prefrontal cortex (PFC)

PFC is connected to NAc and VTA by both dopaminergic as well as glutamatergic projections. The dopaminergic neurons originating from the VTA project dopaminergic inputs to the PFC and these projections mediate the initial rewarding effects associated with drugs of abuse (D'Souza, 2015; Geisler and Zahm, 2005; Watabe-Uchida et al., 2012). In contrast, VTA and NAc receive glutamatergic projections originating from the PFC (Filip and Cunningham, 2003; Kalivas et al., 2009). The glutamatergic projections from PFC to VTA regulate the dopaminergic transmission in the VTA and therefore can regulate rewarding effects associated with drugs of abuse (Overton and Clark, 1997). However, the glutamatergic projections from the PFC into the NAc are implicated in the initiation of drug dependence as well as learning associated with drug-seeking behavior (Goodwani et al., 2017; Kalivas and Volkow, 2005; Moussawi and Kalivas, 2010). Apart from its interconnections with VTA and NAc, PFC also is innervated by glutamatergic projections from the hippocampus and amygdala, which are critical in the development of behavioral

responses of learning-associated with drug use (Kalivas and Volkow, 2005; McDonald, 1996; Parent et al., 2010).

In addition to the possible effects of alcohol on the glutamate homeostasis in the PFC, alcohol was found also to affect the neuronal activity of the PFC. Exposure to alcohol decreased the neuronal activity in PFC *in vivo* and in neuronal slice cultures. This decrease in neuronal activity was further characterized by alcohol's diminishing effects on the amplitude and duration of the spontaneous membrane depolarizations in the PFC (Tu et al., 2007). In humans, alcohol exposure is associated with a decrease in prefrontal cortical excitability (Kahkonen et al., 2003). In addition to its effect on glutamatergic system, chronic exposure to alcohol also led to loss of dopamine receptors (D2 and D4) signaling in the medial PFC (Trantham-Davidson et al., 2014). Glutamate release dysregulation was observed in the medial PFC of adult rats following acute intraperitoneal alcohol injection (1 g/kg) (Mishra et al., 2015). While another study found that alcohol craving is associated with significant increase in glutamate levels in the dorsolateral PFC (Frye et al., 2016b). Pyramidal glutamatergic neurons in the medial PFC are involved in the development of alcohol withdrawal (George et al., 2012). The dysregulation of glutamatergic projections from and into the PFC might be involved in the development of alcohol dependence, craving, withdrawal and relapse.

5.1 Role of glutamate receptors in the PFC in alcohol dependence

Several studies have reported compelling evidence confirming involvement of glutamatergic receptors in the PFC in maintenance of dependence to alcohol (Gass et al., 2014; Pickering et al., 2007). A study employing a mGluR5 positive allosteric modulator CDPPB [3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)] reported that activation of mGluR5 facilitated alcohol-extinction as well as decreased cue-induced reinstatement to alcohol seeking. Conversely, infusion of mGluR5 antagonist, 3-((2-methyl-4-thiazolyl)ethynyl) pyridine in the infralimbic cortex, but not prelimbic cortex, inhibited CDPPB facilitation of extinction (Gass et al., 2014). Moreover, this study found an increase in amplitude of NMDA currents in the PFC during the extinction period of alcohol seeking, and this effect was partially abolished after administration of mGluR5 positive allosteric modulator. Since long-term alcohol exposure increases ratio of NMDAR to AMPAR current as well as NMDAR subunits expression without affecting AMPAR subunit in the PFC (Kroener et al., 2012), these findings indicate that the expression of NMDAR is more sensitive to chronic alcohol exposure as compared to AMPAR.

Further supporting the role of iGluRs in the PFC, alcohol exposure dampens NMDAR-mediated excitatory postsynaptic currents reversibly at toxic concentrations. These data indicate that NMDA -mediated excitatory postsynaptic responses in the PFC maybe susceptible to acute high dose exposure to alcohol. Interestingly, there was no notable effect of alcohol on the AMPAR-mediated electrical activity in the PFC (Weitlauf and Woodward, 2008). However, a positive correlation between alcohol consumption and AMPA GluR1 receptor subunit mRNA expression in the PFC was reported in rats (Pickering et al., 2007). Taken together, targeting mGluRs or iGluRs in the PFC could provide important therapeutic benefits for potential treatment of alcohol addiction.

5.2 Role of glutamate transporters in the PFC in alcohol dependence

It is suggested that motivational effects of chronic exposure to drug of abuse may lead to a significant decrease in GLT-1 expression in the NAc but not in the PFC (Reissner and Kalivas, 2010). In line with this, we reported that exposure to alcohol for five weeks did not affect GLT-1 protein levels in the PFC (Alhaddad et al., 2014; Sari and Sreemantula, 2012). However, several studies from our laboratory showed that β-lactam antibiotics (e.g. ceftriaxone) attenuated chronic and relapse-like alcohol drinking in part by upregulatory effects on GLT-1 and xCT expression in the PFC (Alasmari et al., 2015a; Alasmari et al., 2016; Alhaddad et al., 2014; Hakami et al., 2016a; Qrunfleh et al., 2013; Rao et al., 2015b; Rao et al., 2015c). Our laboratory also reported that β-lactam antibiotics increased levels of pAkt and NF-κB in PFC and consequently reduced alcohol consumption (Rao et al., 2015b; Rao et al., 2015c) (Figure 4). Furthermore, ceftriaxone, a β-lactam antibiotic, was able to reduce the level of IκBα, an inhibitor of NF-κB, in the PFC (Rao et al., 2015c) (Figure 4). Importantly, stimulation of xCT antiporter can increase intracellular cystine level, which is further involved in the biosynthesis of glutathione (GSH) via γ-GlutamylCysteine synthetase and glutathione synthetase enzymes sequentially (Lewerenz et al., 2009). It is important to note that ceftriaxone treatment for seven days increased GSH contents in the brain (Amin et al., 2014). Moreover, GSH induces neuroprotective and antioxidant effects through inhibitory effects on reactive oxygen species (Lewerenz et al., 2009) (Figure 3).

6. Effects of alcohol on the glutamate homeostasis in the striatum

Several studies have investigated the effects of alcohol exposure on the dysregulation of glutamate homeostasis in the striatum. For example, withdrawal from chronic alcohol exposure resulted in a marked increase in extracellular glutamate levels in the striatum, an effect found to be a consequence of increased glutamate release (Rossetti and Carboni, 1995). In addition, NT69L, an analog of neurotensin (NT) (8–13), reduced alcohol consumption and preference and induced alterations in glutamatergic and dopaminergic systems in mouse striatum (Li et al., 2011). The latter study reported that this compound was able to reduce alcohol-increased dopamine and glutamate extracellular levels in mice striatum.

6.1 Role of glutamate receptors in the striatum in alcohol dependence

One of the earlier studies examining the effect of alcohol withdrawal on glutamatergic neurotransmission reported an increase in the extracellular glutamate levels in the striatum after withdrawal from chronic alcohol, an effect associated with activation of NMDAR (Rossetti et al., 1999; Rossetti and Carboni, 1995). This increase in the activity of NMDAR led to the stimulation of positive feedback process, and consequently resulted in the increase in glutamate release (Rossetti et al., 1999). These findings were further substantiated by use of NMDAR antagonist, dizocilpine, which counteracted the effect of alcohol withdrawal on the extracellular glutamate levels (Rossetti et al., 1999). Furthermore, alcohol inhibits NMDAR-dependent LTP while facilitating LTD, thereby modifying the corticostriatal circuitry involved in habit-learning process (Yin et al., 2007). Interestingly, acute *ex-vivo* exposure of the dorsal striatum to alcohol resulted in an increase in a NMDAR subunit phosphorylation. This finding was associated with long-term facilitation of the GluN2B-

containing NMDARs in the dorsal striatum (Wang et al., 2007). Conversely, operant self-administration of alcohol was attenuated by GluN2B NMDAR antagonist infused directly into the dorsal striatum (Wang et al., 2007). In another study from the same laboratory, it was reported that repeated alcohol exposure induced long-lasting increase in phosphorylation and membrane localization of GluN2B NMDAR in the dorsomedial striatum (DMS). This effect was confirmed with the electrophysiological and biochemical findings observed in DMS (Wang et al., 2010). In the next study, the authors examined the role of GluR1 and GluR2 in alcohol-facilitated LTP in the DMS (Wang et al., 2012). Not surprisingly, this study reported that *ex-vivo* and systemic exposure to alcohol led to facilitation of LTP in the DMS. Interestingly, this LTP induction was accompanied by increased synaptic localization of GluA1 and GluR2, AMPAR subunits, in the DMS. Furthermore, microinjection of AMPAR blocker in the striatum attenuated alcohol self-administration but not sucrose (Wang et al., 2012). These findings suggest that alcohol facilitates LTP through alterations in the AMPAR subunits.

