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Ethanol-Associated Changes in Glutamate Reward Neurocircuitry: A Minireview of Clinical and Preclinical Genetic Findings

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Contents

1. Alcoholism and Genetics	42
2. Central Glutamatergic Activity	44
2.1 Metabotropic Glutamate Receptors	45
2.2 Ionotropic Glutamate Receptors	47
2.3 Glutamate Transporters and Carriers	47
2.4 Glutamate Synthesis and Metabolism	48
2.5 Glutamate and the Postsynaptic Density	48
3. Central Glutamate Activity and Alcohol Dependence	50
3.1 Alcohol's Effects on Glutamate Activity and Extracellular Levels	50
3.2 Metabotropic Glutamate Receptors and Alcohol	52
3.3 Ionotropic Glutamate Receptors and Alcohol	54
3.4 Glutamate Transporters/Carriers and Alcohol	55
3.5 Glutamate-Associated Enzyme Activity and Alcohol	56
3.6 The Postsynaptic Density and Alcohol	56
4. Glutamate-Associated Genetic Variations and Alcoholism	57
5. Alcohol and Glutamate Gene Expression	59

6. Alcohol-Associated Changes in Gene/Protein Expression of P Rats	60
6.1 Metabotropic Glutamate Receptor Expression Differences	61
6.2 Ionotropic Glutamate Receptor Expression Differences	63
6.3 Expression Differences for Glutamate Transporters, Enzymes, and Postsynaptic Density	66
7. Conclusions	72
Acknowledgments	73
References	73

Abstract

Herein, we have reviewed the role of glutamate, the major excitatory neurotransmitter in the brain, in a number of neurochemical, -physiological, and -behavioral processes mediating the development of alcohol dependence. The findings discussed include results from both preclinical as well as neuroimaging and postmortem clinical studies. Expression levels for a number of glutamate-associated genes and/or proteins are modulated by alcohol abuse and dependence. These changes in expression include metabotropic receptors and ionotropic receptor subunits as well as different glutamate transporters. Moreover, these changes in gene expression parallel the pharmacologic manipulation of these same receptors and transporters. Some of these gene expression changes may have predated alcohol abuse and dependence because a number of glutamate-associated polymorphisms are related to a genetic predisposition to develop alcohol dependence. Other glutamate-associated polymorphisms are linked to age at the onset of alcohol-dependence and initial level of response/sensitivity to alcohol. Finally, findings of innate and/or ethanol-induced glutamate-associated gene expression differences/changes observed in a genetic animal model of alcoholism, the P rat, are summarized. Overall, the existing literature indicates that changes in glutamate receptors, transporters, enzymes, and scaffolding proteins are crucial for the development of alcohol dependence and there is a substantial genetic component to these effects. This indicates that continued research into the genetic underpinnings of these glutamate-associated effects will provide important novel molecular targets for treating alcohol abuse and dependence.



1. ALCOHOLISM AND GENETICS

Over half of adult Americans have a family history of alcoholism or alcohol (ethanol) abuse and one in four Americans have had an alcohol use disorder (AUD) during their lifetime, costing the US economy an estimated \$225 billion per year (Research Society on Alcoholism).^{1,2} AUDs continue to be ranked as the third leading cause of preventable death by the Centers for Disease Control and Prevention.³ Moreover, research supports a causal relationship between AUDs and at least 50 different medical conditions.⁴⁻⁶

The well-documented familial incidence of alcoholism indicates that heredity contributes significantly to a predisposition toward, and the development of, AUDs.⁷⁻⁹ In fact, family history positive (FHP) individuals are at a three- to sevenfold increased risk to develop alcoholism, relative to those who are family history negative (FHN).¹⁰ This genetic proposal has been confirmed by multiple gene studies (e.g., the Collaborative Study On the Genetics of Alcoholism (COGA), the Study of Addiction: Genes and Environment (SAGE), and the European research project on risk-taking behavior in teenagers (IMAGEN)) examining the association between diagnostic criteria for alcohol dependence/addiction and the presence of single nucleotide polymorphisms (SNPs) in alcohol-dependent individuals.¹¹⁻¹⁷

Similar to humans, different lines of heterogeneous stock rats display a wide-range of ethanol-consumption levels.¹⁸ The very early work by Williams and coworkers,¹⁹ as well as Mardones and coworkers,²⁰ resulted in the hypothesis that ethanol intake in rodents is also under substantial genetic control. From their early work and that of four other international sites, bidirectional selective breeding has resulted in at least five high alcohol-consuming versus their respective low alcohol-consuming rat lines.²¹ One of these selectively bred high alcohol-consuming rat lines is the alcohol-preferring P rat. Essentially, starting from a closed colony of Wistar rats, the highest alcohol drinkers were mated together and the lowest alcohol drinkers were mated together, which resulted in the P and NP lines, respectively.^{21,22} The selectively bred alcohol-preferring P rat meets all of the criteria put forth for a valid animal model^{23,24} of alcoholism.^{21,22} It also meets the more recently proposed criteria including relapse-like,²⁵ binge-like,^{21,22,26,27} and early/adolescent-onset of excessive drinking, which results in blood alcohol concentrations (BACs) greater than the threshold (80 mg%) of NIAAA's²⁸ definition for binge drinking.^{26,29} By the nature of selective breeding, P rats represent multigenerational FHP subjects and their counterparts, the selectively bred alcohol-nonpreferring NP rats, represent multigenerational-FHN individuals. Regarding the point about representing FHP individuals, the P rat has some neurochemical, neurophysiological, and behavioral characteristics similar to those seen clinically in FHP individuals.^{21,26,30}

Some of these neurochemical characteristics of P rats involve the glutamatergic system and these findings parallel clinical findings in both FHP individuals and chronic alcoholics. The present minireview will focus on the central glutamatergic system and its role in alcohol dependence. The basic structure and function of the glutamatergic system will be outlined; as it

pertains to its activity in the brain, its receptors, and transporters as well as the excitatory synapse. A summary of the existing literature on how ethanol affects the structure and function of the central glutamatergic system will be presented in the context of both preclinical and clinical research. A synopsis of the genetic influence on the development and/or expression of alcohol dependence will be described. An overview of the current knowledge regarding how the ethanol–glutamate interaction affects gene and/or protein expression will be presented, again from both preclinical and clinical perspectives. This will be followed by a compilation of our findings with the P rat as it pertains to innate differences in gene and/or protein expression, relative to its NP counterpart, as well as ethanol’s modulation of gene and/or protein expression in subregions of the mesocorticolimbic reward circuitry. This minireview will then be concluded with some closing thoughts on some limitations observed in the existing literature.



2. CENTRAL GLUTAMATERGIC ACTIVITY

The amino acid glutamate is the primary excitatory neurotransmitter in the central nervous system (CNS). Therefore, it is not surprising that glutamate receptors are located throughout the brain (see Fig. 1 for glutamatergic projections). In addition, given the ubiquitous distribution of glutamate and its receptors, its function as the primary excitatory neurotransmitter is crucial for many processes, especially those mediating neuroplasticity, learning and memory.^{31–33} Glutamate interacts with both metabotropic mGlu1–mGlu8 (Grm1–Grm8 = mGluR1–mGluR8) and ionotropic receptors, which include those that can bind to *N*-methyl-*D*-aspartate (NMDA) (subunits: GluN1 (Grin1 = NR1); GluN2a–GluN2d (Grin2a–Grin2d = Nr2a–Nr2d); GluN3a–GluN3b (Grin3a–Grin3b = NR3a–NR3b), those that can bind to α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) subunits: GluA1–GluA4 (Gria1–Gria4 = GluR1–GluR4) or kainite subunits GluK1–GluK4 (Grik1–Grik4 = GluR5–GluR7 + KA1–KA2); these nomenclatures reflect IUPHAR, HUGO, and “old” symbols respectively.^{34–36} Due to glutamate’s role in excitotoxicity, extracellular glutamate must be tightly controlled.^{34,37,38} This is accomplished, for the most part, by multiple glutamate transporters.³⁴ The human excitatory amino acid transporter 2 (EAAT2) and its rodent analog glutamate transporter 1 (GLT1) appear to be the main transporters performing this function centrally.^{34,37}

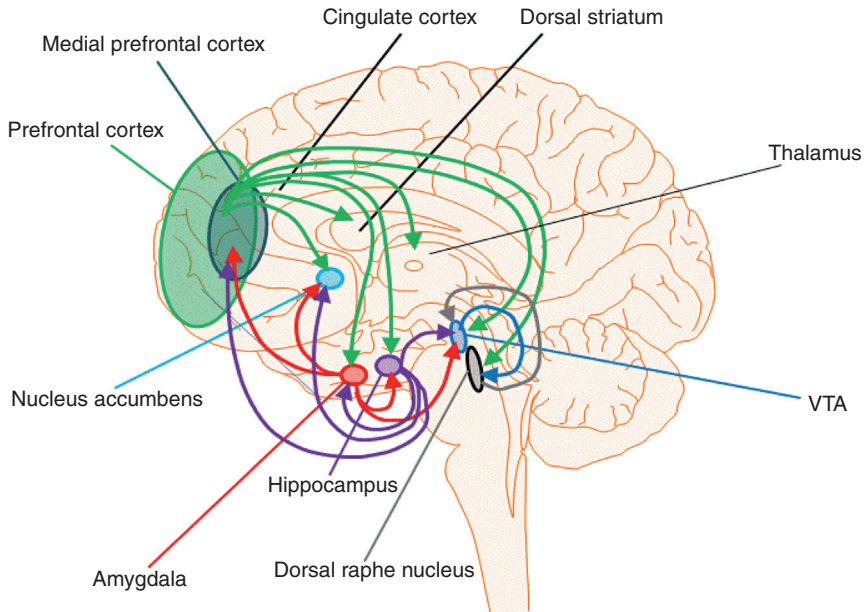


Figure 1 A simplified diagram depicting glutamatergic projections of the mesocorticolimbic, extended amygdala, and brainstem reward neurocircuitry. The online version includes color coding of these projections; with green representing the PFC/mPFC, red representing the amygdala, purple representing the hippocampus, blue representing the VTA, and gray representing the DRN.

