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A Molecular Mechanism of Ethanol Dependence: The Influence of the Ionotropic Glutamate Receptor Activated by N-Methyl-D-Aspartate

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1. Introduction

The World Health Organization (WHO) estimates that there are about 2 billion people world-wide who consume alcoholic beverages (WHO, 2004). Alcohol use is related to a wide range of physical, mental, and social detriments. Additionally, alcohol affects almost every organ in the human body as well as the central nervous system (CNS) (Spanagel, 2009). There are several theories as to how alcohol affects the CNS. They are classified into two main groups depending on the primary target of ethanol. These two groups are lipid and protein theories (Goldstein, 1986). Before the 1990s, different lipid theories postulated that alcohol acted via some perturbation of the membrane lipids in CNS neurons. In particular, the effects on membrane fluidity and the disordering of the bulk lipid phase of membranes were originally attractive hypotheses for alcohol action. However, recently the protein hypothesis has become the predominant theory (Lovinger, 1997). This hypothesis predicts that alcohol acts specifically on membrane proteins such as receptors and ion channels. The main reason for a shift towards the protein theory originates from evidence that alcohol, at concentrations in the 10–20 mM range, directly interferes with the function of several ion channels (K^+, Ca^{2+}) and receptors (Lovinger et al., 1989). These ethanol effects are mediated through a number of neural transmitter systems including γ-aminobutyric acid (GABA) and glutamate (Takadera et al., 2008; Murail et al., 2011).

The GABA receptor is involved in GABA signalling and the ionotropic glutamate receptor complex activated by *N*-methyl-D-aspartate (iGluR-NMDA) is involved in glutamate signalling. The GABA and NMDA receptors have competing roles in neural excitability and transmission. Activation of GABA receptors results in a decrease in neural activity. In contrast, activation of iGluR-NMDA results in an increase in neural activity. Alcohol has been shown to have opposite effects on these two types of receptors. Alcohol administration leads to increases in GABA receptor activity and decreases in iGluR-NMDA activity (Suzdak et al., 1986; Tsai et

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al., 1995). The GABA receptor is a key inhibitory neurotransmitter receptor in the CNS (Figure 1). There are two types of GABA receptors. The $GABA_A$ receptor is a ligand-gated ion channel receptor and the $GABA_B$ receptor is a G coupled-protein receptor. Both are associated with the influx of chloride ions into the cell upon activation by GABA. Under normal conditions GABA binds to the GABA receptor and the chloride channel opens (Figure 1). This allows negatively charged chloride ions to enter the cell and inhibit neuronal cell activity. The GABA receptor is affected by low concentrations of alcohol (Suzdak et al., 1986). Also, ethanol has been shown to reduce the number of $GABA_A$-receptor sub-units, and GABA receptor polymorphisms have been associated with several alcoholic phenotypes (Mihic et al., 1997; Sander et al., 1999). The effects of alcohol are not limited to the modulation of GABA receptor activity; they also modulate iGluR-NMDA activity.

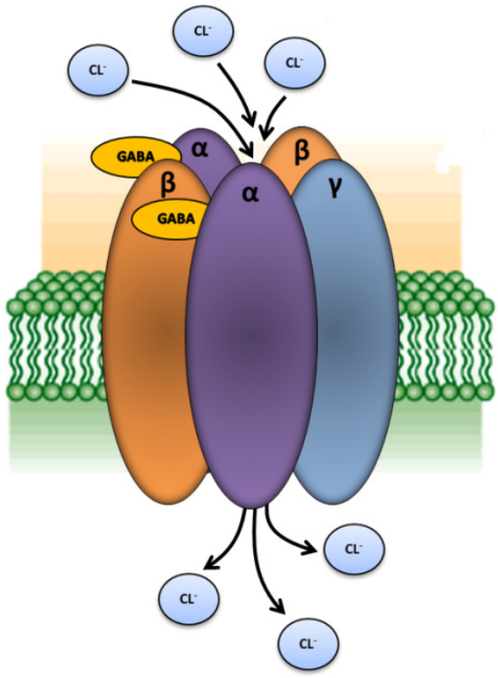

Fig. 1. The GABA receptor composition and potential GABA binding sites. Adapted from Belelli, 2005.

Most GABA receptors are believed to assemble as pentamers with two α subunits, two β subunits, and one γ subunit. The figure shows the influx of Cl^- ions into the cell during GABA activation of the GABA receptor. The GABA molecules bind to interfaces between the α and β subunits.

The iGluR-NMDA is one of the most active molecules in the central nervous system that is involved in learning and memory. It has been extensively studied during the last 30 years. iGluR-NMDA function is inhibited by ethanol in a concentration-dependent manner over the range of 5–50 mM. This is also the concentration range that produces intoxication and that is linearly related to the intoxication potency (Ron, 2004). This suggests that ethanol-induced inhibition of responses to the iGluR-NMDA activation may contribute to the neural and the cognitive impairments associated with alcohol intoxication. However, the mechanism(s) of ethanol interference on NMDA receptor function remains in question.

The iGluR-NMDA is a ligand-gated ion channel with a heteromeric assembly of GluN1, GluN2 (A-D), and GluN3 subunits. The GluN1 and GluN2 subunits contain the co-agonist and agonist binding sites for glycine and glutamate respectively. The GluN3 subunit has some modulatory functions on channel activity especially under pathological conditions (Paoletti, 2011; Traynelis et al., 2010). Electrophysiological studies demonstrated ethanol interactions with domains that influence channel activity. This suggested that residues within the transmembrane (TMD) domains were involved. In the search for these possible binding sites of alcohol in the iGluR-NMDA, several putative binding sites were discovered. Utilizing site-directed mutagenesis, several studies reported putative binding sites in the TM3 and TM4 domains of the GluN1 and GluN2 subunits, respectively. Furthermore, the substitution of an alanine for a phenylalanine residue in the TM3 domain of the GluN1 subunit strongly reduced ethanol sensitivity in recombinant iGluR-NMDAs (Ren et al., 2003, Ren et al., 2008).