6.2 Role of glutamate transporters in the striatum in alcohol dependence

Similar to the NAc, withdrawal from alcohol results in downregulation of GLT-1 in striatum (Abulseoud et al., 2014). Conversely, ceftriaxone restored alcohol-downregulated GLT-1 expression in the striatum and this elevation in GLT-1 levels after ceftriaxone treatment lasted at least 7-days after ceftriaxone treatments (Abulseoud et al., 2014). In another study, ceftriaxone-induced upregulation of GLT-1 in striatum also led to reduced alcohol drinking in mice (Lee et al., 2013). This latter study suggested that the modulatory role of GLT-1 in alcohol drinking behavior is mediated by type 1 equilibrative nucleoside transporter (ENT1) in the striatum. Another study from our laboratory showed that repeated oral high dose of alcohol induced a significant decrease in striatal GLT-1, but not xCT or GLAST, in Wistar rats (Alshehri et al., 2017). However, there is little known about the role of striatal glutamate transporters on the development of alcohol withdrawal symptoms as well as reinstatement of alcohol seeking. Further studies are warranted to evaluate the effects of upregulation of striatal glutamate transporters in the attenuation of alcohol seeking.

7. Effects of alcohol on the glutamate homeostasis in the amygdala

Substantial preclinical evidence confirms the role of amygdala in the development of dependence to drugs, including alcohol (Aal-Aaboda et al., 2015; Caine et al., 1995; Di Ciano and Everitt, 2004; Schroeder et al., 2008; Sinclair et al., 2012). The glutamatergic projections from the amygdala innervating the NAc regulate alcohol-seeking behavior (Gass et al., 2011; Keistler et al., 2017). Glutamate released from the amygdala into the NAc plays a key role in sensitization and seeking behaviors associated with exposure to drugs of abuse (Kalivas et al., 2009) (Figure 1). Interestingly, the amygdala receives glutamate inputs from PFC (Hubner et al., 2014). A microdialysis study reported a significant increase in the glutamate release in the amygdala after chronic alcohol treatment (Roberto et al., 2004). oreover, a significant increase in glutamate neurotransmission in basolateral amygdala was detected in rats exhibiting a high rate of alcohol self-administration (Gass et al., 2011). Furthermore, synaptic glutamate concentration and presynaptic glutamate function in the amygdala were increased in rats withdrawing from chronic alcohol exposure (Christian et

al., 2013). Another study found that acute and chronic exposure to alcohol alters glutamate homeostasis in part through cannabinoid receptor-1 in the amygdala (Robinson et al., 2016). Thus, reports suggest that dysregulated glutamate neurotransmission in the amygdala after alcohol exposure has a critical role in the development of alcohol dependence, as well as reinstatement of alcohol use.

7.1 Role of glutamate receptors in the amygdala in alcohol dependence

Direct inhibition of mGluR5 in the basolateral amygdala reduced cue-induced reinstatement of alcohol-seeking behavior (Sinclair et al., 2012). Several studies reported that chronic alcohol exposure increased the mRNA and protein expression of NMDAR as well protein expression of group 1 mGluRs in the amygdala (Obara et al., 2009; Roberto et al., 2006). Furthermore, stimulating mGluR2 attenuated cue-induced alcohol-seeking behavior and this was associated with alteration in *c-fos* expression levels in the amygdala (Zhao et al., 2006). In addition, the mGluR2/3 agonist (LY379268) attenuated conditioned reinstatement of alcohol. This was associated with a significant increase in GTPγS binding in the amygdala (Kufahl et al., 2011). Another study showed that binge alcohol consumption increased the expression of mGluR1 and mGlu 5 as well as their signaling. This effect was suggested to be a compensatory mechanism to maintain excessive alcohol consumption (Cozzoli et al., 2012; Cozzoli et al., 2014; Obara et al., 2009; zumlinski et al., 2008). The effect of alcohol on group I mGluRs was further substantiated by using intra-amygdala infusion of mGluR1 and mGluR5 blockers, which decreased binge alcohol consumption (Cozzoli et al., 2014). Chronic alcohol exposure increased the expression levels of group 1 mGluRs as well as NMDA in the amygdala (Cozzoli et al., 2014). Thus, presynaptic mGluR2/3 agonist or post-synaptic mGluR5 antagonist in the basolateral amygdala might provide a fruitful avenue to inhibit binge-alcohol intake as well as relapse to alcohol. Additionally, chronic alcohol exposure increased iGluR (AMPA and NMDA receptors) function and expression in the amygdala (Christian et al., 2012; Floyd et al., 2003; Roberto et al., 2006). Moreover, microinjection of AMPA/kainate receptor antagonist, CNQX, in the amygdala reduced alcohol induced-CPP in rats (Zhu et al., 2007). These data provide strong evidence about the potential implication of amygdala iGluRs and mGluRs in the development of alcohol dependence.

7.2 Role of glutamate transporters in the amygdala in alcohol dependence

A study from our laboratory investigated the effects of alcohol consumption for five weeks on the expression of GLT-1 and xCT in amygdala (Aal-Aaboda et al., 2015). As highlighted in Figure 2A and2B, the study found that GLT-1 and xCT in the amygdala are downregulated in male P rats exposed to alcohol for five weeks as compared to alcohol naïve group (Aal-Aaboda et al., 2015). Moreover, MS-153 upregulated both GLT-1 and xCT in amygdala and consequently reduced alcohol drinking and preference (Aal-Aaboda et al., 2015). Another study from our laboratory found that ceftriaxone reduced alcohol consumption in part by upregulating xCT and GLT-1 in the amygdala (Rao and Sari, 2014). Together, these studies demonstrate that targeting GLT-1 and xCT in the amygdala might provide promising therapeutic effect for potential attenuation of alcohol seeking behavior.

8. Effects of alcohol on the glutamate homeostasis in the hippocampus

The role of memory formation on manifesting alcohol-seeking behavior is important in alcohol dependence. The contribution of hippocampus, the memory center, in promoting dependence to alcohol and other drugs of abuse is discussed across the literature (Aal-Aaboda et al., 2015; Adcock et al., 2006; Delgado and Dickerson, 2012). It is important to note that hippocampus is anatomically connected by its glutamatergic projections into NAc, PFC and amygdala (Brittetal.,2012)(Figure 1). Moreover, several studies have reported that exposure to alcohol was associated with increased extracellular glutamate levels in the hippocampus (Moghaddam and Bolinao, 1994; Ward et al., 2009). Interestingly, one study reported that lower doses of alcohol increased extracellular glutamate levels, while exposure to higher doses of alcohol lead to a decrease in extracellular glutamate (Moghaddam and Bolinao, 1994). here have been consistent reports of elevated extracellular glutamate levels in hippocampus following withdrawal from chronic alcohol exposure (Dahchour and Witte, 1999; Dahchour and De Witte, 2003). Another study examining the effect of alcohol on the hippocampus reported death of newly formed neurons in the dentate gyrus (a subregion of hippocampus) of the adult rats after six weeks of moderate doses of alcohol (Herrera et al., 2003). In the same study, authors also reported a relationship between decreased neurogenesis following alcohol exposure and impaired hippocampal-dependent cognitive functions. Similar findings were found in another study where chronic alcohol exposure disrupted hippocampal neurogenesis by decreasing neural progenitor cell proliferation, inhibiting cell survival and altering morphological maturation of newborn neurons (He et al., 2005). In line with these reports, a reversible reduction in hippocampal volumes was demonstrated in human alcoholics using magnetic resonance imaging (MRI) (White et al., 2000). Taken together, these findings indicate that alcohol exposure can impair the hippocampal neuronal activity and development in adult and newborns.