2.1 Metabotropic Glutamate Receptors (Fig. 2)

The mGlu receptors are G-protein-coupled protein receptors (GPCRs) located at the neuronal synapse, extrasynaptically as well as on glial cells (Fig. 2). These receptors are divided into three groups. Group I mGluRs (mGlu1 and mGlu5) are predominately postsynaptic and engage in slow excitatory neurotransmission; Group II mGluRs (mGlu2 and mGlu3) are predominately presynaptic, with some postsynaptic and glial localization (Fig. 2) where they engage in slow inhibitory neurotransmission; and Group III mGluRs (mGlu4, mGlu6, mGlu7, and mGlu8) are generally restricted to the presynaptic terminal and, like Group II mGluRs, engage in slow inhibitory neurotransmission. The mesocorticolimbic and associated reward circuitry express high levels of mGlu1, mGlu2, mGlu3, and/or mGlu5, notably in the Acb, caudate nucleus, cortex, lateral septum, dorsal striatum, amygdala, and hippocampus.^{39–43}

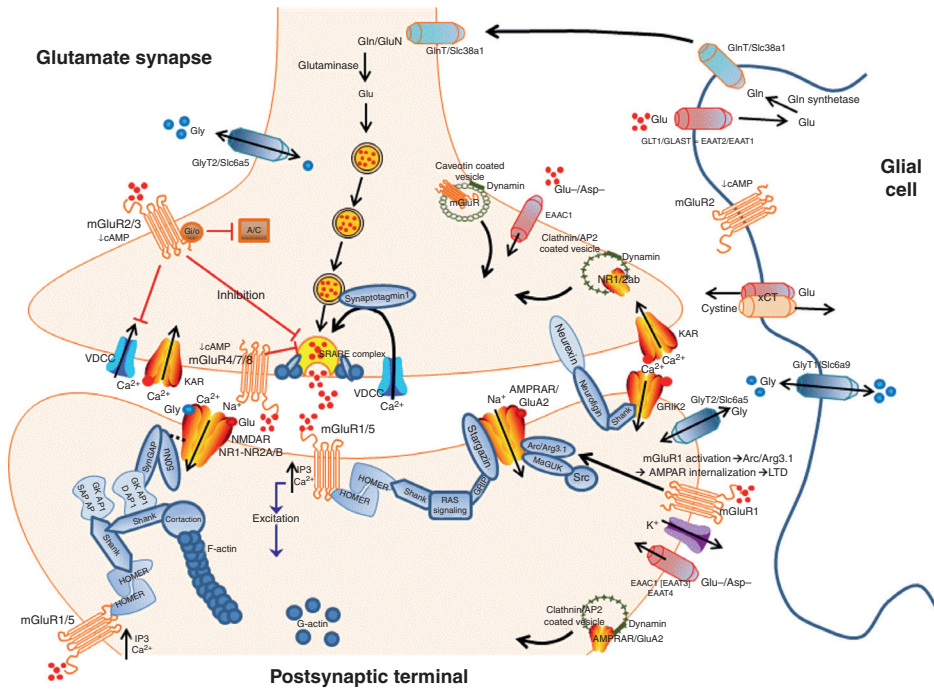


Figure 2 A simplified diagram of a prototypic glutamatergic synapse in the brain. The figure depicts some of the intra-, inter-, and extracellular activities of glutamate-associated plasticity. Abbreviations: AC, adenylate cyclase; AP2, adaptor protein 2; Arc, activity-regulated cytoskeleton-association protein; Asp, aspartate; GKAP/DAP1, disks large associated protein 1 (part of PSD); Gln or GluN, glutamine; GlnT, glutamine transporter; GLT, glutamate transporter; Glu, glutamate; Gly, glycine; GlyT, glycine transporter; LTD, long-term depression; nNOS, neuronal nitric oxide synthase; Src, a tyrosine kinase; VDCC, voltage dependent calcium channel. Other abbreviations are in the text and tables.

2.2 Ionotropic Glutamate Receptors (Fig. 2)

Ionotropic glutamate receptors are ligand-gated ion channels involved in fast excitatory transmission in the CNS. There are several types of glutamate ionotropic receptors including AMPA, kainate, and NMDA-receptor subunits. Most ionotropic glutamate receptors are located postsynaptically, although some are located presynaptically and on glia cells as well (Fig. 2). Similar to the mGlu receptors, they are found throughout the brain including cortical regions, hippocampus, amygdala, basal ganglia, midbrain, hindbrain, and brainstem nuclei.^{44–49} A recent functional addition to the ionotropic glutamate receptors are the delta1 and delta2 subunits symbolized by *Glud1* and *Glud2*. In the past these subunits were considered orphans and not functional but more recent findings indicate that they are indeed functional, by modulating LTD and prepulse inhibition of the acoustic startle response (sensorimotor gating), and their localization is not restricted to the cerebellum but includes cortical and limbic regions as well.^{50–54}

2.3 Glutamate Transporters and Carriers (Fig. 2)

As discussed earlier, excessive glutamate in the synapse (e.g., the addiction-related hyperglutamatergic state) can lead to excitotoxicity and neuronal death. Thus, glutamate uptake/transport from the synapse and perisynapse is required to prevent the plasticity associated with addiction to become excitotoxicity.^{34,38} There are several transporters that regulate extracellular glutamate levels including the EAAT1 (glutamate aspartate transporter: GLAST or *Slc1a3*), EAAT2 (GLT1), EAAT3 (excitatory amino acid carrier 1: EAAC1 or *Slc1a1*), EAAT4 (*Slc1a6*), and EAAT5; where EAAT indicates the human homolog.^{55–57} There are also intracellular vesicular glutamate transporters (VGLUT1–3 of the *Slc17* family of genes) that mediate the uptake of glutamate into synaptic vesicles. Intracellular glutamate carriers, other than the vGLUTs, include the *Slc25a* family of genes. Since the NMDAR NR1 subunit contains the glycine-binding site, it is important to recognize the role of bidirectional glycine transporters (GlyT1 which is primarily glial and GlyT2 which is primarily neuronal) at the excitatory synapse (GlyTs are represented by gene families *Slc6a* and *Slc7a*). Given the increased interest in *N*-acetyl-cysteine's role in mental health, it is equally important to recognize the cystine–glutamate exchanger's (xCT = *Slc7a11*) role in reversing damage induced by excessive extracellular glutamate. The xCT, generally located on glial cells, takes up cystine and releases glutamate molecules. Cystine is then converted to cysteine, which is used to synthesize glutathione and other proteins. Glutathione is a key antioxidant and

important in reversing neuronal damage induced by excitotoxicity and oxidative stress.⁵⁸

2.4 Glutamate Synthesis and Metabolism (Fig. 2)

Another biologic method for preventing excessive glutamate in the synapse is glutamate's synthesis from glutamine intracellularly.³⁴ Glutamine can be transported into and out of the synapse without inducing neurotoxicity. The metabolism of glutamate to glutamine occurs primarily in glial cells via the enzyme glutamine synthetase. Glutamine is then transported (glutamine transporter (GlnT), which is common to both glia and neurons and encoded by the *Slc38a* gene family) out of the glial cell. In turn, glutamine is transported out of the perisynapse and into the excitatory presynaptic compartment. Glutamine is then converted to glutamate by glutaminase and transported to the synaptic membrane, or shunted into GABA synthesis via glutamate decarboxylase (GAD). Glutamate is also metabolized by glutamate dehydrogenase—yielding alpha-ketoglutarate, which enters the TCA/Krebs cycle.

2.5 Glutamate and the Postsynaptic Density (Fig. 2)

The postsynaptic density (PSD) is a cytoskeletal specialization that is located beneath the postsynaptic membrane and directly contiguous with the presynaptic “active zone” of excitatory synapses. Glutamate receptors, synaptic proteins, scaffolding proteins, kinases, and other downstream-signaling proteins are located within this PSD. There are several scaffolding proteins within the PSD including membrane-associated guanylate kinases (MAGUKs), Shanks, and Homers.^{59–61} Scaffolding proteins can be defined as molecules binding at least two other signaling proteins together. These scaffolding proteins are crucial for synaptic plasticity (e.g., learning and memory) by (1) acting as platforms where signaling molecules can assemble; (2) localizing signaling molecules at specific intracellular sites; (3) coordinating positive and negative feedback signals to modify intra- and extra-cellular signaling pathways; as well as (4) protecting these signaling pathways from inactivation, generally by preventing and/or disrupting phosphorylation.^{62–64} In general, scaffolding proteins act as signaling proteins for neuromodulator receptors and anchor these receptors (e.g., glutamate receptors) to the synaptic membrane.⁶⁵ Given their role in receptor anchoring, dysregulation of these scaffolding proteins can lead to a number of neurological diseases.⁶⁶