The iGluR-NMDA functions as a modulator of synaptic response and a molecular coincidence detector. At resting membrane potentials, iGluR-NMDAs are inactive. This is due to a voltage-dependent block of the channel pore by magnesium ions. This prevents ion flow. For example, the depolarization of the post-synaptic cell occurs through a train of impulses arriving at the pre-synaptic terminal. These impulses sustain the activation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. AMPA receptors are a non-NMDA-type ionotropic transmembrane receptor for glutamate. This depolarization caused by the influx of sodium ions into the post-synaptic cell leads to the repulsion of magnesium ions in the iGluR-NMDAs. This repulsion of magnesium ions releases the channel inhibition and allows for iGluR-NMDA activation. However, this is not the only factor necessary for iGluR-NMDA channel function. Other factors include the agonist (glutamate) and the co-agonist (glycine) that allows the channel to open. Unlike GluA2-containing AMPA receptors, NMDA receptors are permeable to calcium ions as well as being permeable to other ions such as sodium and potassium. Therefore, iGluR-NMDA activation leads to a calcium influx into the post-synaptic cell. This event is important in the activation of a number of signaling cascades. Depending on the specific impulse received, the iGluR-NMDA is responsible for a wide range of post-synaptic functions that are involved in physiological processes such as long-term potentiation (LTP) and synaptic plasticity (Van Dongen, 2009). These processes have essential functions in learning and memory (Traynelis et al., 2010). In addition, the iGluR-NMDA is involved in different neurodegenerative diseases such as Alzheimer's, Huntington's, and Parkinson's (Ułas et al., 1994; Hallett et al., 2005; Levine et al., 2010; Saft et al., 2010). It is also involved in psychiatric disorders and pathophysiological conditions such as neuropathic pain (Javitt & Zukin, 1991; Collins et al., 2010). Recently, the iGluR-NMDA has been proposed as an important factor that can be altered in several addictions such as drug and alcohol addiction.

Ethanol inhibits iGluR-NMDA activity at very low concentrations that are typically found during alcohol dependence and abuse (Vengeliene et al., 2005). Several studies have investigated the direct involvement of the N-methyl D-aspartate receptor subtype 2B (NR2B)-containing iGluR-NMDA in ethanol dependence. Narita et al. demonstrated that the protein levels of NR2B subunits in the limbic forebrain but not the cerebral cortex were significantly increased during chronic ethanol dependence in mice. These findings suggest that the up-regulation of NR2B subunits during chronic ethanol exposure may be implicated in the initial development of physical dependence on ethanol (Narita et al., 2007). Sheela et

al. reported that NR2B mRNA was significantly elevated in cultured mouse cortical neurons during chronic ethanol exposure (intermittent and non-intermittent) and remained elevated 5 days after withdrawal (Sheela et al., 2006). Studies such as these and others demonstrate that ethanol is a potent inhibitor of the iGluR-NMDA in a number of brain regions (Lovinger, et al., 1989; Lovinger, 1995; Weitlauf & Woodward, 2008). The ability of ethanol to inhibit responses to the iGluR-NMDA is dependent on the subunit combination of the iGluR-NMDA. The N-methyl D-aspartate receptor subtypes 1/2A (NR1/NR2A) and 1/2B (NR1/NR2B) combinations are preferentially sensitive to ethanol inhibition (Otton et al., 2009).

Structural information about the putative alcohol-binding sites on proteins such as the iGluR-NMDA continues to be discovered (Peoples & Weight, 1992; Mirshahi & Woodward, 1995). The functional impact of these binding sites also remains to be elucidated. Substitution studies have shown that a complete substitution for ethanol is exerted by iGluR-NMDA antagonists and certain GABA-mimetic drugs acting through different sites within the $GABA_A$ receptor complex. It has been consistently shown in mice, rats, and monkeys that noncompetitive antagonists of the iGluR-NMDA such as dizocilpine (MK-801), phencyclidine (PCP), ketamine, or memantine (which all act as an ion channel blockers) result in a generalized ethanol response. However, competitive iGluR-NMDA antagonists have often shown only partial substitution for ethanol. Moreover, it has been demonstrated that ketamine produced dose-related ethanol-like subjective effects in detoxified alcoholics. This suggests that NMDA receptors mediate the subjective effects, at least in part, of ethanol in humans (Ren et al., 2003).

In recent years the iGluR-NMDA has emerged as one of the most important and relevant molecules in all neural processes and a key structure in all excitable tissues. The iGluR-NMDA participates in almost all physiological, pathological, and pharmacological processes of the postsynaptic neural membrane. In addition, the iGluR-NMDA is a major target of alcohol (ethanol) in the brain and has been implicated in acute tolerance, long-term facilitation (LTF), sensitization, dependence, withdrawal, and craving (Nagy & László, 2002; Trujillo & Akil, 1995; De Witte, 2004). This chapter's focus is to present in a coherent and comprehensive approach as to why the iGluR-NMDA is one of the most important therapeutic targets in alcohol addiction. It will provide important information for understanding the effects produced by ethanol on the iGluR-NMDA such as the signaling pathways involved and the physiological consequences. It will also summarize information regarding the potential use of different iGluR-NMDA modulators as therapeutic treatments for the adverse effects of alcoholism. In addition, the review will summarize key results obtained from preclinical research such as *in vivo* animal models, *in vitro* cellular models, and *ex vivo* organotypic/acute brain slice models that are currently used to investigate CNS addictions.

2. Structural and functional aspects of the iGluR-NMDA

The iGluR-NMDA is a post-synaptic receptor involved in most neural functions that include fundamental processes such as learning, memory, and possibly consciousness (Lebel et al., 2006; Lareo & Corredor, 2007). The NMDARs are heteromeric complexes composed of three major types of subunits: NR1, with eight isoforms generated by the alternative splicing of the Grin1 gene (Perez-Otano et al., 2001); four NR2 subunits (A–D) generated by the genes

Grin2A–D (Sun et al., 2000); and two NR3 subunits generated by the genes *Grin3A* and *Grin3B* (Andersson et al., 2001). The stoichiometry of the NMDAR remains unknown. It is also not clear whether the NMDAR is a trimeric, tetrameric, or pentameric subunit complex (Ferrer-Montiel & Montal, 1996; Laube et al., 1998; Rosenmund et al., 1998; Hawkins et al., 1999; Nusser, 2000). However, it is known that the various cellular, biophysical, and pharmacological properties of NMDARs are dependent on the splice variants and the composition of these subunits within the receptor complex (Cull-Candy & Leszkiewicz, 2004; Paoletti & Neyton, 2007). The NMDAR is differentially distributed throughout the CNS and has been shown to mediate the fast synaptic action of the major excitatory neurotransmitter L-glutamate (Cochilla & Alfors, 1999; Nusser, 2000). These receptors are multimodulated. Glycine, polyamines (spermine and spermidine), histamine, and cations can act as positive modulators (McBain & Mayer, 1994; Hirai et al., 1996; Kashiwagi et al., 1997; Paoletti et al., 1997). The NMDA receptors are coupled to high conductance cationic channels that are permeable to Ca^{+2}, K^{+}, and Na^{+} ions (Cushing et al., 1999).