8.1 Role of glutamate receptors in the hippocampus in alcohol dependence

Hippocampus widely expresses mGluRs and iGluRs (Khakpai et al., 2013; Pomierny-Chamioło et al., 2014). Importantly, chronic exposure to alcohol induces upregulation of MDA subunit (GluN1, GluN2A and GluN2B) expression as well as function in rat hippocampus (Smothers et al., 1997; Trevisan et al., 1994). Moreover, a non-competitive NMDAR antagonist, memantine, which is widely reported to attenuate alcohol seeking, also prevented alcohol-associated increases in NMDAR expression in the hippocampus (Maler et al., 2005). However, the exact order of sequence is unknown, whether the NMDAR normalization led to attenuation of alcohol seeking or vice-a-versa. Several reports in the literature demonstrated that hippocampal NMDAR are sensitive to inhibitory effects of alcohol (Criswell et al., 2003; Randoll et al., 1996; Yang et al., 1996), which eventually leads to attenuation of hippocampus-mediated LTP (Blitzer et al., 1990; Givens and McMahon, 1995; Morrisett and Swartzwelder, 1993). Another study revealed that alcohol significantly reduced hippocampal LTP at concentrations required to inhibit response to NMDA (Blitzer et al., 1990).

In addition to NMDAR, alcohol exposure may also affect AMPA and KA receptors in the hippocampus. More specifically, exposure to alcohol has been reported to have an inhibitory

effect on AMPA and KA receptors in the hippocampus (Carta et al., 2003; Costa et al., 2000; Crowder et al., 2002; Martin et al., 1995; Weiner et al., 1999). In hippocampus, chronic alcohol exposure increased the expression of AMPAR GluR-C subunit, without any changes in GluR-A and GluR2 subunits, in an in-situ hybridization study (Brückner et al., 1997). In addition, deletion of GluR-C AMPAR subunit in the mesocorticolimbic areas including hippocampus reduced cue-induced reinstatement of alcohol seeking behavior as well as alcohol deprivation effect in mice (Sanchis-Segura et al., 2006).

Although most of the studies on the effects of alcohol on hippocampus have been focused on iGluRs, there is substantial evidence demonstrating that alcohol also affects mGluRs signaling in the hippocampus. For example, examining the effect of chronic alcohol exposure on hippocampus in rats revealed a sub-region and receptor sub-type specific changes in mGluR mRNA expression (Simonyi et al., 2004). This study found that chronic alcohol led to a decrease in mGluR3 and mGluR5 mRNA expression in dentate gyrus. However, the same study found that chronic alcohol exposure reduced mGluR1, mGluR5 and mGluR7 mRNA expression with no effects on mGluR2, nGluR4 and mGluR8 in the CA3 subfield. It is important to note that stimulation of mGluR2 reduced cue-induced reinstatement of alcohol-seeking behavior, an effect mediated in part by increased *c-fos* gene expression in the hippocampus (Zhao et al., 2006). Moreover, it has been reported an increase in phosphoinositide hydrolysis through activating mGluR in hippocampal membranes isolated from offspring of rats treated with alcohol (Valles et al., 1995). This effect was associated with reduced binding activity of 3H-MK-801 to NMDAR suggesting an interaction between mGluRs and NMDAR in the hippocampus following alcohol exposure in offspring. We suggest here that alcohol consumption as well as the reinstatement of alcohol exposure alter glutamate receptors expression and function in the hippocampus.

8.2 Role of glutamate transporters in the hippocampus in alcohol dependence

As mentioned above, several studies reported an increase in the extracellular glutamate levels in the hippocampus following exposure to alcohol (Moghaddam and Bolinao, 1994; Ward et al., 2009). However, this effect could be attributed to either its diminished clearance of glutamate by the synaptic glutamate transporters or due to heightened release from the presynaptic glutamatergic terminals. Interestingly, a recent study from our laboratory reported that chronic exposure to alcohol decreased the expression of GLT-1 and xCT in the hippocampus (Aal-Aaboda et al., 2015) (Figure 2B). Accordingly, MS-153 reduced alcohol drinking and preference at least in part by increasing both GLT-1 and xCT expression in the hippocampus (Aal-Aaboda et al., 2015). In another study, repeated high doses of alcohol, given orally, reduced the expression of hippocampal GLT-1 but not xCT, in the Wistar rats (Alshehri et al., 2017). Although these findings point towards diminished glutamatergic clearance associated with chronic alcohol exposure in the hippocampus, further studies are warranted to unravel the mechanisms involved in downregulatory effects of chronic alcohol exposure on hippocampal glutamate transporters.

9. Effects of alcohol on the glutamate homeostasis in the ventral tegmental area (VTA)

Dopaminergic connections between VTA and the other brain regions such as NAc and PFC indicate the involvement of the VTA in the process of alcohol dependence (Brodie et al., 1990; Ding et al., 2012; Gatto et al., 1994). Additionally, VTA is innervated by the glutamatergic projections from the PFC, amygdala, pedunculopontine tegmentum, and laterodorsal tegmentum (Geisler et al., 2007; Omelchenko and Sesack, 2007) (Figure 1). Importantly, glutamatergic neurons are also found in the VTA (Yamaguchi et al., 2007). There has been controversial reporting on the effect of alcohol on the extracellular glutamate levels in the VTA. For example, in one study, alcohol administration in naïve alcohol-preferring rats had no effect on extracellular glutamate levels in the VTA (Kemppainen et al., 2010). In a different study, a biphasic response in extracellular glutamate levels in the VTA after alcohol exposure was observed in rats. Lower dose of alcohol (0.5 g/kg) resulted in an increase in the extracellular glutamate levels in the VTA (Ding et al., 2012). This effect was not observed with moderate dose (1 g/kg) of alcohol in drug-naïve rats, while higher doses of alcohol (2 g/kg) led to a significant decrease in the extracellular glutamate levels in the VTA in both alcohol-naïve as well as alcohol experienced rats (Ding et al., 2012). In yet another study, a challenge dose of alcohol in rats repeatedly treated with morphine had no effect on extracellular glutamate levels (Ojanen et al., 2007). Finally, alcohol at clinically relevant doses increased extracellular glutamate levels in the VTA in midbrain slices of rats. This effect was attributed to an increase in glutamatergic modulation of dopaminergic neurons in the VTA (Xiao et al., 2009b). Importantly, alcohol self-administration directly into the VTA is higher in P rats as compared to NP rats (Gatto et al., 1994). Overall, the balance of the evidence suggests that alcohol exposure is associated with significant alterations of glutamate neurotransmission in the VTA.

9.1 Role of the glutamate receptors in the VTA in alcohol dependence

Alcohol administration or voluntary consumption increased the ratio of AMPA to NMDA currents in the VTA. This effect was associated with an increase in AMPAR mediated excitatory synaptic transmission or a decrease in NMDAR mediated currents, secondary to changes in receptors expression (Stuber et al., 2008). Moreover, long-term alcohol exposure induced upregulation of GluN1 subunit of MDA as well as GluR1 subunit of the AMPAR in the VTA (Ortiz et al., 1995). This effect was not observed with the short-term alcohol exposure. These results were in agreement with a previous study which reported that long-term alcohol exposure activates VTA neurons (Ortizetal.,1995). In addition, exposure to alcohol enhanced NMDA plasticity in the VTA (Bernier et al., 2011). Furthermore, alcohol's inhibitory effect on synaptic transmission in the VTA is attributed to its inhibitory effect on NMDAR-mediated excitation (Stobbs et al., 2004). Alternatively, pharmacologically blocking AMPA/K receptors by intra-VTA microinjections with CNQX, an AMPA/KA receptor antagonist, attenuated alcohol seeking without affecting sucrose-intake in rats (Czachowski et al., 2012). This indicates that glutamatergic neurotransmission in the VTA is implicated in the development of alcohol-seeking behavior. Despite the important role of iGluRs in the VTA on the alcohol dependence process, little is known about the role of VTA mGluRs in alcohol dependence.