MAGUKs are expressed widely throughout the central nervous system. They are the scaffolding proteins closest to the surface of the postsynaptic

membrane and they contain multiple PDZ domains.⁶⁷ The PSD-95 protein is one of the most studied MAGUK scaffolding proteins and is involved in postsynaptic stability as well as excitatory receptor insertion.⁶⁸ PSD-95 binds to numerous proteins associated with AMPAR and NMDAR complexes. Schnell *et al.*⁶⁹ found that interaction between PSD-95 and the AMPA receptor-interacting protein, Stargazin, determines the density of AMPARs at the synapse and through this interaction can regulate synaptic maturation.⁷⁰ The PSD-95 anchors NMDARs to the postsynaptic membrane and it acts as a signaling scaffold mediating the activation of neuronal nitric oxide synthase (nNOS) by calcium-calmodulin activity following, for example, entry of calcium through NMDAR channels.⁷¹ Differences between AMPA and NMDA receptors influence on synaptic events is due, in part, to their respective cytosolic C-terminal binding sites to the PSD-95/discs large/zona occludens-1 (PDZ) domain-containing scaffolding proteins.⁷²⁻⁷⁴ The PDZ domain's function is to regulate protein-protein interactions by binding to the C-terminus of each respective target protein; thus, highlighting its crucial role in neuroplasticity, dendritic growth, and dendritic arborization.^{68,75}

The ionotropic NMDA and AMPA receptors are primarily concentrated in the PSD but there are distinct differences in synaptic regulation of these receptors.^{76,77} Additionally, NMDARs initiate synaptic plasticity by interacting with other components of the PSD.⁷⁸⁻⁸² Similarly, AMPARs are involved in rapid synaptic transmission and these receptors cycle on and off in a manner tightly controlled by neuronal activity/plasticity in the PSD.⁷⁸⁻⁸² The recycling (i.e., insertion and removal) of these AMPARs at the synapse modulates synaptic efficiency and thus, like NMDARs, influence learning and memory.^{76,77}

Considerable evidence indicates the crucial role of Shank and Homer proteins in neuroplasticity, as well as alcohol and drug dependence.⁸³⁻⁸⁹ Shank and the Homer scaffolding proteins are located deeper within the PSD than the MAGUKs (i.e., toward the cytoplasm).⁷⁰ Three genes encode for Shank proteins (Shank1, Shank2, and Shank3) and Shank scaffolding proteins bind to neuroligins and neurexins, as well as NMDAR complexes in the PSD.⁷⁰ Shanks are capable of binding other scaffolding molecules within the PSD (e.g., Homers) and thus are capable of linking mGlu receptors, as well as AMPA and kainate ionotropic receptors to NMDARs. Therefore, scaffolding proteins present in the PSD mediate a number of plasticity-associated events through reorganization of PSD-associated proteins, regulation of membrane protein trafficking and activity, as well as the

maintenance of associated epithelial cell polarity and morphology.^{90–92} Moreover, the connection between Shank proteins and neuroligins/neurexins indicates that the PSD may bridge with the “active zone” of the presynaptic terminal. The Homer family of scaffolding proteins is encoded by 3 genes (*Homer1*, *Homer2*, and *Homer3*). Homer scaffolding proteins interact with the C-terminus of Group I mGluRs, bind to Shank/PSD-95/NMDA-receptor complexes, and can also interact with a number of downstream effectors of mGlu1/5 including: IP3 receptors, diacylglycerol lipase-2, and PI3K enhancer (PIKE). Homer proteins are best characterized for their role in regulating mGlu1/5 trafficking, PSD localization, and signaling of mGlu1/5 and NMDA receptors, but are also critical in the regulation of actin and dendritic morphology.⁹³ Furthermore, through their ability to associate with Shank, Homers facilitate cross-talk between mGlu1/5 and NMDA receptors and the integration of their calcium-dependent intracellular events underpinning synaptic plasticity.⁹⁴



3. CENTRAL GLUTAMATE ACTIVITY AND ALCOHOL DEPENDENCE

3.1 Alcohol's Effects on Glutamate Activity and Extracellular Levels

Substantial preclinical evidence indicates that glutamatergic activity mediates natural as well as alcohol- and drug-associated reward through direct and indirect interactions with other neurotransmitter/neuromodulatory systems within the mesocorticolimbic, extended amygdala, and associated reward neurocircuitry (Fig. 1).^{95–102} A key hypothesis that has received considerable attention postulates that the mesocorticolimbic and extended amygdala reward circuits, in the presence of alcohol, lose homeostasis between excitatory and inhibitory transmission and revert to a hyperglutamatergic/hyperexcitatory state resulting in the development and expression of alcohol/drug dependence.^{37,98,102–107}

In general, ethanol consumption and/or exposure to low or moderate doses of ethanol elevate glutamatergic transmission and/or extracellular levels of glutamate in the nucleus accumbens (Acb)¹⁰⁸; Acb shell (AcbSh)^{88,89,109–111}; basolateral amygdala (BLA)¹¹²; cortex¹¹³; Hippocampus^{114,115}; ventral tegmental area (VTA)¹¹⁶; and posterior VTA (pVTA).^{110,117} It has also been shown that genetics influence ethanol-induced increases in extracellular glutamate

within the Acb and/or PFC, such that rats with a predisposition for higher ethanol intake (P and Lewis rats) display greater elevations in glutamate relative to rats with a predisposition for lower ethanol intake (NP and F344 rats).^{118,119} Interestingly, similar relations have been observed between alcohol-preferring versus nonpreferring inbred strains of mice.^{86,120} In addition, these elevations in glutamatergic activity can be conditioned to the environment in which the animal had access to ethanol, with glutamatergic increases seen in the Acb core (AcbCo) or basolateral Amyg (BLA).^{103,121,122}

A recent comprehensive preclinical study provides an excellent example of glutamate's role in the development and expression of alcohol dependence.¹²³ Griffin and coworkers¹²³ evaluated whether free-choice ethanol access would increase Acb extracellular glutamate levels and found that dependence-induced (chronic intermittent access via ethanol-vapor chambers) ethanol-drinking doubled Acb glutamate levels over those seen in nondependent mice. Moreover, these authors reported that this doubling of glutamate was observed a week later indicating that these increases in glutamate activity were not dependent upon ethanol withdrawal itself. Consistent with prior neuropharmacologic results in studies of low versus high alcohol-consuming inbred mice,¹²⁰ Griffin *et al.*¹²³ showed that pharmacologic elevation of glutamate in the Acb, with a pan-glutamate-reuptake inhibitor (Threo-beta-benzyloxyaspartate, TBOA), increased the ethanol intake of nondependent mice to the levels observed in dependent animals, with TBOA also increasing ethanol intake further in the dependent mice. Also consistent with the results of Kaposova and Szumlinski,¹²⁰ when the mGlu2/3 autoreceptor agonist LY379268 was microinjected into the Acb to lower glutamate levels, ethanol intake was decreased in both the dependent and nondependent mice.¹²³ These latter results parallel earlier work indicating that manipulations of extracellular glutamate, including mGlu2/3 activation, actively regulate ethanol intake in multiple animal models of alcoholism (discussed later in the chapter).

Finally, evidence for altered glutamate neurotransmission within the previously mentioned brain regions, as well as the anterior cingulate cortex (ACC), has been reported in clinical studies of alcohol-dependent individuals as well.¹²⁴⁻¹³¹ For example, a proton magnetic resonance spectroscopy (MRS) study examining the role of hippocampal glutamate in major depression and risky alcohol drinking revealed that elevated glutamate levels in the hippocampus were directly associated with both the presence of major depression and self-reported risky drinking.¹³² These authors noted that the major depression and risky drinking group did not differ from the control

group in age-of-first alcohol use, Alcohol Use Disorders Identification Test (AUDIT) survey scores or smoking behavior; but, this group did have significantly more FHP individuals (approximately six-fold) indicating a possible confound. Another recent MRS study provided support for differences in glutamate activity of FHP versus FHN individuals.¹³³ These authors reported that glutamate/glutamine ratios increased significantly between adolescence and emerging adulthood in FHN, but not FHP, individuals. This suggests that having a familial history of AUDs may genetically predispose an individual for abnormal developmental changes in glutamatergic neurotransmission across periadolescence.¹³³

3.2 Metabotropic Glutamate Receptors and Alcohol

It has been shown that ethanol-binge drinking by mice upregulates mGlu1/5-Homer2 signaling in several mesocorticolimbic structures including the Acb^{83,84} and the central amygdala (CeA)⁸⁵ and activation of either or both of these Group I mGluRs is required for mice to manifest binge-drinking behavior.^{83–85}

Several studies have examined the effects of mGlu5 antagonists on operant ethanol self-administration behaviors in P rats as well. Systemic administration of the mGlu5 antagonist 2-methyl-6-(phenylethynyl)pyridine (MPEP) can reduce operant ethanol self-administration,¹³⁴ reduce ethanol breakpoint without affecting sucrose breakpoint or locomotor activity,¹³⁵ and block the repeated alcohol-deprivation effect (ADE).¹³⁴ The effects of MPEP in rats were extended to mice by demonstrating that this mGlu5 antagonist interfered with the acquisition and maintenance of ethanol drinking by C57BL/6J mice as well,^{136,137} which appears to depend upon a protein kinase C-epsilon (PKC-epsilon) pathway.¹³⁸ A subsequent study using P rats¹³⁹ examined the effects of systemic MPEP on the extracellular signal-regulated kinase (ERK1/2) pathway,¹⁴⁰ which is downstream of mGlu5 and implicated in addiction. MPEP attenuated cue-induced reinstatement of ethanol-seeking behavior, which was associated with decreased phosphorylated (p)ERK1/2 immunoreactivity (IR) in the BLA, but not CeA, and AcbSh, but not AcbCo.¹³⁹ These findings support a role for ERK1/2 phosphorylation in the BLA and AcbSh in mediating cue-induced reinstatement of ethanol-seeking behavior. A third study from this laboratory¹⁴¹ confirmed a role for mGlu5 within the AcbCo in ethanol self-administration; such that local application of MPEP into the AcbCo reduced ethanol operant responding without affecting locomotor activity, sucrose or water responding. In contrast, MPEP infused into the dorsomedial caudate

nucleus or mPFC did not alter operant ethanol self-administration.¹⁴¹ In a study that examined the effects of mGlu5 antagonist 3-[(2-methyl-1,3-tiazol-4-yl)ethynyl]-pyridine (MTEP) on ethanol self-administration by two high alcohol-consuming rat lines (inbred P (iP) and Fawn-Hooded (FH)) reported that MTEP significantly decreased intake.¹⁴² However, these authors also reported that MTEP induced mild sedative effects in iP but not FH rats. Together, these findings indicate that mGlu5 receptors play an important role in regulating different aspects of alcoholism-related behavior in both rat and mouse models.