NMDAR subunits contain a long extracellular N-terminal domain, three true transmembrane segments, a re-entrant pore loop, and an intracellular C-terminal domain of variable length (Mayer, 2005). The C-terminus of both NR1 and NR2 subunits interact with several intracellular scaffolding proteins and are subject to phosphorylation. As such, they are involved in the regulation of receptor trafficking and function (Salter & Kalia, 2004; Lau & Zukin, 2007). Glutamate, an agonist, binds to the NR2 subunits while the co-agonist glycine binds to the NR1 subunit. The N-terminal domain of the NR2 subunit is subject to allosteric inhibition by compounds such as ifenprodil and zinc (Figure 2) (Perin-Dureau et al., 2002; Hatton & Paoletti, 2005). Synaptic NMDA receptors are localized in the post-synaptic density where they are structurally organized into large macromolecular complexes that interact with signaling molecules such as kinases and phosphatases. They also interact with other transmembrane proteins such as adhesion proteins and metabotropic glutamate receptors (mGluRs) (Husi et al., 2000). Membrane export and synaptic insertion of NMDA receptors involves intrinsic trafficking signals specific for each subunit, splice variant, and complex interaction between NMDA receptors and a variety of interacting proteins. These interacting proteins include the post-synaptic density protein (PSD95), Drosophila disc large tumor suppressor (Dlg1), and zonula occludens-1 protein (zo-1) also known collectively as PDZ-domain proteins. Membrane insertion and regulated endocytosis of NMDA receptors are also tightly controlled by phosphorylation events (Chen & Roche, 2007; Lau & Zukin, 2007). The synaptic activity of NMDARs influence the number and the subunit composition of other synaptic membrane receptors (Zhou & Baudry, 2006; Lau & Zukin, 2007).

The NMDAR requires simultaneous activation by glutamate and glycine for channel opening (Dingledine et al., 1999). Ion passage also requires depolarization because magnesium directly blocks the ion channel in a voltage-dependent manner. Although the physiological significance remains unknown, the receptor is also modulated by polyamines such as spermine and spermidine in a biphasic manner (Figure 2) (Lynch & Guttmann, 2002). At low micromolar concentrations, polyamines promote channel opening by increasing the affinity of the receptor for glycine as well as by removing tonic proton inhibition (Dingledine et al., 1999). In contrast, polyamines at high concentrations that are probably not achievable *in vivo* block the channel in a voltage dependent manner. Three

other types of endogenous compounds (zinc, redox modulators, and nitric oxide) also inhibit the NMDA receptor allosterically through different sites (Lynch & Guttmann, 2002). Several compounds such as haloperidol, amitriptyline, and amantidine have been characterized for their ability to inhibit NMDA receptors. These diverse pharmacological antagonists produce different effects when given to animals which suggest that the NMDA receptor population within the brain is heterogeneous.

2.1 NMDA receptor complexes: Structure and function

The NMDA type of glutamate receptor is thought to play a role in long-term potentiation, memory formation, and controlling brain development (MacDonald et al., 2006; Ewald & Cline, 2009; Vastagh et al., 2012). NMDA receptor-mediated neurotoxicity is implicated in neurodegeneration associated with epilepsy, ischemia, Huntington's chorea, Alzheimer's disease, and AIDS encephalopathy (Durand et al., 1993; Reyes et al., 2006).

Three gene families encoding NMDA receptor subunits have been identified in rat brain. One family is composed of the NR1 gene. The NR1 gene encodes RNA that undergoes alternate splicing to yield at least eight receptor variants. These variants arise from the splicing of three alternative exons which have been designated as N1, C1, and C2. Exon N1 encodes 21 amino acids that can be inserted into the N-terminal domain. Exons C1 and C2 are adjacent and encode the last portion of the C-terminal domain. Exon C1 encodes 37 amino acids and exon C2 encodes 38 amino acids before reaching a stop codon followed by an additional 239 nucleotides from the 3' non-coding region. The splicing out of exon C2 removes the first stop codon. This yields an open reading frame that encodes an unrelated sequence C2' which consists of 22 amino acids before a second stop codon is reached. The NR1 subunit is essential for channel activity and has glycosylated and de-glycosylated functionally active forms (Reyes et al., 2006).

There are four subtypes (A–D) of the NR2 subunit which bind glutamate (Figure 2). These subunits confer the majority of pharmacological and biophysical properties associated with NMDA receptor (NMDAR) subtypes (Chen & Wyllie, 2006). Since the cloning of NMDAR subunits, the identification of many native NMDARs has been elucidated by comparing the properties of native receptors with those of known recombinant subunit compositions. These studies determined that NR2A and NR2B-containing NMDARs are widely expressed throughout the CNS while NR2C-containing NMDARs are mainly expressed in the cerebellum. Expression levels of NR2D subunits peak around the first week of postnatal development and are thought to be retained in certain neurons that express receptors with properties indistinguishable from recombinant receptors containing only NR1 and NR2D subunits (Monyer et al., 1992; Momiyama et al., 1996; Misra et al., 2000). Activation of NR1/NR2D NMDARs at synaptic sites are thought to produce long lasting synaptic events since recombinant forms deactivate with a time constant of several seconds following rapid synaptic-like glutamate application (Vicini et al., 1998; Wyllie et al., 1998; Wyllie, 2008). The NR2 subunits contain divergent sequences that regulate unique protein-protein interactions and distinct receptor trafficking properties. For example, the NR2A and NR2B intracellular C-terminal domains contain trafficking motifs that regulate NMDAR endocytosis and intracellular trafficking (Tang et al., 2010).