9.2 Role of the glutamate transporters in the VTA in alcohol dependence

It has been reported that alcohol during maintenance as well as deprivation reduced GLAST expression but not xCT and GLT-1 in posterior VTA of female P rats (Ding et al., 2013). Since most studies reported a significant reduction in GLT-1 and xCT expression in other brain regions, studies are needed to verify the effect of alcohol on GLT-1 and xCT in the VTA.

10. Genetic aspect of alcohol associated changes in glutamate and reward circuitry

Studies have found that alcohol use is correlated to changes in gene expression of iGluRs, mGluRs and glutamate transporters [For review see (Bell et al., 2016)]. It has been found that the variation of alcohol response level is regulated mainly by glutamatergic signaling genes (Joslyn et al., 2010). For instance, polymorphism of *GRIN2A* promotor region of NMDAR has been associated with alcoholism (Domart et al., 2012). Another study reported that a polymorphism of GluN2B subunit of NMDAR is found in alcoholics (Schumann et al., 2008). Increase in neural activity has been associated with a single nucleotide polymorphism (SNP) in KAR in abstinent alcoholics in the PFC and OFC (Bach et al., 2015). Furthermore, AMPAR, NMDAR and KAR mRNA expression were increased in the hippocampus of alcohol-addicted subjects (Jin et al., 2014). Additionally, SNP of *GRM3* gene subunit of mGluR3 has also been reported in alcoholics (Xia et al., 2014). The important role of mGluRs gene polymorphism in alcohol dependence was investigated in a study that revealed two SNPs of *GRM8* gene of mGluR8 in alcohol-dependent European American (Long et al., 2015). It is important to note that severe alcohol exposure reduced *Grm2* expression (mGluR2 gene) in the infralimbic cortex (Meinhardt et al., 2013). These observations indicate that polymorphism of both iGluRs and mGluRs genes may be associated with UDs. Further studies are warranted to explore the differential role of family history of alcohol addiction on the development of alcohol dependence [For review see (Bell et al., 2016)]. However, there is little known about the degree of glutamate receptors gene changes in alcohol-addicted adolescent, young adult and adult.

Importantly, a recent study from our laboratory found that gene expression of glutamate transporters, GLT-1 and xCT, are decreased in animal exposed to alcohol and cocaine, but not in alcohol alone group, in the NAc in P rats (Hammad et al., 2017). This indicates that the downregulatory effects of chronic alcohol and cocaine exposure on glutamate transporters involve the transcriptional pathways. It has been shown that the expression of vesicular glutamate transporters genes, *Slc17a6* and *Slc17a7*, were increased in postmortem VTA of smokers (Flatscher-Bader et al., 2007). These effects were abolished in VTA tissues of individuals exposed to both nicotine and alcohol. Therefore, alcohol and nicotine may induce differential effects on the gene expression of vesicular glutamate transporters in the VTA. Furthermore, preadolescent rat binged alcohol showed an increase in gene expression of vesicular glutamate transporter-1, *Vglut1*, and reduced *Vglut2* and *Vglut3* gene expression in the dorsal raphe nucleus (DRN) in P rats [For review see (Bell et al., 2016)]. It has been found that chronic and severe exposure to alcohol is associated with a SNP in *vglut1* in female (Comasco et al., 2014). genetic variant of GLT-1 was associated with

dysregulated behaviors and cirrhosis in alcoholics (Foley et al., 2004; Sander et al., 2000). However, more studies are required to investigate the association between astrocytic glutamate transporters gene polymorphism and alcohol dependence.

11. The interplay between glutamate and other neurotransmitter system in AUDs:

Several studies have found an interaction between glutamate and other neurotransmitters in animals that developed AUDs (Sari et al., 2011; Valenzuela, 1997; Xiao et al., 2009a). A prior study shows that alcohol-induced increase in glutamate release can lead to increase in the firing of dopaminergic neurons in the VTA (Xiao et al., 2009a). Additionally, it has been suggested that exposure to alcohol for 14 weeks altered the innervation ratio of glutamate to dopamine in the NAc in alcohol preferring rats (Zhou et al., 2006). Moreover, chronic alcohol exposure increased the activity of glutamatergic system and blocked the activity of GABAergic system leading to potentiated dopaminergic activity in the central reward brain areas, including NAc and VTA [For review see (Rao et al., 2015a)]. It is noteworthy that the development of relapse-like alcohol-seeking behavior was mediated by glutamate receptors of neurons expressing dopamine transporter or dopamine receptor-1 (Eisenhardt et al., 2015). This latter study found that memantine, NMDAR antagonist, infusion into the NAc or VTA attenuated alcohol deprivation effect in mice, which suggest the critical role of glutamatergic inputs into the dopaminergic neurons in alcohol relapse. Taken together, interactions of the dopaminergic and glutamatergic systems play a key role in the development of alcohol dependence, withdrawal and relapse.

Additionally, the biosynthesis of GABA and glutamate might be considered as key factor in the development of alcohol dependence. A clinical study suggested that the synthesis of glutamate and GABA are increased and decreased, respectively during withdrawal period in alcoholics (Brousse et al., 2012). Moreover, chronic alcohol exposure induced depressing effects leading to elevated glutamatergic activity and decreased GABAergic activity to maintain the balance between the excitatory and inhibitory neurotransmitters systems (Valenzuela, 1997)]. Alcohol exposure inhibits the electrical function of GABAergic neurons through inhibition of NM AR (Stobbs et al., 2004). However, less is known about the role of the interaction of GABAergic neurons and glutamate receptors, including NMDAR, on the effects of alcohol dependence, craving, withdrawal and reinstatement.

Serotonin (5-HT) has been found to modulate the activity of glutamatergic system in the NAc and PFC [For review see (Sari et al., 2011)]. It has been found that 5-HT stimulated GABAergic and cholinergic interneurons, an effect associated with reduced glutamatergic projections into the medium spiny neurons (Blomeley and Bracci, 2005; Blomeley and Bracci, 2009; Pakhotin and Bracci, 2007). Additionally, 5-HT might interact with NMDAR and consequently enhanced alcohol tolerance in rats (Khanna et al., 1994). Study showed that 5-HT decreased NMDAR-mediated currency in the PFC neurons (Zhong et al., 2008). Furthermore, 5-HT has been found to modulate AMPAR and its signaling pathways. For instance, stimulating 5-HT receptor-1A could inhibit AMPA-mediated CAMKII cascade in the prefrontal pyramidal neurons (Cai et al., 2002).

In summary, the interaction of glutamate with other neurotransmitters appears to be critical in multiple phases of AUDs. However, studies are warranted to explore the interaction of the neurotransmitters and glutamatergic transporters in the central reward brain regions.