Other mGluR ligands have also been tested for their effects on excessive ethanol drinking or ethanol reinforcement. Earlier studies revealed that systemic pretreatment with the selective mGlu1 antagonist, (–)-ethyl (7E)-7-hydroxyimino-1,7a-dihydrocyclopropa[b]chromene-1a-carboxylate (CPCCOEt), produced inconsistent effects on operant ethanol self-administration in P rats¹³⁴ versus C57BL/6J mice,¹³⁷ such that intra-Acb infusions of CPCCOEt were unable to alter binge drinking in mice.⁸³ These inconsistent effects of CPCCOEt on measures of alcohol intake likely reflect its relative insolubility. For instance, the more soluble, highly selective, mGlu1 antagonist JNJ 16259685 lowers operant ethanol self-administration and ethanol breakpoint in P rats when administered systemically^{135,143} and reduces binge drinking when infused into the AcbSh of mice.¹⁴⁴ However, systemic JNJ 16259685 pretreatment has nonselective effects in that it also reduces locomotor activity,^{135,143} which may reflect the high abundance of mGlu1 receptors in the cerebellum and their effects on its control of motor movement.^{134,135,143}

Studies have also examined the effects of targeting mGlu2/3 receptors within the contexts of operant ethanol self-administration. When given systemically, the mGlu2/3 antagonist LY341495 did not alter operant responding for ethanol by P rats,¹³⁴ although systemic pretreatment with the mGlu2/3 receptor agonist LY404039 reduced ethanol-seeking and -relapse-like behavior.¹⁴⁵ However, these latter authors reported that LY404039 did not alter the maintenance of operant ethanol self-administration in these animals.¹⁴⁵ Interestingly, the local application of the mGlu2/3 agonist LY379268 in the AcbCo was sufficient to reduce operant ethanol self-administration by P rats,¹⁴¹ in a manner akin to the aforementioned studies of mice drinking under free-access conditions in the home cage.^{120,123} While understudied, the effects of mGlu2/3 agonists appear to depend upon the route of administration or the experimental procedures employed. However, as observed with mGlu1 antagonists,^{134,135,143} the

effect of intra-AcbCo mGlu2/3 agonism on operant ethanol drinking by P rats is also not specific due to effects on locomotor activity.¹⁴¹ Other lines of evidence support an important role for mGlu2 receptors in drug addiction as well.^{97,102,146}

3.3 Ionotropic Glutamate Receptors and Alcohol

As noted earlier, ionotropic glutamate receptors play an important part in the development of alcohol abuse and dependence, with many of ethanol's effects mediated by these receptors. For instance, Enoch and coworkers¹⁴⁷ as well as Jin and coworkers¹⁴⁸ have reported that numerous ionotropic glutamate receptor subunit expression levels are significantly altered in chronic alcoholics and cocaine addicts, relative to control samples. Nevertheless, there has been limited behavioral–pharmacology research on the involvement of ionotropic receptors in excessive ethanol intake by P rats. Pretreatment with aniracetam, a selective positive allosteric modulator of AMPA receptors, increased operant responding for ethanol and cue-induced reinstatement of operant responding for ethanol by P rats, while not altering locomotor activity or operant responding for sucrose.¹⁴⁹ These authors confirmed the involvement of AMPA receptors by demonstrating that the AMPA receptor antagonist 6,7-dinitroquinoxaline-2,3-dione blocked aniracetam-induced increases in ethanol self-administration. Besides the P rat, the selectively bred ALKO alcohol-accepting (high ethanol-consuming rat line from Finland) has also been used to assess the role of AMPA/kainate receptors in excessive ethanol intake, with systemic administration of the antagonist CNQX significantly reducing operant ethanol-seeking behavior by these rats.¹⁵⁰ In addition, systemic administration of the mixed NMDA/glycine receptor antagonist L-701,324 also significantly reduces operant ethanol-seeking behavior in AA rats.¹⁵⁰ While MTEP is an mGlu5 receptor antagonist and decreases ethanol self-administration, it also decreases mRNA expression for both *Glua2* and *Glun1* in the cingulate cortex of iP and FH rats.¹⁴² Thus, the effects of mGlu-receptor activity on ethanol intake are paralleled by its regulation of ionotropic glutamate receptor subunit gene expression underscoring the interaction of these two classes of glutamate receptors. Although there has been limited research on ionotropic glutamate receptors regarding alcohol- and/or drug-intake, -seeking, etc.¹⁵¹; there is clear evidence that NMDA and AMPA receptors are affected by ethanol, which in turn affects neuroplasticity, learning and memory.^{31,32,95,151,152}

3.4 Glutamate Transporters/Carriers and Alcohol

Chronic ethanol-drinking or ethanol exposure significantly reduces glutamate uptake in the brain through downregulation of glutamate transporter, or antiporter, expression^{153–156} and reductions in glutamate transporter expression have been confirmed in postmortem evaluation of brains from alcoholics.¹²⁸ Recent studies have examined the effects of modulating GLT-1, glial EAAT2, and the cystine–glutamate antiporter (xCT) on ethanol intake. Ceftriaxone, a beta-lactam antibiotic, increases glutamate reuptake by upregulating GLT1 expression.¹⁵⁷ Given the general hyperglutamatergic state in alcohol/drug dependence, it is not surprising that this compound also decreases ethanol consumption, ethanol dependence (ED)-associated withdrawal signs, and withdrawal-associated escalation of ethanol intake in P rats.^{158–162} These authors reported a consistent upregulation of GLT1 expression in the Acb and mPFC, which was negatively associated with the observed reductions in ethanol consumption. Moreover, these authors have shown that ceftriaxone, or its analogs, significantly reduces ethanol intake and reverses chronic ethanol-induced downregulation of GLT1 expression in the mPFC, Acb, Amyg, and hippocampus, as well as reversing downregulation of xCT in some brain regions.^{153,163–166}

Given that chronic ethanol induces excitotoxicity and oxidative stress, it is noteworthy that ethanol-induced increases in *Slc7a11* (the protein product being xCT, the cystine–glutamate antiporter) are not dependent upon excitotoxicity or oxidative stress.¹⁶⁷ These authors reported that ethanol itself can inhibit octamer-binding transcription factor 1's (OCT-1) repression of the *Slc7a11* promoter *in vitro*, which in turn elevates *Slc7a11* transcription. This increase in xCT would putatively increase the import of cystine into glial cells, where it would be converted to cysteine and subsequently into glutathione resulting in increased glutathione and decreased neurotoxicity.⁵⁸ Regarding intracellular glutamate transport, an early study¹⁶⁸ examined the effects of continuous ethanol exposure versus exposure interspersed with repeated deprivations on these vesicular glutamate transporters. These authors reported that repeated deprivations increased vGLUT2-immunostained terminals in the AcbSh compared to the water control group. However, ethanol exposure did not alter the level of vGLUT1-immunostained terminals in this brain region. These results suggest that the presence of multiple withdrawal episodes preferentially increases vGLUT2 expression in glutamate terminals in the AcbSh of P rats.¹⁶⁸

3.5 Glutamate-Associated Enzyme Activity and Alcohol

Early work revealed that chronic ethanol exposure decreased glutamine synthetase while increasing glutamate and GAD in the cortex of rats.¹⁶⁹ A more recent preclinical study found decreased GAD-67 expression levels, in the BLA, 2 months after 3 weeks of ethanol-diet initiated in adulthood, but not adolescence.¹⁷⁰ Another study reported that chronic ethanol consumption decreased glutamine synthetase in the striatum (dorsal vs. ventral was not delineated) but not cortex of rats.¹⁷¹ A contemporary study also reported reduced glutamine synthetase in the brain, although the area of the brain was not identified, after chronic ethanol consumption, which started at the beginning of adolescence.¹⁷² A postmortem study indicated that glutamine synthetase was downregulated in the hippocampus of alcoholics without hepatic pathology.¹⁷³ These consistent reductions in glutamine synthetase following ethanol exposure or consumption suggest the presence of astrocytic pathology and, by extension, increased neurotoxicity. Regarding glutamate dehydrogenase, which metabolizes glutamate; adolescent binge-like drinking by rats resulted in a 40% decrease in hippocampal glutamate dehydrogenase 1, which was not seen in rats that received the same protocol during adulthood.¹⁷⁴ Given this finding, it is noteworthy that ethanol inhibits NMDA excitation and LTP to a greater extent in hippocampal slices from adolescent versus adult rats.^{175,176} Therefore, significant differences in ethanol's effects on glutamatergic activity occur across periadolescence and adulthood (see later in the chapter).