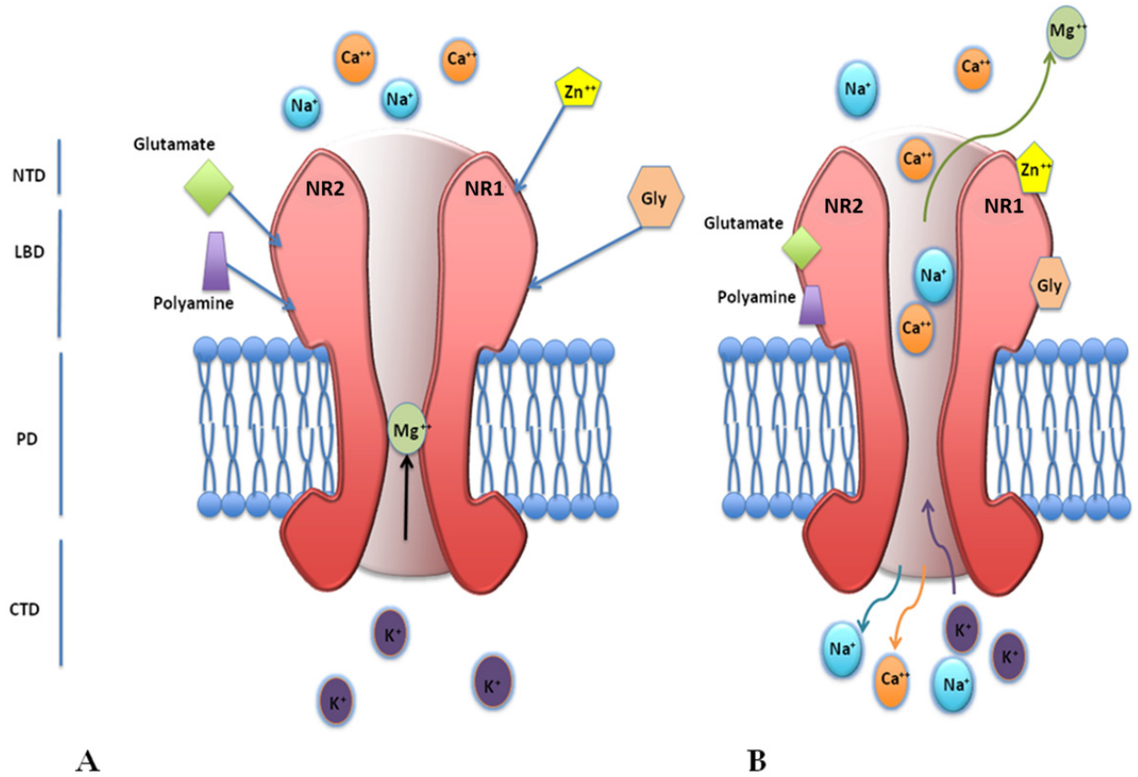

Fig. 2. NMDA receptor composition and potential ligand binding sites.

Most NMDARs are believed to assemble as tetramers that associate two NR1 and two NR2 subunits in a "dimer of dimers" quaternary structure. The diagram shows an assembly with the heterodimer NR1/NR2. For clarity, only one of the two NR1/NR2 heterodimers is shown. The extracellular region is composed of the N-terminal domain (NTD) and ligand binding domain (LBD). Allosteric modulators such as zinc interact with the NTD. Competitive agonists such as glycine, glutamate, and polyamines interact with the LBD. The intermembrane region is composed of the Pore Domain (PD). The Mg^{2+} ion is the endogenous pore blocker of the PD. The intracellular region is composed of the C-terminal domain (CTD). The CTD is involved in receptor trafficking and cell signaling processes.

The successful cloning of NR3, the third subunit of the NMDA receptor, has taken the complexity of NMDA receptors to a new level. NR3 subunits have been identified in rat brains in two variants known as NR3A and NR3B (Méndez et al., 2008; Ciabarra et al., 1995; Sucher et al., 1995). The NR3 subunits have been reported in GenBank as L34938 and U29873, respectively. NR3A has 27% similarity to the other NMDA receptor subunits and 23% similarity to other non-NMDA receptor proteins. Despite this low homology, NR3A was grouped under the NMDA receptor because the CTD and the region upstream of M1 are structurally related to other NMDA receptor subunits (Ciabarra et al., 1995; Moreno et al., 2010; Vargas et al., 2010). The NR3B subunit was initially discovered in 1995. Its complete characterization was published later by other groups (Forcina et al., 1995;

Sevarino et al., 1996; Matsuda et al., 2003; Méndez, 2008). NR3B is also the most similar to NR3A with 47% similarity in amino acid sequence, but it has only 17-21% similarity to NR1 and NR2. There is greater similarity between NR3 and NR1 than with NR2 (Andersson et al., 2001). The mouse homolog of NR3B has 1003 residues whereas the rat homolog is one residue shorter (Chatterton et al., 2002; Nishi et al., 2001; Low & Wee, 2010). NR3 subunits have been reported to be expressed differentially in space and in time. Méndez et al. reported that the NR3A subunit is expressed in different proportions between 1 day postnatal and adult rats, while NR3B has the same expression at both age groups (Méndez et al., 2008).

The "dimer of dimers" quaternary structure of the NMDAR contains at least 2 glutamate binding sites and 2 glycine-binding sites (Figure 2). NMDARs can also assemble with 2 different NR1 splice isoforms and 2 different NR2 subunits. Studies on the AMPA receptor (AMPAR), another member of the ionotropic glutamate receptor family, have given insights into the structure of the NMDA receptor. Crystallographic analyses coupled with electrophysiologic studies indicate a tetrameric structure similar to AMPARs. In the NMDAR, regions of NR2 and NR1 subunits are necessary for transmitting allosteric signals between the glutamate and glycine-binding sites that are analogous to the areas of dimer interactions in AMPARs. This suggests that the NMDARs have similar dimer-dimer interactions. Therefore, collected research suggests that functional NMDAR complexes are tetramers of 2 NR1 and 2 NR2 subunits with an evolutionary link between glutamate receptors and potassium channels (Figure 2). The actual process of assembly of the individual subunits into the functional channel has not been well characterized. However, critical residues in this process are known to be located in the N-terminal domain of the NMDAR (Prybylowski & Wenthold, 2004).

2.2 Stoichiometry

The stoichiometry of NMDA receptors has not been completely established, but the consensus is that they are mostly tetramers composed of two NR1 subunits and two NR2 subunits (Paoletti & Neyton, 2007; Ulbrich & Isacoff, 2008) (Figure 3). NMDARs assemble from two glycine-binding NR1 subunits with two glutamate-binding NR2 subunits to form glutamate-gated excitatory receptors that mediate synaptic transmission and plasticity (Figure 2, 3). The role of glycine-binding NR3 subunits is less clear. In *Xenopus laevis* oocytes, two NR3 subunits co-assemble with two NR1 subunits to form a glycine-gated receptor; such a receptor has yet to be found in mammalian cells. The NR1, NR2, and NR3 appear to co-assemble into tri-heteromeric receptors in neurons, but it is not clear whether this occurs in oocytes (Figure 3). To test the rules that govern subunit assembly in NMDA receptors, Ulbrich and Isacoff developed a single-molecule fluorescence co-localization method. They found that NR1, NR2, and NR3 follow an exclusion rule that yields separate populations of NR1/NR2 and NR1/NR3 receptors on the surface of oocytes. In contrast, co-expression of NR1, NR3A, and NR3B yields tri-heteromeric receptors with a fixed stoichiometry of two NR1 subunits with one NR3A subunit and one NR3B subunit (Figure 3). Therefore, at least part of the regulation of subunit stoichiometry appears to be caused by internal retention. Cell-to-cell differences in these rules may help sculpt distinct physiological properties (Ulbrich & Isacoff, 2008).