12. Human Studies

One of the most critical aspects in the field of alcohol addiction has been the learning behaviors-associated with alcohol-dependent and alcohol-abstinent humans. In consistent with pre-clinical studies, plethora of studies have reported about the neurobiological changes in the glutamatergic system in human alcoholics. Magnetic Resonance Spectroscopic (MRS) analyses in human subjects revealed that acute alcohol withdrawal increased extracellular glutamate levels in the prefrontocortical regions compared to the healthy control subjects (Hermann et al., 2012). Study employing Proton-MRS demonstrated that higher glutamate levels were found in the NAc in alcohol-dependent subjects compared to controls. Moreover, the combined glutamate and glutamine levels in the NAc and exhibited appositive correlation with craving, measured using the Obsessive Compulsive Drinking Scale (Bauer et al., 2013). However, the ACC glutamate levels are known to revert back to normal during post-withdrawal period (Mon et al., 2012). Intriguingly, a study comparing heavy-drinking with light-drinking subjects found a negative correlation between glutamate content in the frontal white matter and the severity of alcohol dependence and loss of control over alcohol intake, suggesting that glutamate plays a critical role during transition from non-dependent heavy drinking to the state of dependence (Ende et al., 2013). It is important to note that glutamate-glutamine cycling in the brain is suggested to be disrupted in alcohol dependent group compared to the control group (Thoma et al., 2011). In addition to the increase in the glutamate levels in the brain, an elevation in glutamate levels was also documented in the cerebrospinal fluid (CSF) of patients abstinent from alcohol for four weeks, an elevation strongly correlated with the severity of alcohol dependence (Frye et al., 2016a; Umhau et al., 2010). Treatment with a NMDAR antagonist, acamprosate, reduced this alcohol-induced increase in CSF glutamate levels (Frye et al., 2016a). However, another study reported no change in glutamate levels in CSF of alcohol-dependent subjects as compared to control subjects (Tsai et al., 1998). In another study examining the alcohol use pattern in humans diagnosed with depression concluded a positive association between glutamate levels in hippocampus and alcohol use (Hermens et al., 2015). These studies provide compelling evidence of dysregulated glutamatergic homeostasis, as demonstrated by altered glutamate levels in brain regions, during and post-withdrawal from AUD.

Changes in glutamate receptors have also been found in humans with AUDs (Enoch et al., 2014; Kryger and Wilce, 2010; Laukkanen et al., 2014). Postmortem whole-hemisphere autoradiographic analysis in Cloninger type 2 alcoholics, as compared to control subjects, revealed an increase in AMPAR expression in the ACC (Kärkkäinen et al., 2013). Another study also reported a decrease in the mRNA as well as protein expression of GluR2 subunit of AMPAR in amygdala of alcoholics (Kryger and Wilce, 2010). An increase in GRIA4 (encoding GluA4) gene expression in alcoholics has also been reported (Enoch et al., 2014). Moreover, a SNP in NMDAR-2B subunit (GRIN2B) confers genetic susceptibility to earlier age at onset of alcohol withdrawal in patients (Pauletal.,2017). Human alcoholics also showed upregulation of GRIN2B (encoding GluN2B) and GRIN2D (encoding for GluN2D)

compared to controls (Enoch et al., 2014). Moreover, abstinent alcoholics with SNP in the GRIN2C subunit of NMDAR demonstrated an increase in alcohol-stimulated neuronal activity in the (which positively correlated with alcohol craving) and PFC (negatively associated with alcohol craving) (Bach et al., 2015). This was further confirmed in another study performed on over 1000 humans which revealed a strong association between genetic variations of GluN2B with development of alcohol dependence (Schumann et al., 2008). Similarly, abstinent alcoholics with SNPs in GRIK1, gene encoding for KARs, exhibited an increase in the neuronal activity in the PFC and OFC (Bach et al., 2015).

A previous study reported a decrease in the relative mRNA expression of GluN1, GluN2A and GluN2B subunit in superior frontal and primary motor cortex in cirrhotic alcoholics compared to non-cirrhotic alcoholics, suggesting the involvement of NMDAR in alcohol-induced cirrhosis (Ridge et al., 2008). A significant increase in GRIK3 (GluA7) gene expression has also been reported in alcoholics compared to controls (Enoch et al., 2014). This study concluded that NMDAR is implicated significantly in the development of alcohol addiction. Like preclinical studies, increase in mGluR1/5 expression has been documented in hippocampus of type 1 alcoholics (non-genetic) as compared to either type 2 alcoholics (genetic) or healthy subjects (Kupila et al., 2013). Interestingly, postmortem analysis of ACC from alcoholics also revealed a reduction in mGluR2 transcript levels (aminMeinhardt et al., 2013), and this decrease in mGluR2-mediated neurotransmission is thought to be critical in relapse to alcohol. Interestingly, in another study where the alcoholic subjects were sub-divided into type 1 and type 2, an increase in mGluR2/3 in type 2 alcoholics was found using a radioligand binding technique (Laukkanen et al., 2014). Human alcoholics also were found to have upregulation in the GRM3 (mGluR3) and GRM4 (mGluR4) (Enoch et al., 2014).

We and others have extensively investigated the effects of alcohol exposure on the glutamate transporters in animals. Not surprisingly, several studies have reported similar alterations in the glutamate transporters in humans as well. For example, microarray analysis of postmortem basolateral amygdala from human alcoholics revealed a significant downregulation of GLT-1 and GLAST (Kryger and Wilce, 2010). In contrast, upregulation of GLT-1 and AAT3 was observed in white blood cells, during early and late withdrawal from alcohol in humans (Ozsoy et al., 2016). Another study reported a marked increase in GLAST expression in the PFC of chronic alcoholics, suggesting that this increase in GLAST protein levels is a compensatory mechanism to combat the increase of glutamate levels (Flatscher-Bader and Wilce, 2006; Flatscher-Bader and Wilce, 2008). The possible reason for the inconsistent alterations in the expression of glutamate transporters in alcoholics is that different cells and brain regions were investigated. Additionally, a genetic variant of GLT-1 in humans, G603A, has been linked with risk-tasking behaviors and cirrhosis associated AUD (Foley et al., 2004; Sander et al., 2000).

13. Limitations on targeting glutamatergic system for potential therapeutic options for the treatment of AUDs

Despite all of the remarkable progress made in understanding the role of the glutamatergic system in the development of AUDs in preclinical models, targeting this system to reverse or stop the development of AUDs remains a challenge in the clinic setting. One of the key challenges in this field has been the inconsistency and contradictory findings around the same targets. For example, pharmacological antagonism of mGluR1 using CPCCOEt resulted in contradictory findings regarding its efficacy in reducing ethanol consumption (1–3), thereby questioning its validity as a target to treat AUDs in preclinical studies. However, pharmacologically targeting mGluR1with a different antagonist EMQMCM appeared to be an effective strategy in reducing ethanol-induced CPP. This suggests that mGluR1 might be a valid target to treat AUDs. On the other hand, a few compounds like LY379268, a mGluR2/3 agonist, when used in preclinical studies have more consistently produced the desired effect on reducing alcohol intake. However, the ability of LY379268 to affect the intake of natural reinforcers, such as sweetened milk, suggests that LY379268's effect on alcohol is nonspecific (4–6). More potent and selective in-vivo tool compounds are required to better understand the role of these targets in AUDs. Secondly, most of preclinical studies targeting proteins of interest by local administration of pharmacological modulators in a specific sub-region of the brain preclude a very well-established fact that most glutamatergic receptors and transporters are globally expressed in more than one sub-regions of the brain. Although these studies are important tools to understand the biological significance of the target receptor or transporter, the findings do not necessarily reproduce in preclinical models using conventional dosing paradigms. This could be attributed to the fact that expression of most of these receptors and transporters is not constrained to one sub-region of the brain, and therefore modulating the glutamatergic system systemically could result in off-target effects. However, with advances in targeted-gene therapy, targeting these glutamatergic receptors and transporters may become a viable option in the future. An additional challenge in the development of therapy that targets the glutamatergic system is the lack of studies comparing preclinical and clinical biomarkers to assess target engagement and efficacy of the treatment. Despite the progress made in development of PET tracers and other CSF biomarkers towards several of these glutamatergic receptors and transporters, very few preclinical studies have utilized these tools to better understand receptor occupancy and efficacy end-points of the treatment. Increased understanding and availability of tools to assess pharmacokinetic and pharmacodynamic relationships in the preclinical models, as well as use of translational biomarkers in preclinical models, should be emphasized in order to better facilitate translation of preclinical findings into the clinic.

14. Concluding remarks and future directions

Several studies have demonstrated alterations in glutamate neurotransmission in the mesocorticolimbic brain regions following alcohol exposure. It is well known that c receives glutamatergic inputs from PFC, amygdala and hippocampus. It is also reported that PFC sends as well as receives glutamatergic projections into VTA and from the hippocampus and amygdala. Amygdala and hippocampus also send glutamatergic projections into the PFC.