3.6 The Postsynaptic Density and Alcohol

Recent research indicates that alcohol and/or drugs of abuse have a profound developmental effect on the PSD as well^{174,177,178}; such that, similar to earlier reports on the vulnerability of the adolescent hippocampus to alcohol and/or drug exposure,^{175,176} there is a differential effect of binge-like ethanol exposure between adolescent and adult rats. Risher *et al.*¹⁷⁷ reported that adolescent intermittent ethanol (AIE) exposure in rats reduced PSD-95 expression levels in the hippocampus, leading to the retention of immature-like dendritic spine phenotypes into adulthood. There was also a reduction in the number of VGlut1/PSD-95 and VGlut1/SAP102 (another MAGUK) colocalized synaptic puncta and these effects were driven by decreases in PSD-95 and SAP102 density with no effect on presynaptic VGlut1 expression levels.¹⁷⁷ In contrast, chronic intermittent ethanol (CIE) during adulthood¹⁷⁸ did not alter PSD-95 expression in the hippocampus as a whole. However, these authors indicated that adult CIE could

alter dendritic complexity in a subregion-specific manner, with a partial return to basal levels after protracted abstinence.¹⁷⁸ Taken together these studies suggest that the PSD-95, and glutamate activity, may be more vulnerable to ethanol-induced changes during adolescence than during adulthood and that adolescent ethanol-induced changes in PSD-95 may interfere with the maturation of dendritic spines. Similarly, considerable evidence indicates a crucial role for Shank and Homer proteins in neuroplasticity as well as alcohol and drug dependence.^{86–89,179} For example, significant increases in both AcbCo and CeA Homer2a/b expression levels were seen 24 h after the removal of ethanol from chronically drinking P rats.¹⁸⁰



4. GLUTAMATE-ASSOCIATED GENETIC VARIATIONS AND ALCOHOLISM

An early Genome Wide Association Study (GWAS) followed by a Gene Set Enrichment Analysis (GSEA) found that when gene variations were analyzed for grouping, neuronal signaling genes dominated other associations with an individual's level of response to alcohol and glutamate was the primary neurotransmitter system implicated.¹⁸¹ These authors also noted that FHP individuals show an altered level of response to alcohol and ketamine (an NMDA antagonist), thus confirming a genetic risk for alcoholism and altered glutamatergic function.^{181,182} Similarly, a pathway analysis of variants in 130 addiction-related candidate genes confirmed a significant role for glutamate signaling in alcohol dependence, with the odds ratio of *mGlu1*-rs2300620 (>1.6) exceeding that of any other significant gene variant.¹⁸³ A contemporary study using pathway analysis revealed the NMDA-dependent AMPA-trafficking cascade centered on the gene encoding the multiple PDZ domain protein (*Mpdz*) was significantly associated with alcohol dependence in a subset of the SAGE study.¹⁸⁴

Regarding particular gene variants, polymorphisms of *Glun2a*, NR2a subunit of the NMDA receptor, are significantly associated with being an FHP individual, an early onset of risky drinking during adolescence as well as the maximum number of drinks in adulthood, with this association having been replicated in a second large sample.¹⁸⁵ A subsequent study also found that a polymorphism in the promoter region of *Glun2a* is significantly associated with alcohol dependence, with this finding replicated in a second sample.¹⁸⁶ A variant of another ionotropic glutamate receptor subunit, *Gluk3*, was found to be associated with alcohol withdrawal-associated

delirium tremens, but not seizures, in a German cohort.¹⁸⁷ Another study reported that a polymorphism in *Gluk1* is significantly associated with alcohol dependence in Caucasians, although many of the alcohol-dependent, but none of the control, subjects were polysubstance users, which may represent a confound in this study.¹⁸⁸

Following earlier work indicating theta event-related oscillations (EROs) are associated with the P300 event-related potential (ERP), whose amplitude is negatively associated with FHP,^{189,190} a Family Based Association Test (FBAT) was carried out on a subset of the COGA sample.¹⁹¹ These authors reported that multiple polymorphisms of the *mGlu8* gene are significantly associated with theta EROs and a genetic risk to develop alcoholism.¹⁹¹ A subsequent study by this group confirmed that polymorphisms of the *mGlu8* gene are associated with a genetic risk to develop alcohol dependence.¹⁹² In another recent study, the *mGlu3*-rs6465084 polymorphism was found to be significantly associated with alcohol dependence in a male Han Chinese cohort.¹⁹³

A recent study examining the association between polymorphisms in vesicular glutamate transporters and severe alcoholism in a female Swedish cohort indicated a nominally significant association with *Slc17a6*-rs2290045.¹⁹⁴ A study evaluating associations between alcohol dependence and GAD genes (*Gad1* and *Gad2*), the enzyme responsible for conversion of glutamate to GABA, in a subset of the Irish Affected Sib Pair Study of Alcohol Dependence sample reported that two polymorphisms in *Gad1* were associated with initial sensitivity to alcohol and a different polymorphism in *Gad1* was associated with age at alcohol dependence onset.¹⁹⁵ An analysis of the relationship between *Gad1* variants and alcohol dependence in an Italian cohort revealed that the *Gad1*-rs11542313 polymorphism was significant.¹⁹⁶ An earlier study evaluating polymorphisms of *Gad2* indicated that the functional promoter *Gad2*-243A>G variant was significantly associated with alcohol dependence in Russian but not European American males.¹⁹⁷ A contemporary study found that three polymorphisms of *Gad1*, but no polymorphisms of *Gad2*, were significantly associated with alcohol dependence in Han Taiwanese men.¹⁹⁸ In a recent genome-wide DNA methylation study, it was reported that methylation of *Gad1* is significantly associated with alcohol dependence in a cohort of Han Chinese men, suggesting epigenetic effects of chronic alcohol abuse.¹⁹⁹ These results indicate that polymorphisms in *Gad1* are significantly associated with alcohol dependence, or related phenotypes, in multiple populations around the world.



5. ALCOHOL AND GLUTAMATE GENE EXPRESSION

Extending the previously mentioned behavioral pharmacologic and neuropharmacologic studies are the results of a very comprehensive study by Meinhardt and coworkers,²⁰⁰ demonstrating that: (1) in ethanol-dependent rats, glutamate-related gene changes were primarily seen in the mPFC, rather than the Acb or amygdala; (2) within the mPFC of ethanol-dependent rats, downregulation of the NMDA-receptor subunits *Glun2a* and *Glun2b*, as well as *mGlu2*, but not *mGlu3*, and *egr1* (early growth response protein 1: *Zif268*, a transcription factor involved in neuroplasticity and vesicular exocytosis at excitatory synapses) were significant in the infralimbic mPFC only, with *mGlu2* displaying the greatest reduction; (3) the AcbSh receives its glutamatergic projections primarily from the infralimbic mPFC, according to retrograde tracing, and these projection neurons displayed significant ethanol-dependence-associated downregulation of *mGlu2*, *Egr2*, and *Egr4*; (4) while basal glutamate in the AcbSh did not differ between ethanol-dependent and control rats, peripheral administration of the mGlu2/3 agonist LY379268 significantly reduced extracellular glutamate in the AcbSh of control but not ethanol-dependent rats, suggesting a downregulation of mGlu2/3 function; (5) ethanol-dependent rats displayed greater relapse (almost twice as many responses) and progressive ratio than controls; (6) lentiviral knockdown of *mGlu2* in the infralimbic mPFC significantly attenuated cue-induced reinstatement of responding in ethanol-dependent but not nondependent rats; (7) *mGlu2* knockdown rats did not differ from control rats in operant relapse responding, operant responding for sweetened condensed milk, or locomotor activity in an open-field test; and (8) a RT-qPCR analysis of postmortem ACC samples revealed that alcoholics had significantly less *mGlu2* mRNA than their respective controls. An early postmortem study revealed that individuals who had abused nicotine, but not alcohol, displayed greater expression of *Slc17a6* and *Slc17a7* vesicular glutamate transporters in the VTA.²⁰¹ However, in individuals who abused both nicotine and alcohol, these increases in *Slc17a6* and *Slc17a7* were significantly reduced.²⁰¹

In another study, Enoch and coworkers¹⁴⁷ reported that hippocampal glutamate receptor subunit gene expression levels differed between alcoholics, cocaine addicts, and healthy controls. Specifically, *Glua4*, *Gluk3*, and *mGlu4* expression was significantly higher in alcoholics, relative to both

controls and cocaine addicts; *Gln2b* expression was higher in both alcoholics and cocaine addicts, relative to control levels; and *Gln2d* and *mGlu3* was upregulated in alcoholics while being downregulated in cocaine addicts, relative to control levels.¹⁴⁷ Substantial changes of glutamate receptor subunit gene expression in the hippocampal dentate gyrus and to a lesser extent orbital frontal cortex, but not the dorsal-lateral prefrontal cortex, of alcoholics versus healthy controls was confirmed in another recent postmortem study.¹⁴⁸ In particular, the data from the dentate gyrus revealed that alcoholics had higher expression levels of *Glua2*, *Glua3*, *Gluk2*, *Gluk3*, *Gluk5*, *Gln1*, *Gln2a*, *Gln2c*, *Gln2d*, and *Gln3a*; whereas only *Gln3a* expression was higher than controls in the orbital frontal cortex.¹⁴⁸



6. ALCOHOL-ASSOCIATED CHANGES IN GENE/PROTEIN EXPRESSION OF P RATS

An examination of protein expression changes in subregions of the Acb and Amyg of chronic ethanol-drinking P rats revealed at 24-h withdrawal that GluN2a expression levels were increased, whereas GluN2b expression levels were decreased in the AcbSh.¹⁸⁰ These authors also reported that Homer2a/b, mGlu1, mGlu5, GluN2a, and GluN2b expression levels were all consistently increased in the AcbCo and CeA.¹⁸⁰ To test the hypothesized genetic role for the mGlu2 receptor in alcohol dependence, a recent RNA and exome sequencing study revealed that a SNP which creates a stop codon in the *mGlu2* gene is present in P, but not NP, rats.²⁰² This stop codon results in the absence of mGlu2 receptors, impaired glutamatergic synaptic transmission, and altered levels of multiple genes associated with synaptic function. These authors also examined F2 rats from a PxNP–NPxP cross and found that *mGlu2* expression levels were significantly and inversely related to ethanol consumption, with decreases in *mGlu2* and mGlu2 receptor expression associated with significant increases in ethanol-drinking behavior.²⁰² Similarly, mGlu2 knockout mice display significantly greater ethanol consumption and preference than their wild-type counterparts.²⁰² As mGlu2 receptors function as autoreceptors, the results indicate an inverse relationship between mGlu2 gene/protein expression and ethanol intake, which is consistent with the aforementioned hypothesis that excessive drinking phenotypes are associated with a hyperglutamatergic state.