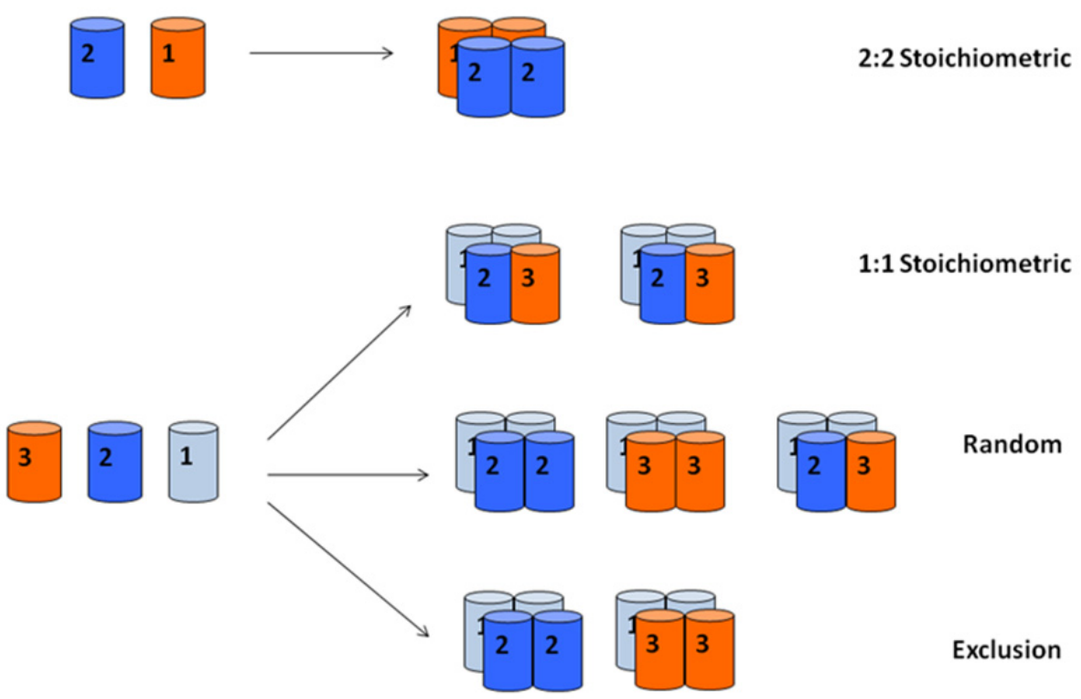

Fig. 3. The different assembly scenarios for NMDA receptors:

Scenario (i) is a 2:2 stoichiometric assembly where two NR1 (orange) and two NR2 (blue) subunits co-assemble. Scenario (ii) is a 1:1 stoichiometric assembly where two NR1 (light blue) subunits assemble with one NR2 (blue) and one NR3 (orange) subunit. Scenario (iii) is a random assembly where two NR1 (light blue) subunits assemble randomly with two NR2 (blue) or two NR3 (orange) subunits, or one NR2 (blue) and one NR3 (orange) subunit. Scenario (iv) is an exclusion rule where two NR1 (light blue) subunits assemble with either two NR2 (blue) or two NR3 (orange) subunits but never form a tri-heteromeric receptor (adapted from Ulbrich & Isacoff, 2008).

3. The impact of alcohol on the NMDA receptor

Alcohol has a complex pharmacology that acts by disrupting distinct receptor or effector proteins via direct or indirect interactions (protein theory). At very high concentrations, it might even change the composition of lipids in the surrounding membrane (lipid theory). At concentrations in the 5–20 mM range, (which constitutes the legal intoxication range for driving in many countries) alcohol directly interferes with and/or influences the function of several membrane receptors. Lovinger et al. showed that NMDA function was inhibited by alcohol in a concentration dependent manner in the range of 5-50 mM. The amplitude of the NMDA-activated current was reduced 61% by 50 mM alcohol (Lovinger et al., 1989). Also, the potency of several alcohols to inhibit the NMDA-activated current is linearly related to their intoxicating potency. This suggests that alcohol-induced inhibition of NMDA receptor activation may contribute to the neural and cognitive impairments associated with intoxication. Several other ionotropic receptors have also been characterized as primary targets of alcohol. These other ionotropic receptors include $GABA_A$ and glycine receptors

that have their functions enhanced by alcohol (Mihic, 1999). Alcohol has also been shown to potentiate the function of other non-ionotropic receptors such as neuronal nicotinic ACh receptor (nAChR) and 5-hydroxytryptamine 3 (5-HT3) that is also known as serotonin (Lovinger, 1999; Narahashi et al., 1999).

The influence of alcohol on ionotropic receptors depends on the alcohol concentration and receptor subunit composition. For example, NMDA receptors composed of either NR1/NR2A or NR1/NR2B subunit complexes are more sensitive to alcohol's inhibitory effects than those composed of NR1/NR2C or NR1/NR2D subunit complexes (Kalluri et al., 1998; Allgaier, 2002). Another example is $GABA_A$. $GABA_A$ receptors are composed of α, β, γ, and δ subunits. Most subunit compositions of $GABA_A$ receptors are deficient in δ subunits and only display responses to alcohol at high concentrations (460 mM). However, $GABA_A$ receptors containing δ subunits are affected by very low concentrations (1–3 mM) of alcohol. Also in α4βδ subunit complexes, $GABA_A$ receptors containing the β3 subunit have been found to be almost 10 times more sensitive to alcohol than receptors containing the β2 subunit (Wallner et al., 2003).

In summary, despite the generally held view that alcohol is a non-specific pharmacological compound recent studies demonstrate that it specifically targets certain receptors such as NMDA, GABA, 5-HT3, and nAChRs. Concentrations as low as 1 mM produce alterations in the function of these ionotropic receptors. The complex interaction of alcohol on these receptors is generally characterized by the inhibition of NMDA receptors and by the inhibition of GABA receptors. This complex interaction of alcohol with different receptors is responsible for the psychotropic effects seen with alcohol consumption. These pathways involved in the effects of alcohol on the brain continue to be elucidated.

3.1 The stages of alcohol effects and NMDAR function

The effects of alcohol can be categorized into several stages. These stages are referred to as initiation of alcohol consumption (acute alcohol effects), maintenance of alcohol consumption (chronic alcohol effects and loss of control), craving and alcohol seeking (withdrawal), and relapse to alcohol use (compulsive alcohol consumption) (Wolffgramm et al., 2000; Heyne et al., 1998; Heyne et al., 2000; Ferko, 1994). The glutamatergic system which is a fast-signaling system important for information processing has been shown to play a pivotal role in these stages. The glutamatergic system is composed of at least three major types of glutamate receptors: the AMPA receptor, the NMDA receptor, and the kainate receptor. The NMDA receptor has been demonstrated to have major influence in the first and last stages of alcohol effects and minor or no influence in the middle stages. The AMPA receptor has been shown to have a major influence in the craving and reinstating of alcohol seeking stage (middle stage).

In the initiation of alcohol consumption stage, glutamate has been shown to enhance the central depressant action of alcohol because glutamate can alter the alcohol induced loss of righting reflex (LORR) (Petrakis et al., 2004). NMDARs are associated with decreases in LORR response time and are primary targets of alcohol. This suggests that altered NMDAR function contributes to the initial pathophysiological response during acute alcohol exposure (Ge et al., 2007). Accordingly, NMDAR antagonists are capable of preventing initial alcohol responses. This was shown by the elimination of alcohol-induced conditioned

place preference in rats during administration of dizocilpine (a non-competitive NMDAR antagonist). It was also shown by the attenuation of alcohol self-administration in a free-choice operant task with administration of 2-amino-5-phosphopentanoic acid (a competitive NMDAR antagonist) microinjections (Biala & Kotlinska, 1999; Rassnick et al., 1992).