The glutamatergic interactions between different central reward brain regions have been predominant focus in alcohol dependence research.

Importantly, significant alterations in mGluRs and iGluRs expression and function have been reported in the mesocorticolimbic areas in animals exposed to alcohol. Moreover, studies have found that pharmacological or genetic modulation of specific glutamate receptors in the NAc, PFC, striatum, amygdala, and hippocampus attenuated alcohol seeking behavior. However, further studies are warranted to understand the contribution of these glutamate receptors, both iGluRs and mGluRs, in each brain region for attenuating alcohol dependence, tolerance, relapse, withdrawal, and craving.

Additionally, chronic exposure to alcohol reduced expression of major astroglial glutamate transporters (GLT-1 and xCT) in the NAc, amygdala and hippocampus but not in PFC. Moreover, attenuation of chronic and relapse-like alcohol drinking has been associated with upregulation of GLT-1 and xCT expression in the NAc, PFC, amygdala, and hippocampus. This indicates that stimulating these transporters in mesocorticolimbic area is critical for attenuating alcohol seeking behavior. However, more studies are needed in the near future to evaluate the effects of long-term exposure to alcohol on the expression of GLT-1andxCTinthestriatum and VTA. This is important to provide evidence about the potential therapeutic effects of glutamate transporters upregulators in striatum and VTA for managing alcohol dependence.

Acknowledgments

The review article was written during the period of fund supported by Award Number R01AA019458 (Y.S.) from the National Institutes on Alcohol Abuse and Alcoholism. Authors would like to thank Rami M. Alzhrani for his technical assistance.

Abbreviations

(GLT-1)	Glutamate transporter 1
(xCT)	cystine-glutamate antiporter
(GLAST)	glutamate/aspartate transporter
(mGluRs)	metabotropic glutamate receptors
(iGluRs)	ionotropic glutamate receptor
(NMDA)	N-methyl-D-aspartate
(Gessa et al.)	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
(NAc)	nucleus accumbens
(PFC)	prefrontal cortex
(VTA)	ventral tegmental area
(ACC)	anterior cingulate cortex

(OFC)	orbitofrontal cotrx
(pAKT)	phosphorylated-AKT
(NF-κB)	nuclear factor-kappa B
(MS-153)	(R)-(−)-5-methyl-1-nicotinoyl-2-pyrazoline
(P) rats	alcohol-preferring
(GSH)	glutathione
(CPP)	conditioned place preference
(EAAT)	Excitatory amino-acid transporters

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SAMHSA, 2015b Substance Abuse and Mental Health Services Administration (SAMHSA). 2015 National Survey on Drug Use and Health (NSDUH). Table 5.6A—Substance Use Disorder in Past Year among Persons Aged 18 or Older, by Demographic Characteristics: Numbers in Thousands, 2014 and 2015 Available at: https://www.samhsa.gov/data/sites/default/files/NSDUH-DetTabs-2015/NSDUH-DetTabs-2015/NSDUH-DetTabs-2015.htm#tab5-6a. Accessed 1/18/17.

SAMHSA, 2015c Substance Abuse and Mental Health Services Administration (SAMHSA). 2015 National Survey on Drug Use and Health (NSDUH). Table 5.6B—Substance Use Disorder in Past Year among Persons Aged 18 or Older, by Demographic Characteristics: Percentages, 2014 and 2015. Available at: https://www.samhsa.gov/data/sites/default/files/NSDUH-DetTabs-2015/NSDUH-DetTabs-2015/NSDUH-DetTabs-2015.htm#tab5-6b. Accessed 1/18/17.

SAMHSA, 2015d Substance Abuse and Mental Health Services Administration (SAMHSA). 2015 National Survey on Drug Use and Health (NSDUH). Table 2.46B—Alcohol Use, Binge Alcohol Use, and Heavy Alcohol Use in Past Month among Persons Aged 12 or Older, by Demographic Characteristics: Percentages, 2014 and 2015 Available at: https://www.samhsa.gov/data/sites/default/files/NSDUH-DetTabs-2015/NSDUH-DetTabs-2015/NSDUH-DetTabs-2015.htm#tab2-46b. Accessed 1/18/17.

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Highlights

- Chronic alcohol exposure elevates glutamate transmission in mescorticolimbic areas.
- Chronic alcohol use alters iGluRs and mGluRs expression in mesocorticolimbic areas.
- Alcohol exposure downregulates GLT-1 and xCT in NAc, amygdala and hippocampus.
- Upregulating GLT-1 and xCT in the mescorticolimbic area attenuates alcohol seeking.
- Blocking iGluRs and mGluRs in the mescorticolimbic area attenuates alcohol seeking.

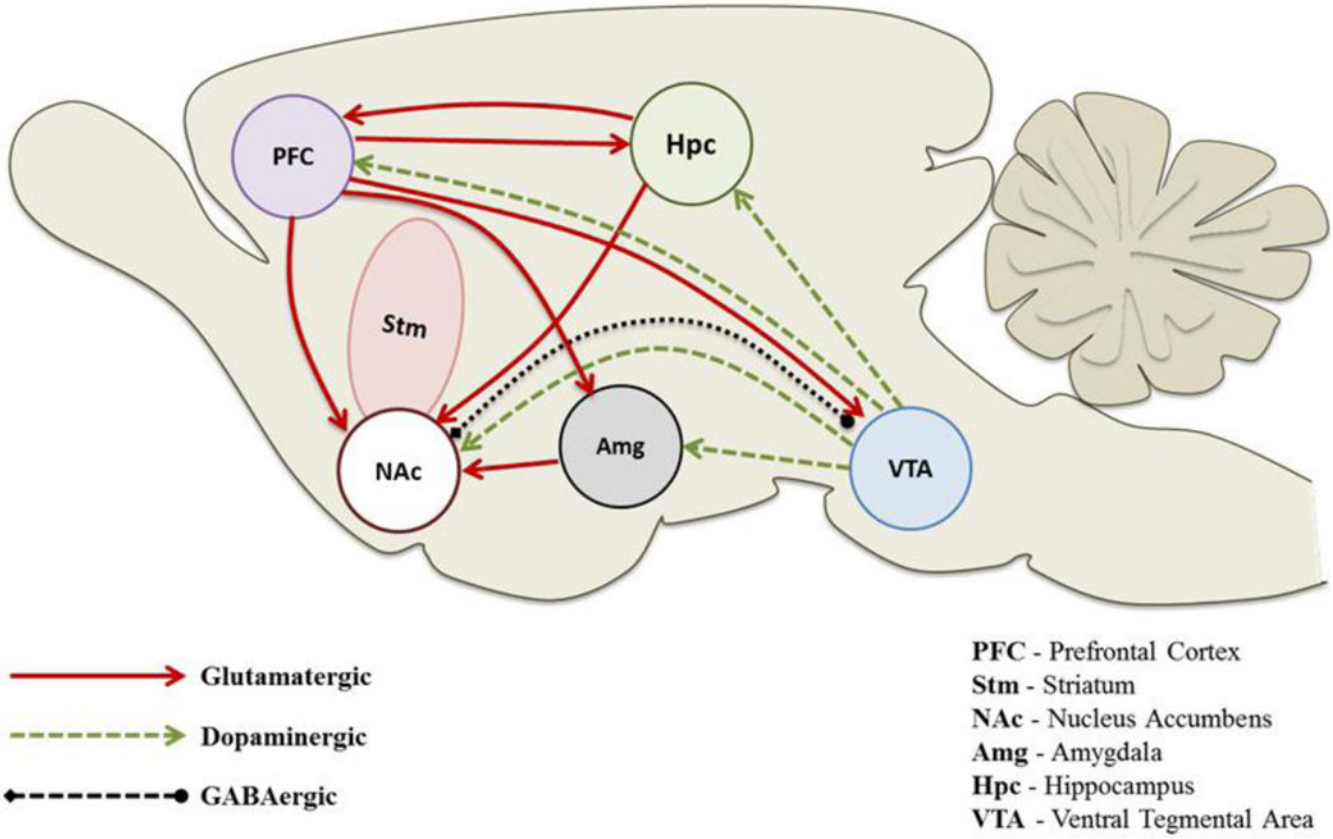

Figure 1. Schematic diagram shows glutamatergic, dopaminergic and GABAergic pathways involved in alcohol dependence in mesocorticolimbic areas.