Our laboratories have published a number of studies that examined central gene and/or protein expression profiles of P rats.^{180,203–215} Here we

highlight alcohol-associated glutamate-related genes whose expression levels have been identified as significantly changed by ethanol self-administration in P rats, or innately different between P and NP rats, in brain regions implicated in alcohol reward and reinforcement (Fig. 1).

6.1 Metabotropic Glutamate Receptor Expression Differences (Table 1)

A recent study revealed that periadolescent binge-ethanol consumption, using our drinking-in-the-dark—multiple-scheduled-access model,^{27,29} resulted in a general elevation of *mGlu1* and *mGlu4* (twice or three times that of controls, respectively) in the dorsal raphe nucleus (DRN) and *mGlu7* (~1.4-fold increase) in the CeA.²¹³ However, there was a significant decrease (~1.7-fold decrease) in *mGlu6* expression within the DRN as well. Given that *mGlu1*, for the most part, is excitatory and the others inhibitory, this suggests a balance between ethanol-induced up- and downregulation of gene expression for these metabotropic receptors.

mGlu2 and *mGlu3* expression levels were significantly lower in the AcbSh and CeA of adult ethanol-naïve P versus NP rats.^{209,211} As discussed earlier, Zhou and coworkers²⁰² reported a stop-codon polymorphism of *mGlu2* in P, but not present in NP rats that appear to predispose them to high ethanol consumption. And, multiple studies^{134,135,141,143,145} have shown that mGlu2/3 agonists, presumably acting at the presynaptic autoreceptor, block ethanol-self-administration, -seeking, and -relapse behavior in P rats. Noteworthy is the fact that *mGlu4*, *mGlu5*, *mGlu7*, and *mGlu8* (all are involved with inhibitory activity, generally at the presynaptic terminal) are also significantly lower in the AcbSh of P versus NP rats. Gene expression for only one mGluR, *mGlu1*, was ~1.4-fold higher in the AcbSh of P versus NP rats, which is a finding consistent with prior results from inbred C57BL/6J versus DBA2/J mice.⁸⁶ Regarding the effects of ethanol, operant ethanol self-administration by adult P rats significantly reduced *mGlu1* (~20% decrease) expression levels in the AcbSh, relative to ethanol-naïve P rats.²¹⁴ Given *mGlu1*'s basal elevation, relative to NP rats, it is possible that ethanol self-administration could reverse these levels; although, it is also possible for mGlu1 protein levels to be elevated by ethanol with mRNA levels responding in the opposite direction. Regarding this hypothesis, our laboratory has shown that extended (6 months) ethanol drinking by P rats does indeed increase mGlu1, and mGlu5, receptor protein expression in the AcbCo and CeA.¹⁸⁰ Again, previous work with P rats found that an mGlu1 antagonist significantly reduced operant ethanol self-administration and

Table 1 Metabotropic Glutamate Receptor (mGluR) Gene (*mGlu*) Expression Differences Between P and NP Rats (i.e., Fold-Change (F-C) P vs. NP) or Changes Induced by Ethanol Consumption in P Rats, Relative to Ethanol-Naive P Rats (i.e., F-C Ethanol (E) vs. Control (C))

Gene ID	Gene Names	F-C P vs. NP	F-C E vs. C	Age of Rat	Brain Region
<i>mGlu7</i>	Glutamate receptor, metabotropic 7 (<i>Grm7</i> : mGluR7)		1.37	Adolescent	CeA
<i>mGlu1</i>	Glutamate receptor, metabotropic 1 (<i>Grm1</i> : mGluR1)		2.06	Adolescent	DRN
<i>mGlu4</i>	Glutamate receptor, metabotropic 4 (<i>Grm4</i> : mGluR4)		2.98	Adolescent	DRN
<i>mGlu6</i>	Glutamate receptor, metabotropic 6 (<i>Grm6</i> : mGluR6)		<i>-1.70</i>	Adolescent	DRN
<i>mGlu1</i>	Glutamate receptor, metabotropic 1 (<i>Grm1</i> : mGluR1)	1.39		Adult	AcbSh
<i>mGlu1</i>	Glutamate receptor, metabotropic 1 (<i>Grm1</i> : mGluR1)		<i>-1.19</i>	Adult	AcbSh
<i>mGlu2</i>	Glutamate receptor, metabotropic 2 (<i>Grm2</i> : mGluR2)	<i>-1.46</i>		Adult	AcbSh
<i>mGlu3</i>	Glutamate receptor, metabotropic 3 (<i>Grm3</i> : mGluR3)	<i>-1.22</i>		Adult	AcbSh
<i>mGlu4</i>	Glutamate receptor, metabotropic 4 (<i>Grm4</i> : mGluR4)	<i>-1.39</i>		Adult	AcbSh
<i>mGlu5</i>	Glutamate receptor, metabotropic 5 (<i>Grm5</i> : mGluR5)	<i>-1.30</i>		Adult	AcbSh
<i>mGlu7</i>	Glutamate receptor, metabotropic 7 (<i>Grm7</i> : mGluR7)	<i>-1.38</i>		Adult	AcbSh
<i>mGlu8</i>	Glutamate receptor, metabotropic 8 (<i>Grm8</i> : mGluR8)	<i>-1.33</i>		Adult	AcbSh
<i>mGlu2</i>	Glutamate receptor, metabotropic 2 (<i>Grm2</i> : mGluR2)	<i>-1.84</i>		Adult	CeA
<i>mGlu3</i>	Glutamate receptor, metabotropic 3 (<i>Grm3</i> : mGluR3)	<i>-1.24</i>		Adult	CeA

Age of rat refers to whether the P rats had access to ethanol during (peri-)adolescence (postnatal days (PNDs) 30–50) or during adulthood > PND75. Brain regions published thus far: CeA, DRN, and AcbSh. To facilitate distinction between directions of expression (e.g., up- vs. downregulation), downregulation, or lower level F-Cs are in italics and are below the findings of upregulation or higher-level F-Cs for that age and brain region.

breakpoint, although motor activity appeared to be affected as well.^{135,143} In general, these findings indicate that the effects of ethanol self-administration on Group1 *mGluR* mRNA expression can be distinguished from effects upon protein expression and/or that different ethanol-drinking experiences/procedures elicit distinct changes in receptor mRNA/protein expression within Acb subregions, with more protracted drinking regimens eliciting changes within the more dorsal AcbCo.

Overall, adolescent binge-like ethanol drinking upregulated two of the three mGlu receptors identified as significantly changed. In the AcbSh of adult animals, P rats had only one of the seven mGlu genes identified as significantly greater than that seen in NP rats. It is noteworthy that the one gene that had higher expression levels in the AcbSh of adult P rats was downregulated following ethanol self-administration. Both genes identified as significantly different in the CeA of adult rats were Group II (mGlu2 and mGlu3) metabotropic receptors and were lower in P versus NP rats.

6.2 Ionotropic Glutamate Receptor Expression Differences (Table 2)

Ionotropic glutamate receptor subunits *GluA3*, *GluA4*, and *Gluk3* were all elevated in the DRN following periadolescent binge-ethanol drinking, but not to the same extent as *mGlu1* and *mGlu4*.²¹³ Gene expression changes were also observed for the “orphan” ionotropic glutamate delta receptor subunits *Glud1* and *Glud2*. Regarding *Glud2*, there was a 3.5-fold increase in *Glud2ip* (delphilin) after periadolescent binge drinking as well;²¹³ *Glud2ip* is a scaffolding protein for *Glud2*. To some extent, this parallels increases in expression levels of *mGlu7*, in the CeA, induced by periadolescent binge-ethanol drinking. In the periadolescent binge-drinking P rats, the *GluN* subunits were generally downregulated, with only the *Glun2c* subunit being upregulated.²¹³

In adult P rats, whereas metabotropic glutamate receptor gene expression levels were consistently lower than those of NP rats; this was not true for *GluA* and *Gluk* gene expression,^{209,211} which was mixed. *GluA1*, *GluA2*, *GluA3*, and *Gluk3* expression levels were all lower in the AcbSh of P versus NP rats; whereas *GluA4* and *Gluk1* expression levels were higher in the AcbSh of P versus NP rats.²¹¹ Ethanol-binge drinking by adult P rats reversed the ~30% deficit of *GluA1* in the AcbSh through a 15% increase, relative to ethanol-naïve controls.²⁰⁸ However, operant ethanol self-administration by adult P rats significantly reduced *GluA2* and *GluA3* expression levels, versus ethanol-naïve controls, in the AcbSh.²¹⁴ Thus, in spite of AcbSh deficits in

Table 2 Ionotropic Glutamate Receptor Subunit Gene Expression Differences Between P and NP Rats (i.e., Fold-Change (F-C) P vs. NP) or Changes Induced by Ethanol Consumption in P Rats, Relative to Ethanol-Naive P Rats (i.e., F-C Ethanol (E) vs. Control (C))