Nitric oxide (NO) production has also been correlated to the initiation of alcohol consumption stage. The glutamatergic/NMDA receptor system is closely linked to NO production. NO is an intracellular and extracellular messenger which is produced by nitric oxide synthase (NOS) (Bredt et al., 1990). The stimulation of NMDARs leads to a calcium influx within the cell and the binding of calcium to calmodulin activates neuronal NOS (nNOS) activity (Spanagel et al., 2002). Other studies have also implicated NOS activity in the modulation of alcohol mediated effects on the CNS (Spanagel et al., 2002; Deng & Deitrich, 2007). Alcohol has been shown to increase inducible NOS (iNOS) activity in glial cells and inhibit nNOS activity in neurons. The link between the NMDA receptor system and NO production implies that both iNOS and nNOS activity within the brain may also be involved in the modulation of acute alcohol effects. NOS activity has also been implicated in the maintenance of alcohol consumption stage (Deng & Deitrich, 2007).

In the maintenance of alcohol consumption stage, adaptive responses occur such as changes in the number and/or affinity of synaptic glutamate receptors or their subunits (Henniger et al., 2003; Vengeliene et al., 2008). These adaptive responses act to counterbalance the acute inhibitory effect of alcohol on iGluR-NMDA function (Nagy et al., 2003; Wu et al., 2010). However, studies suggest that iGluR-NMDA has no influence during this stage. For example, NR2A subunit deletion in mice does not affect voluntary alcohol intake (Boyce-Rustay & Holmes, 2006). Also studies utilizing knockout mice (GluR1 and GluR3 deletions) did not have any effect on either home cage alcohol drinking or operant self-administration (Cowen et al., 2003; Sanchis-Segura & Spanagel, 2006). In contrast, two studies did report that antagonists against the non-ionotropic receptor mGluR5 were capable of reducing alcohol-reinforced responding in mice and alcohol-preferring P/Fawn-Hooded rats (Cowen et al., 2005; Schroeder et al., 2005). This suggests a role for non-ionotropic receptors during this stage of alcohol effects.

In the craving and alcohol seeking stage, adaptive responses in the glutamatergic system cause hyper-excitability in the Central Nervous System (CNS) during withdrawal or conditioned withdrawal. Animal studies have shown that the overactivation of glutamate receptors contributes to the generation of hyper-excitability (Grant et al., 1990; Gulya et al., 1991; Davidson et al., 1995; Grant, 1999). Human studies have supported this hyper-excitability by demonstrating that excitatory neurotransmitters were elevated in the cerebrospinal fluid of alcohol-dependent patients (Tsai & Coyle, 1998). These adaptive responses may represent one mechanism that causes alcohol cravings (Gass & Olive, 2008). Both NMDA receptors and non-NMDA ionotropic glutamate receptors such as AMPA receptors have major roles during this stage (Bachteler et al., 2005; Sanchis-Segura et al., 2006). More specifically, as in the previous stage, mGluRs have also been implicated in this alcohol-craving/seeking behavior. For example, mGluR5 receptor antagonists have been effective in attenuating alcohol cravings (Bäckström et al., 2004). In regards to the NMDA receptor, ethanol withdrawal has been shown to potentiate NMDA-induced damage to the hippocampus by increases in mRNA expression of the NR2 subunit which is correlated with withdrawal seizures (Davidson et al., 1993; Follesa & Ticku, 1996). Also, the competitive

NMDA receptor antagonist, CGP-39551, is a potent inhibitor of withdrawal seizures and hyperexcitability (Liljequist, 1991; Ripley & Little, 1995).

In the last stage, relapse to alcohol use, one major hypothesis proposes that the glutamatergic system is critically involved (Gass & Olive, 2008). Several studies have demonstrated a major role for the NMDA receptors during this stage. For example, the clinical drug acamprosate, known to attenuate hyper-glutamatergic activity, was capable of reducing the alcohol deprivation effect (ADE) in Wistar rats under home cage and operant conditions (Spanagel et al., 1996; Spanagel et al., 2005; Heyser et al., 1998). Furthermore, Hölter et al. demonstrated that chronic treatment with a non-competitive NMDA receptor antagonist selectively abolished the increased alcohol intake during the ADE (Hölter et al., 2000). Similarly, reduction of relapse-like alcohol drinking after a deprivation phase was reported during the administration of competitive and non-competitive antagonists of the NMDA receptor (Vengeliene et al., 2005).

3.2 Acute and chronic alcohol exposure

Acute and chronic effects of alcohol exposure on NMDARs have been observed in hippocampal brain slices in which resistance develops 5-15 min after exposure to ethanol (100 mM) (Miyakawa et al., 1997; Yaka et al., 2003; Nelson et al., 2005). However, the mechanisms of this resistance are not fully understood. Wu et al. proposes that time and dose dependent effects of ethanol produce adaptive changes in the NMDAR which may also occur during exposure to ethanol in *ex vivo* conditions. These changes may be the basis for the functional adaptation of these receptors to alcohol exposure (Wu et al., 2011). It has been shown that changes in the process of adaptation can also occur as a result of NMDAR overexpression, or by other signaling mechanisms that are mediated by selective dephosphorylation of the NMDAR after acute or chronic alcohol exposure (Roberto et al., 2004; Lack et al., 2007; Clapp et al., 2010; Wu et al., 2011).

The NMDAR is considered one of the primary molecular targets of ethanol in the brain. Ethanol inhibits NMDAR function via a non-competitive mechanism and induces the dephosphorylation of NR2 subunits (Wirkner et al., 2000; Suvarna et al., 2005; Wang et al., 2007). For example, NR2A and NR2B in hippocampal and cortical brain slices were characterized after acute ethanol exposure. They exhibited a decrease in tyrosine phosphorylation levels. Both the inhibition of NMDAR function and the decrease in tyrosine phosphorylation of NR2 subunits produced by acute ethanol exposure were blocked by protein tyrosine phosphatases (PTP) inhibitors (Alvestad et al., 2003; Ferrani-Kile et al., 2003). This suggests that ethanol's inhibition of NMDAR function is a result of a decrease in tyrosine phosphorylation of NMDARs by ethanol enhancement of PTP activity (Mahadev & Vemuri, 1999).