The mesocorticolimbic brain regions are composed of six major components – nucleus accumbens (NAc), prefrontal cortex (PFC), amygdala (Amy), hippocampus (Hpc), striatum (Stm) and ventral tegmental area (VTA). PFC, Amy and HIPP send glutamatergic projections into NAc. In addition, PFC and Amy as well as PFC and HIPP send and receive glutamatergic inputs from and into each other. Glutamatergic pathways from PFC into VTA have been found to be critical in alcohol dependence.

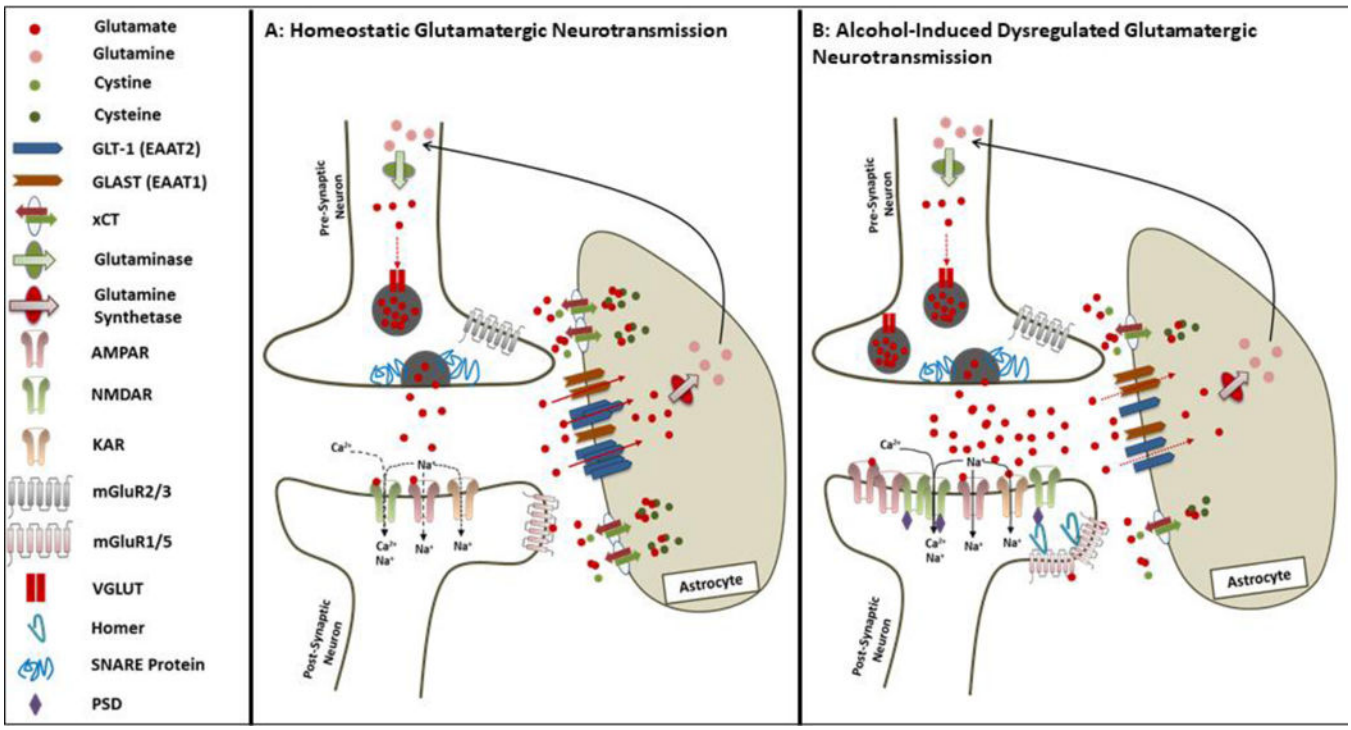

Figure 2. Schematic diagram shows the effects of alcohol on glutamatergic system.

A) Normal release of glutamate neurotransmitter from presynaptic glutamatergic neurons in the central nervous system. In addition, normal expression of glutamate transporter-1 (GLT-1), glutamate aspartate transporter (GLAST) and cystine/glutamate antiporter (xCT) in water naïve model. **B)** Chronic exposure to alcohol decreased GLT-1 and xCT expression but not GLAST in the mesocorticolimbic areas. This effect is associated with marked increased in extracellular glutamate levels.

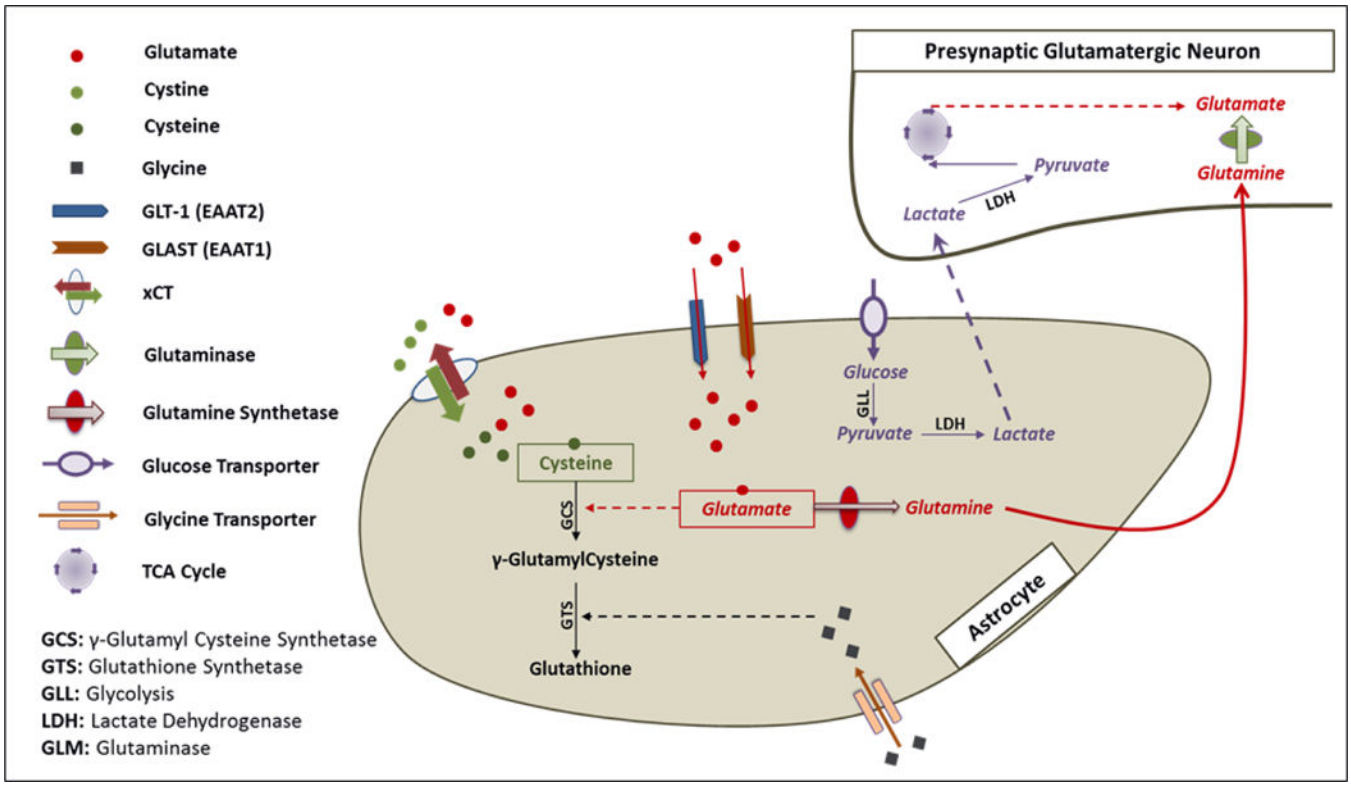

Figure 3.