Gene ID	Gene Names	F-C P vs. NP	F-CE vs. C	Age of Rat	Brain Region
<i>Glua3</i>	Glutamate receptor, ionotropic (<i>Gria3</i> : AMPA3)		1.29	Adolescent	DRN
<i>Glua4</i>	Glutamate receptor, ionotropic (<i>Gria4</i> : AMPA4)		1.31	Adolescent	DRN
<i>Glud1</i>	Glutamate receptor, ionotropic (<i>Grid1</i> : delta1)		1.20	Adolescent	DRN
<i>Glud2</i>	Glutamate receptor, ionotropic (<i>Grid2</i> : delta2)		2.31	Adolescent	DRN
<i>Glua3</i>	Glutamate receptor, ionotropic (<i>Grik3</i> : kainate3)		1.35	Adolescent	DRN
<i>Gln2c</i>	Glutamate receptor, ionotropic NMDA2c (<i>Grin2c</i> : NR2c)		2.61	Adolescent	DRN
<i>Gln2b</i>	Glutamate receptor, ionotropic, NMDA2b (<i>Grin2b</i> : NR2b)		-1.56	Adolescent	DRN
<i>Gln2d</i>	Glutamate receptor, ionotropic, NMDA2d (<i>Grin2d</i> : NR2d)		-1.40	Adolescent	DRN
<i>Gln3a</i>	Glutamate receptor, ionotropic, NMDA3a (<i>Grin3a</i> : NR3a)		-1.39	Adolescent	DRN
<i>Gln11a</i>	Glutamate receptor, ionotropic, NMDA1a like (<i>Grin11a</i> : NR1a like)		-1.32	Adolescent	DRN
<i>Glu1</i>	Glutamate receptor, ionotropic (<i>Gria1</i> : AMPA1)		1.15	Adult	AcbSh
<i>Glua4</i>	Glutamate receptor, ionotropic (<i>Gria4</i> : AMPA4)	1.26		Adult	AcbSh
<i>Glud2ip</i>	Glutamate receptor, ionotropic (<i>Grid2ip</i> : delta2 interacting protein)		3.49	Adult	AcbSh
<i>Gluk1</i>	Glutamate receptor, ionotropic (<i>Grik1</i> : kainate1)	1.73		Adult	AcbSh
<i>Gln1</i>	Glutamate receptor, ionotropic, NMDA1 (<i>Grin1</i> : NR1)		1.09	Adult	AcbSh
<i>Glu1</i>	Glutamate receptor, ionotropic (<i>Gria1</i> : AMPA1)	-1.32		Adult	AcbSh
<i>Glua2</i>	Glutamate receptor, ionotropic (<i>Gria2</i> : AMPA2)	-1.21		Adult	AcbSh
<i>Glua2</i>	Glutamate receptor, ionotropic (<i>Gria2</i> : AMPA2)		-1.16	Adult	AcbSh
<i>Glua3</i>	Glutamate receptor, ionotropic (<i>Gria3</i> : AMPA3)	-1.52		Adult	AcbSh
<i>Glua3</i>	Glutamate receptor, ionotropic (<i>Gria3</i> : AMPA3)		-1.19	Adult	AcbSh

<i>Gluk2</i>	Glutamate receptor, ionotropic (<i>Grik2</i> : kainate2)	-1.33		Adult	AcbSh
<i>Glua1</i>	Glutamate receptor, ionotropic (<i>Gria1</i> : AMPA1)		1.36	Adult	CeA
<i>Glua2</i>	Glutamate receptor, ionotropic (<i>Gria2</i> : AMPA2)		1.34	Adult	CeA
<i>Glua3</i>	Glutamate receptor, ionotropic (<i>Gria3</i> : AMPA3)		1.57	Adult	CeA
<i>Gluk2</i>	Glutamate receptor, ionotropic (<i>Grik2</i> : kainate2)		1.14	Adult	CeA
<i>Gluk5</i>	Glutamate receptor, ionotropic (<i>Grik5</i> : kainate5)		1.30	Adult	CeA
<i>Glun3a</i>	Glutamate receptor, ionotropic, NMDA3a (<i>Grin3a</i> : NR3a)		1.23	Adult	CeA

Refer to [Table 1](#) for details.

Glua2 and *Glua3* of adult P versus NP rats,²¹¹ operant ethanol self-administration appears to exacerbate this condition with further decreases in expression levels.

Surprisingly, despite no adult CeA *Glua*, *Gluk*, or *Glun* gene expression differences between P and NP rats; ethanol-binge drinking by adult P rats elevated *Glua1*, *Glua2*, *Glua3*, *Gluk2*, *Gluk5*, and *Glun3a* expression levels, with no significant downregulation of ionotropic glutamate receptor genes in this region.²⁰⁸

Overall, periadolescent binge-like drinking decreased gene expression for certain NMDA receptor subunits in the DRN, but increased particular AMPA, delta, and kainate receptor subunits within this region as well. Ethanol self-administration by adult P rats did not downregulate any NMDA receptor subunits in the AcbSh but did upregulate an AMPA, delta, and NMDA-receptor subunit.

6.3 Expression Differences for Glutamate Transporters, Enzymes, and Postsynaptic Density (Table 3)

One of the most striking findings is that periadolescent binge-like drinking significantly downregulated gene expression for only three ancillary proteins (*Mpp5*, a MAGUK subfamily member, by ~30%; as well as *Vglut2* and *Vglut3* by ~2-fold in the DRN); while upregulating expression levels for *Homer3* and *Slc1a1* in the CeA along with nine genes from the PSD (e.g., *Homer3* ~4-fold and *Tjp3* ~2.5-fold), as well as all three Shanks, two glutamate transporters (e.g., *Slc1a6* ~3-fold), and two glutamate carriers in the DRN.²¹³

In the adult AcbSh, P rats had lower gene expression of *Homer1* than NP rats.²¹¹ However, operant self-administration of ethanol by adult P rats upregulated *Homer1* by 3.5-fold in the AcbSh.²¹⁴ These data are interesting as studies of rodents with free-access to ethanol^{83–85,89,180} or rodents injected repeatedly with alcohol^{86,88} have consistently detected increases in Homer2 protein expression within Acb and amygdala structures, without detecting significant changes in Homer1 protein expression. This raises the possibility that ethanol-induced changes in Homer1 mRNA/protein expression may depend upon nonpharmacologic factors associated with the act of ethanol taking, which has recently been demonstrated to be the case with respect to intravenous cocaine taking.²¹⁶ Again in the AcbSh, P rats had higher expression levels of *Shank1*, whereas NP rats had higher expression levels of *Shank2* and *Shank3*.²¹¹ Our data suggest a dissociation between the effects of home-cage ethanol drinking which decreased expression of *Shank1* by ~15%²⁰⁴ as opposed to operant ethanol self-administration which

Table 3 Gene Expression, for Ancillary Proteins of the Excitatory Synapse, Differences Between P and NP Rats (i.e., Fold-Change (F-C) P vs. NP) or Changes Induced by Ethanol Consumption in P Rats, Relative to Ethanol-Naive P Rats (i.e., F-C Ethanol (E) vs. Control (C))

Gene ID	Gene Names	F-C P vs. NP	F-C E vs. C	Age of Rat	Brain Region
<i>Homer3</i>	Homer homolog 3		1.15	Adolescent	CeA
<i>Slc1a1</i>	Solute carrier family 1 (neuronal high affinity glutamate transporter), member 1		1.14	Adolescent	CeA
<i>Dlg1/Sap97</i>	Discs, large homolog 1 (Sap97, AMPAR trafficking)		1.28	Adolescent	DRN
<i>Homer3</i>	Homer homolog 3		3.75	Adolescent	DRN
<i>Mpp4</i>	Membrane protein, palmitoylated 4 (MAGUK p55 subfamily member 4)		1.68	Adolescent	DRN
<i>Mpp6</i>	Membrane protein, palmitoylated 6 (MAGUK p55 subfamily member 6)		1.23	Adolescent	DRN
<i>Pdlim5</i>	PDZ and LIM domain 5		1.28	Adolescent	DRN
<i>Shank1</i>	SH3 and multiple ankyrin repeat domains 1		1.41	Adolescent	DRN
<i>Shank2</i>	SH3 and multiple ankyrin repeat domains 2		1.79	Adolescent	DRN
<i>Shank3</i>	SH3 and multiple ankyrin repeat domains 3		1.82	Adolescent	DRN
<i>Slc1a3</i>	Solute carrier family 1 (glial glutamate transporter), member 3		1.70	Adolescent	DRN
<i>Slc1a6</i>	Solute carrier family 1 (glial glutamate transporter), member 6		2.79	Adolescent	DRN
<i>Slc17a7/Vglut1</i>	Solute carrier family 17, member 7 (vesicular glutamate transporter)		5.06	Adolescent	DRN
<i>Slc25a18</i>	Solute carrier family 25 (mitochondrial glutamate carrier), member 18		1.45	Adolescent	DRN
<i>Slc25a22</i>	Solute carrier family 25 (mitochondrial glutamate carrier), member 22		1.30	Adolescent	DRN
<i>Tjp3</i>	Tight junction protein 3 (zona occludens 3)		2.41	Adolescent	DRN

(Continued)

Table 3 Gene Expression, for Ancillary Proteins of the Excitatory Synapse, Differences Between P and NP Rats (i.e., Fold-Change (F-C) P vs. NP) or Changes Induced by Ethanol Consumption in P Rats, Relative to Ethanol-Naive P Rats (i.e., F-C Ethanol (E) vs. Control (C))—cont'd.