NMDARs have also been strongly implicated in synaptic development and cellular models of learning and memory such as long-term potentiation (LTP) and long-term depression (LTD) (Medina et al., 2001; Malenka & Bear, 2004). It has been shown that ethanol inhibits the induction of several forms of neural plasticity such as LTP in the hippocampus, dorsal striatum, and bed nucleus of the stria terminalis while enhancing LTD in the hippocampus (Blitzer et al., 1990; Morrisett et al., 1993; Pyapali et al., 1999; Hendricson et al., 2002; Weitlauf et al., 2004; Hendricson et al., 2007; Yin et al., 2007). Such mechanisms of synaptic

plasticity could subsequently lead to the reorganization of neural circuitry by altering gene and protein expression of neuronal receptors such as NMDAR. LTP and LTD have thus become important candidate mechanisms for alcohol induced alterations of neural circuit function in alcohol addiction (Hyman & Malenka, 2001). These studies have proposed the intriguing possibility that disruptions and subsequent adaptive changes in glutamate signaling through NMDARs may contribute to adaptations in brain function. These adaptions in return may produce ethanol tolerance and/or dependence similar to processes involved in experience-dependent plasticity.

The ability of ethanol to inhibit NMDAR function is dependent on various factors including the NR1 splice variant that is co-assembled with NR2 subunits (Jin & Woodward, 2006). While homomeric NR1 subunits form an active ion channel that conducts Na^+ and Ca^{2+} currents, the incorporation of NR2 subunits allows this channel to be modulated by the Src family of kinases (SFKs), phosphatases, and other small molecules such as ethanol. Therefore, NMDAR complexes containing subunits NR1/NR2A or NR1/NR2B are more sensitive to the inhibitory effects of alcohol than complexes that contain the subunits NR1/NR2D or NR1/NR2C. Additionally, given the differential distribution of NMDAR subunits in the brain, alcohol affects certain brain regions more than others. For example, the NR1/NR2B subtype that is mainly expressed in forebrain regions is more sensitive to the inhibitory effects of ethanol (Allgaier, 2002; Smothers et al., 2001; Popp, 1998).

Recently, it has been found that acute ethanol exposure inhibits NMDAR function by modifying STriatal enriched protein tyrosine phosphatase (STEP) activity. STEP is a brain-specific protein that is thought to play a critical role in synaptic plasticity (Fitzpatrick & Lombroso, 2011). The genetic deletion of STEP61, the active form of STEP within the brain, leads to marked attenuation of acute ethanol inhibition of NMDAR currents. Also, STEP61 negatively regulates Fyn and p38 mitogen-activated protein kinase (p38 MAPK). Both of these proteins are members of the NMDAR super molecular complex. The adaptation of NMDAR responses to acute alcohol is associated with 1) a partial inactivation of STEP61, 2) an activation of p38 MAPK, and 3) a requirement for NR2B activity. Together this data indicates that altered STEP61 and p38 MAPK signaling contributes to the modulation of ethanol inhibition of NMDAR activity in brain neurons (Wu et al., 2011).

The functional activity of the NMDAR is increased by SFKs, but its activity is also regulated by protein tyrosine phosphatases (Pelkey et al., 2002; Salter & Kalia, 2004; Snyder et al., 2005; Paul et al., 2007). STEP61 co-immunoprecipitates with NMDARs suggesting a strong physical association between these two molecules as a signaling unit (Pelkey et al., 2002; Braithwaite et al., 2006; Xu et al., 2009). Inhibition of STEP61, the only actively expressed isoform of STEP in the hippocampus, has been shown to enhance NMDAR function and to attenuate ethanol inhibition of the NMDA receptor (Pelkey et al., 2002; Hicklin et al., 2011). Acute ethanol exposure has been shown to decrease phosphorylation at the tyrosine (Y) 1472 phosphorylation site of the NR2B subunit without altering its protein levels (Alvestad et al., 2003; Wu et al., 2010). Y-1472 is a site in the C-terminal tail of the NR2B subunit where STEP61 has been shown to interact. This suggests that acute ethanol treatment activates STEP61 which is involved in the dephosphorylation of the Y-1472 site (Paul et al., 2007; Braithwaite et al., 2006). In accordance, several studies have also shown that the inhibition of NMDAR currents by the action of ethanol requires the participation of STEP61. When STEP61 activity is repressed, ethanol's ability to inhibit NMDAR current is attenuated

(Alvestad et al., 2003; Wu et al., 2010; Hicklin et al., 2011). During the adaptive response increased levels of STEP33 and phospho-p38 mitogen-activate protein kinase (pp38 MAPK) along with decreased levels of STEP61 were correlated with the failure of acute alcohol exposure to inhibit NMDAR currents (Wu et al., 2010; Wu et al., 2011). STEP33 is produced by the cleavage of STEP61. This cleavage process may be one of the mechanisms involved in the partial inhibition of STEP61 during the adaptive phase of alcohol exposure. Studies such as these have suggested that the adaptive resistance of NMDAR currents to acute ethanol inhibition likely involves NR2B subunit activity.

The mechanism of resistance to acute and chronic alcohol exposure during the adaptive response is not well understood. Several studies have reported increases in the expression level of several subunits of the NMDAR such as NR1, NR2A, and NR2B during the adaptive phase under chronic alcohol exposure conditions (Snell et al., 1996; Roberto et al., 2004; Roberto et al., 2006; Lack et al., 2007). Other studies have reported increases in the accumulation of synaptic NMDARs during this phase as well (Carpenter-Hyland et al., 2004; Clapp et al., 2010). For example, hippocampal neurons exposed to ethanol chronically for 7 days demonstrated an increase and accumulation of synaptic NMDARs that was quickly reversed once ethanol exposure ceased (Clapp et al., 2010). This suggests that alcohol inhibition of NMDAR activity regulates the expression and accumulation of NMDARs. In contrast, other studies reported no changes in the expression levels of the NMDAR or its subunits. Instead these studies showed an increased inhibition of NMDAR activity using NR2B antagonists and concluded that resistance may be attributed to increases in NR2B activity (Ferreira et al., 2001; Wu et al., 2010). Even though increases in NR2B activity have not been directly verified, increases in STEP33 and pp38 as mentioned previously are characteristic of excessive NMDAR activation (Floyd et al., 2003; Hardingham, 2009; Xu et al., 2009).

Other mechanisms involved in the adaptive response have been proposed but proven to be untrue. For example, several studies proposed that tolerance could occur in presynaptic neurons, postsynaptic neurons, or both (Wu et al., 2001). Thus, the inhibitory actions of ethanol on postsynaptic glutamate receptors could be counteracted by an increase in presynaptic glutamate release. This hypothesis was tested via a paired pulse facilitation (PPF) experiment. The PPF ratio proves to be inversely proportional to the amount of neurotransmitter release in neurons (Dobrunz & Stevens, 1997; Dittman et al., 2000; Wu et al., 2001). No significant effects of chronic ethanol in the PPF ratio were seen. This suggested that chronic ethanol did not significantly alter the pre-synaptic mechanisms of NMDA neurotransmission. Another group of studies proposed that alterations in the Mg^{2+} blockade were responsible for the alcohol resistance seen during the adaptive phase. However, acute ethanol inhibition of NMDAR currents did not significantly differ in low Mg^{2+} and control Mg^{2+} conditions. This demonstrated that alterations in the Mg^{2+} blockade were unlikely to be responsible for this adaptive response as well (Alvestad et al., 2003; Hicklin et al., 2011; Wu et al., 2011).