Schematic diagram shows the fate of glutamate after its uptake by astrocytic glutamate transporters GLT-1 and GLAST. After being released by presynaptic terminal into the synapse, excess glutamate is taken up by the astrocytic glutamate transporters GLT-1 and GLAST. Some of the glutamate in the astrocytes is then converted into glutamine by glutamine synthetase. The synaptic glutamate levels are also maintained by another astrocytic transporter known as cystine/glutamate antiporter (xCT). xCT transports the glutamate into the synapse from the astrocytes in exchange for cystine. Cystine, inside astrocyte, is normally converted to cysteine, which is further converted into γ-GlutamylCysteine by γ-GlutamylCysteine synthetase with contribution of astrocytic glutamate. Glutathione synthetase enzyme and glycine convert γ-GlutamylCysteine to glutathione. Increased intracellular glutathione content has been shown to have a neuroprotective role as well as decreases in oxidative stress effects. Glutamine from the astrocytes is then transported back into the presynaptic terminal of the neuron whereby it is converted into glutamate. The neuronal glutamate is taken up by the vesicles and released into the synapse thereby completing the cycle.

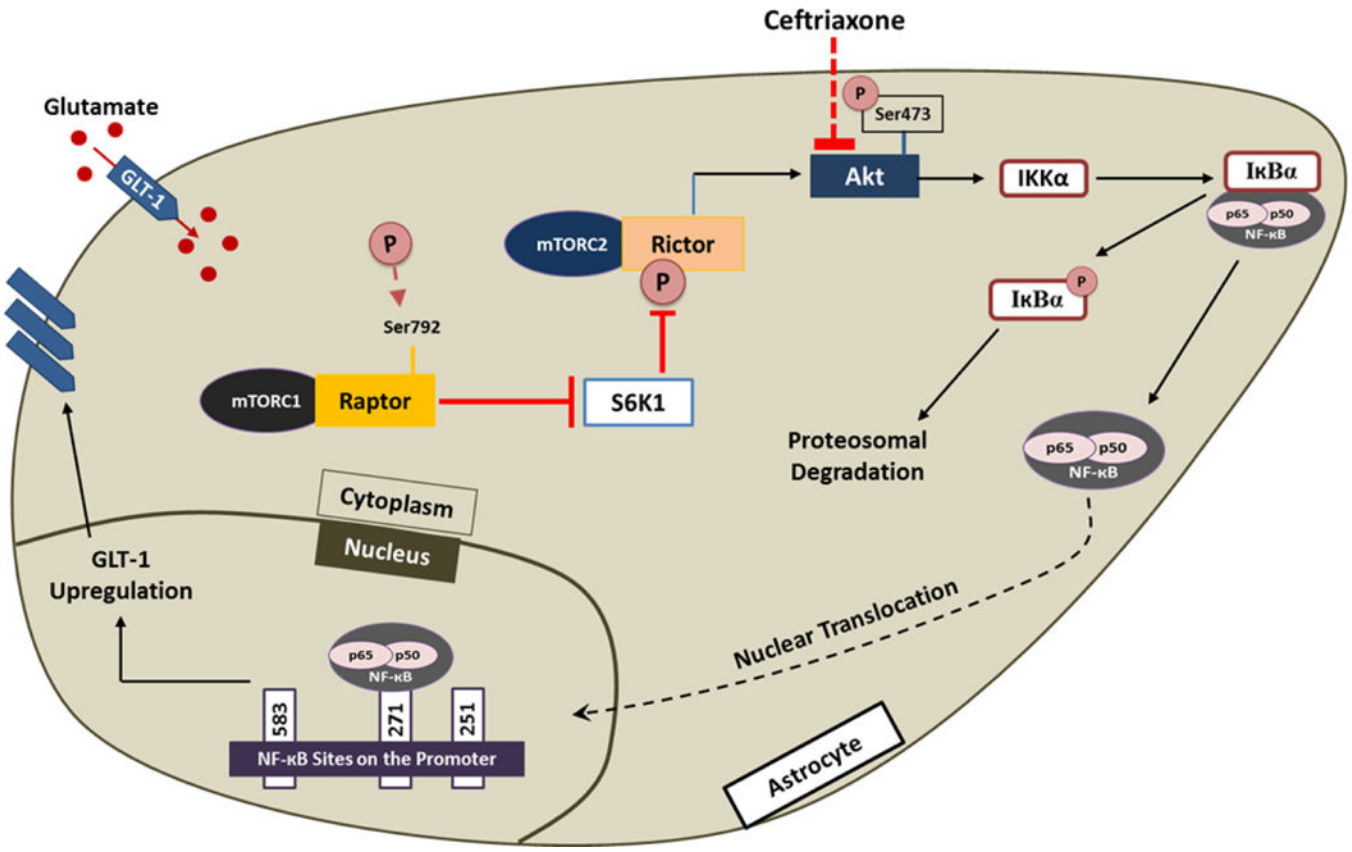

Figure 4.

Schematic diagram shows the effect of β-lactam antibiotics on major signaling pathways involved in stimulation of glutamate transporter-1 (GLT-1) gene expression. Treatment with β-lactam antibiotics leads to direct or indirect phosphorylation of Akt (pAkt). Under normal conditions, NF-kB is sequestered by IκBα and maintained in inactive state. Upon activation by phosphorylated Akt through IKKα, IκBα undergoes proteosomal degradation thereby releasing and activating NF-κB, which then translocates into the nucleus. In the nucleus, NF-κB increases GLT-1 gene transcription, consequently upregulating GLT-1 protein levels.

Table 1:

Summary of studies targeting specific receptors in specific sub-region of the brain along with the tested ligands.

Target	Brain Region	Ligand	Pharmacological Class	Alcohol Drinking Paradigm	References
mGluR2/3	NAc	LY379268	Agonist	Decreased alcohol self-administration	(Besheer et al., 2010), (Bäckström and Hyytiä, 2005), (Griffin et al., 2014)
mGluR2/3	NAc	LY404039	Agonist	Decreased alcohol-seeking relapse	(Rodd et al., 2006)
mGluR2/3	NAc	LY341495	Antagonist	No effect	(Schroeder et al., 2005).
mGluR5	NAc	MPEP	Antagonist	Decreased alcohol self-administration	(Besheer et al., 2010).
mGluR5	NAc	MTEP	Antagonist	Decreased reinstatement to alcohol-seeking	(Sinclair et al., 2012).
mGluR1	NAc	JNJ-16259685	Negative-allosteric modulator	Decreased alcohol consumption	(Lum et al., 2014).
NMDAR	NAc	2-amino-5-phosphopen-tanoic acid	Antagonist	Decreased alcohol self-administration and CPP	(Rassnick et al.,1992) (Gremel and Cunningham, 2009).
AMPAR	NAc	Aniracetam	Positive allosteric modulator	No affect alcohol reinforcement	(Cannady et al., 2017)
mGluR1	Amygdala	JNJ-16259685	Antagonist	decreased binge alcohol consumption	(Cozzoli et al., 2014).
mGluR5	Amygdala	MTEP	Antagonist	decreased binge alcohol consumption	(Cozzoli et al., 2014).
mGluR5	Amygdala	MTEP	Antagonist	Decreased Reinstatement to alcohol-seeking	(Sinclair et al., 2012).
NMDAR	Amygdala	2-amino-5-phosphopen-tanoic acid	Antagonist	Decreased alcohol self-administration and CPP	(Gremel and Cunningham, 2009).
AMPAR/KAR	Amygdala	CNQX	Antagonist	Decreased CPP	(Zhu et al., 2007).
AMPAR/KAR	Amygdala	Aniracetam	Positive allosteric modulator	Increased alcohol self-administration	(Cannady et al., 2017)
AMPAR/KAR	VTA	CNQX	Antagonist	Decreased alcohol seeking	(Czachowski et al., 2012)