Gene ID	Gene Names	F-CP vs. NP	F-CE vs. C	Age of Rat	Brain Region
<i>Mpp5</i>	Membrane protein, palmitoylated 5 (MAGUK p55 subfamily member 5)		-1.27	Adolescent	DRN
<i>Slc17a6/Vglut2</i>	Solute carrier family 17, member 6 (vesicular glutamate transporter)		-1.82	Adolescent	DRN
<i>Slc17a8/Vglut3</i>	Solute carrier family 17, member 8 (vesicular glutamate transporter)		-1.97	Adolescent	DRN
<i>Dlg1/Sap97</i>	Discs, large homolog 1 (Sap97, AMPAR trafficking)		1.28	Adult	AcbSh
<i>Dlg2/Psd93</i>	Discs, large homolog 2 (Chapsyn-110/PSD-93 a MAGUK)		1.24	Adult	AcbSh
<i>Gls2</i>	Glutaminase 2	1.40		Adult	AcbSh
<i>Glul</i>	Glutamate-ammonia ligase (glutamine synthase)		1.14	Adult	AcbSh
<i>Homer1</i>	Homer homolog 1		3.49	Adult	AcbSh
<i>Mpdz</i>	Multiple PDZ domain protein	1.22		Adult	AcbSh
<i>Mpp6</i>	Membrane protein, palmitoylated 6 (MAGUK p55 subfamily member 6)	1.71		Adult	AcbSh
<i>Shank1</i>	SH3 and multiple ankyrin repeat domains 1	1.26		Adult	AcbSh
<i>Shank1</i>	SH3 and multiple ankyrin repeat domains 1		1.19	Adult	AcbSh
<i>Shank2</i>	SH3 and multiple ankyrin repeat domains 2		1.17	Adult	AcbSh
<i>Slc6a9</i>	Solute carrier family 6 (glycine transporter), member 9	1.40		Adult	AcbSh
<i>Slc7a10</i>	Solute carrier family 7 (glycine transporter), member 10 (glial)	1.42		Adult	AcbSh
<i>Slc17a6/Vglut2</i>	Solute carrier family 17, member 6 (vesicular glutamate transporter)	2.25		Adult	AcbSh
<i>Slc25a13/Citrin</i>	Solute carrier family 25 (mitochondrial glutamate carrier), member 13	1.23		Adult	AcbSh

<i>Dlg1/Sap97</i>	Discs, large homolog 1 (Sap97, AMPAR trafficking)	−1.09	Adult	AcbSh
<i>Dlg2/Psd93</i>	Discs, large homolog 2 (Chapsyn-110/PSD-93 a MAGUK)	−1.17	Adult	AcbSh
<i>Dlg2/Psd93</i>	Discs, large homolog 2 (Chapsyn-110/PSD-93 a MAGUK)	−1.38	Adult	AcbSh
<i>Dlg4/Psd95</i>	Discs, large homolog 4 (PSD95 or Sap90)	−1.25	Adult	AcbSh
<i>Gls</i>	Glutaminase	−1.35	Adult	AcbSh
<i>Homer1</i>	Homer homolog 1	−1.68	Adult	AcbSh
<i>Mpp4</i>	Membrane protein, palmitoylated 4 (MAGUK p55 subfamily member 4)	−1.08	Adult	AcbSh
<i>Mpp7</i>	Membrane protein, palmitoylated 7 (MAGUK p55 subfamily member 7)	−1.58	Adult	AcbSh
<i>Pdlim7</i>	PDZ and LIM domain 7	−1.25	Adult	AcbSh
<i>Shank1</i>	SH3 and multiple ankyrin repeat domains 1	−1.14	Adult	AcbSh
<i>Shank2</i>	SH3 and multiple ankyrin repeat domains 2	−1.22	Adult	AcbSh
<i>Shank3</i>	SH3 and multiple ankyrin repeat domains 3	−1.30	Adult	AcbSh
<i>Slc1a3</i>	Solute carrier family 1 (glial glutamate transporter), member 3	−1.30	Adult	AcbSh
<i>Cask</i>	Calcium/calmodulin-dependent serine protein kinase (MAGUK family)	1.14	Adult	CeA
<i>Homer1</i>	Homer homolog 1	2.19	Adult	CeA
<i>Pdlim3</i>	PDZ and LIM domain 3	1.28	Adult	CeA
<i>Slc25a22</i>	Solute carrier family 25 (mitochondrial glutamate carrier), member 22	1.12	Adult	CeA
<i>Gls2</i>	Glutaminase 2	−1.17	Adult	CeA
<i>Homer2</i>	Homer homolog 2	−1.16	Adult	CeA
<i>Mpp3</i>	Membrane protein, palmitoylated 3 (MAGUK p55 subfamily member 3)	−1.45	Adult	CeA
<i>Tgm2</i>	Transglutaminase 2, C polypeptide	−1.30	Adult	CeA

(Continued)

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Gene ID	Gene Names	F-C P vs. NP	F-C E vs. C	Age of Rat	Brain Region
<i>Glul</i>	Glutamate-ammonia ligase (glutamine synthetase)	1.08		Adult	VTA
<i>Dlg2/Psd93</i>	Discs, large homolog 2 (Chapsyn-110/PSD-93 a MAGUK)	<i>-1.17</i>		Adult	VTA
<i>Mpp6</i>	Membrane protein, palmitoylated 6 (MAGUK p55 subfamily member 6)	<i>-1.10</i>		Adult	VTA
<i>Pdlim7</i>	PDZ and LIM domain 7	<i>-1.12</i>		Adult	VTA
<i>Tgm2</i>	Transglutaminase 2, C polypeptide	<i>-1.38</i>		Adult	VTA

Age of rat refers to whether the P rats had access to ethanol during (peri-)adolescence (PNDs, 30–50) or during adulthood >PND75. Brain regions published thus far: CeA, DRN, AcbSh, and VTA. To facilitate distinction between directions of expression (e.g., up- vs. downregulation), downregulation or lower level F-Cs are in italics and are below the findings of upregulation or higher-level F-Cs for that age and brain region.

increased expression levels of *Shank1* by $\sim 20\%$ ²¹⁴ in the AcbSh. Some support for this dissociation can be deduced from the fact that operant ethanol self-administration also increased *Shank2* expression levels by $\sim 20\%$ in the AcbSh.²¹⁴

In the adult CeA, operant ethanol self-administration upregulated *Homer1* expression levels by over two-fold; although operant ethanol self-administration also downregulated *Homer2* expression by $\sim 20\%$, relative to controls.²¹⁴ This latter result is peculiar given that chronic free-choice access to ethanol upregulates CeA *Homer2* protein expression in P rats¹⁸⁰ and C57BL/6J mice,⁸⁵ which, again, may be due to nonpharmacologic factors related to the operant-conditioning procedures employed in the mRNA study.

Glycine transporter expression levels in the adult AcbSh were higher and glutamate transporter expression levels were lower in P rats compared to NP rats.²¹¹ Additionally, whenever ethanol intake modified membrane glutamate transporter expression levels, once each in the adult or adolescent CeA and multiple times in the adolescent DRN, it was always for an increase relative to control levels.^{208,213} However, when examining vesicular glutamate transporter expression levels, *Vglut1* was increased by five-fold, whereas *Vglut2* and *Vglut3* were reduced by \sim two-fold in the DRN following peri-adolescent binge-like drinking.²¹³ Also, in the adult AcbSh *Vglut2* expression levels were more than two-fold higher than that seen in NP rats.²¹¹

In general, adolescent binge-like drinking upregulated 14 of the 17 glutamate ancillary genes identified as significantly changed, suggesting that ethanol induces substantial increases in DRN glutamatergic activity during this stage of development. Of the genes identified as significantly different between P and NP rats, in the AcbSh of adult animals, half were higher and half were lower. In the CeA (9 of 17) and VTA (4 of 5), adult P rats generally had lower expression levels of glutamate ancillary genes than their NP counterparts. Regarding ethanol exposure, 6 of the 10 genes identified as significantly different relative to ethanol-naïve controls were upregulated in the AcbSh of adult P rats. In ethanol-drinking/self-administering adult P rats, two of the five genes identified as significant in the CeA were downregulated. Overall, these findings of glutamate transporter and cytoskeleton/scaffolding protein gene expression level changes induced by ethanol parallel the existing literature indicating that ethanol exposure alters glutamate clearance from the synapse and induces neuroplastic changes in the PSD.



7. CONCLUSIONS

The findings discussed in this review include results from both pre-clinical as well as neuroimaging and postmortem clinical studies. Expression levels for a number of glutamate-associated genes and/or proteins are modulated by alcohol abuse and dependence. These changes in expression include metabotropic receptors and ionotropic receptor subunits as well as different glutamate transporters. Moreover, these changes in gene expression parallel pharmacologic manipulation of these same receptors and transporters. Some of these gene expression changes may have predated alcohol abuse and dependence, because a number of glutamate-associated polymorphisms are related to a genetic predisposition to develop alcohol dependence. Other glutamate-associated polymorphisms have been linked to age at the onset of alcohol-dependence and/or initial level of response/sensitivity to alcohol. Finally, findings of innate and/or ethanol-induced glutamate-associated gene expression differences/changes observed in a genetic animal model of alcoholism, the P rat, are highlighted. Overall, the existing literature indicates that changes in receptors, transporters, enzymes, and scaffolding proteins are crucial for the development of alcohol dependence and there is a substantial genetic component to these effects.

This review reveals that there are presently key limitations to our understanding of glutamate's role in the development of alcohol dependence and the impact that genetics has on this process. First, there are no studies examining glutamate-associated gene and/or protein expression changes across the juvenile, adolescent, emerging adult, and full adult stages of development. This information is crucial given the fact that the risky drinking age-of-onset is inversely associated with the probability of developing alcohol dependence (i.e., earlier onset leads to higher risk of developing alcoholism). And, while there is some evidence for an association between being a FHP individual and initiating risky drinking at a younger age, findings comparing the effects of ethanol on glutamate function between FHP individuals/models and FHN individuals/models are very limited. Second, studies thus far have been limited to gross examinations of regions and/or subregions of major structures in the central reward neurocircuitry. Also related to this point, a third limitation is the lack of publications examining multiple regions (i.e., putative circuits) within a single study. Despite these limitations, substantial progress has been made

with new targets for medications development/screening identified, such as the GLT1/EAAT2 glutamate transporter or PKC- ϵ 's modulation of mGlu5 activity. Nevertheless, the research community still has much to do in unraveling the role of glutamate-associated genes in the development of alcohol dependence, especially as it relates to pharmacogenomics and personalized pharmacologic interventions.

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