Studies in humans have shown that individuals with low initial sensitivity (high resistance) to acute ethanol effects on cognition are at greater risk for becoming alcohol dependent (Schuckit & Smith, 2001). However, other studies have shown that those individuals that develop greater acute ethanol tolerance (low initial resistance) also have a greater risk for alcohol dependence (Newlin & Renton, 2010). Even though the underlying mechanisms for

alcohol dependence are not clear, the general consensus is that STEP61 and p38 MAPK activities have critical roles in the modulation of acute and chronic alcohol exposure. These studies also suggest that NR2B subunit antagonists are likely to be effective in regulating the acquisition of functional tolerance to the acute and chronic inhibitory effects of ethanol.

3.3 Mechanisms of alcohol-induced brain damage

The mechanisms of alcohol-induced brain damage and abstinence-induced regeneration are complex (Crews et al., 1998; Farber et al., 2004). The extent of neurodegeneration and potential regeneration varies by brain region and is dependent on many factors including pattern of intake (Crews & Nixon, 2009). Alcoholics display cycles of excessive ethanol intake, abstinence, and relapse behavior. For example, Bell et al showed that high alcohol drinking rats consumed significantly more alcohol upon re-exposure than control rats after a period of alcohol abstinence (Bell et al., 2008). Another studied showed that repeated alcohol deprivation cycles increased the severity of relapse within rats (Rodd et al., 2008). These studies suggest a strong correlation between abstinence and relapse that perpetuate the detrimental cycle of alcohol consumption.

Chronic exposure to ethanol causes an adaptive increase in NMDA receptor sensitivity both *in vivo* and *in vitro.* This leads to an increased vulnerability to the glutamate-induced cytotoxic response (excitotoxicity) (Dodd et al., 2000). This sensitization of neuronal cells is one of the most important factors in the mechanism underlying ethanol-induced brain damage. Increased calcium influx through NMDA receptors, as a result of hyper-sensitivity, is tightly coupled to the increase in calcium influx within the mitochondria. This results in the increased production of reactive oxygen species and oxidative damage that eventually attenuates mitochondrial function. Primary inhibition of the mitochondrial respiratory chain can also indirectly induce further NMDA receptor stimulation and damage (Matsumoto et al., 2001).

Various studies show the effect of alcohol on the induction of brain damage. Cell culture models *in vitro* suggest that chronic ethanol intake inhibits the NMDARs which over time results in a hyper-sensitivity that is alleviated by alcohol withdrawal (Chandler et al., 1993; Chandler et al., 2006, Chandler et al., 1999). These studies suggest that neurotoxicity occurs through NMDARs during withdrawal (Butler et al., 2008; Smith et al., 2008). However, other studies *in vivo* using different NMDAR antagonists such as MK801 (dizocilpine), memantine, and DNQX failed to reduce binge ethanol neurotoxicity. Surprisingly, some doses even increased neurodegeneration (Collins et al., 2010; Corso et al., 1998; Crews et al., 2004; Hamelink et al., 2005). These studies suggest that the mechanism of ethanol-induced brain damage is not glutamate excitotoxicity. Therefore, ethanol-induced brain damage in the binge model occurs during intoxication. Other studies also support the hypothesis that alcohol-induced neurodegeneration occurs primarily during intoxication and is related to increased oxidative stress and pro-inflammatory signaling (Qin et al., 2008). Abstinence after binge ethanol intoxication results in brain cell genesis that could contribute to the return to normal brain function and structure found in abstinent humans (Crews & Nixon, 2009).

Additionally, transcription factors such as the cAMP responsive element-binding protein (CREB) and the nuclear factor κB (NF-κB) regulate the gene expression that increases plasticity and survival of damaged neurons (Walton & Dragunow, 2000; Mabuchi et al.,

2001; Hara et al., 2003). In the presence of ethanol changes can be seen in DNA binding protein activities such as increased DNA binding by NF-κB and reduced DNA binding by CREB. NMDAR activation by synaptic glutamate release is associated with decreased DNA binding by CREB as a result of a decrease in CREB phosphorylation (pCREB) (Papadia & Hardingham, 2007). This decrease in pCREB has also been observed in an *in vivo* model of alcohol consumption where rats were treated with ethanol. Therefore, pCREB is reduced during intoxication (Bison & Crews, 2003). NMDAR inhibition is caused by saturation with ethanol and is expected to enhance neurodegeneration and inhibit neurogenesis. During ethanol withdrawal there is a notable increase in pCREB, 3 days post-withdrawal, which is consistent with the neurogenesis observed (Bison & Crews, 2003). Therefore, it is possible that during abstinence NMDAR activity recovers leading to an increase in the pCREB activated transcription of genes involved in plasticity, cell growth, cell proliferation, and neurogenesis. However, oxidative stress and pro-inflammatory cytokines could attenuate the regeneration process due to imbalances generated by brain cell damage (Collins & Neafsey, 2011; Qin et al., 2008).

3.3.1 Alcoholic neurodegeneration and glial cells

Studies in nonhuman primate adolescents show alcohol-induced changes during neurogenesis in the hippocampus. Alcohol significantly reduced the number of different neural progenitor cell types 1, 2a, and 2b as well as glial progenitor cells (Taffe et al., 2010). This suggests that alcohol interferes with the division and migration of progenitor cells in the hippocampus preneuronal region. Thus, the effect of alcohol decreases neurogenesis and increases degeneration. These results demonstrate that the neurogenic niche of the hippocampus during adolescence is very vulnerable to alcohol. It also demonstrates that alcohol decreases the turnover of neurons in the hippocampus by altering the process of neural development. This effect diminishes slowly and can be seen two months after alcohol abstinence. These findings could explain the deficit in the hippocampus associated with cognitive tasks that may be associated with increased DNA binding of NF-kB and reduced DNA binding of CREB (Fulton et al., 2009, Taffe et al., 2010).

Alcohol-related neuronal loss has been documented in specific regions of the cerebral cortex (superior frontal association cortex), hypothalamus, and cerebellum (Harper et al., 2003; Baker et al., 1999). Glial cells, also contribute to neurodegeneration because astroglial degeneration has been reported during ethanol exposure (Miguel-Hidalgo et al., 2006, Miguel-Hidalgo & Rajkowska, 2003). Glial cells are non-neuronal cells that provide physical and functional support for neurons and are essential for normal neuronal cell function. The loss of glial cells results in a deficiency of metabolic and trophic support for neuronal cells. For example, loss of glial cells leads to the inactivation of neurotransmitters such as glutamate and loss of ionic homeostasis, particularly K+ (Bezzi & Volterra, 2001; Volterra & Meldolesi, 2005; Obara et al., 2008). This loss directly enhances the deleterious effects of ethanol on neurons. An increase in the glial fibrillary acidic protein (GFAP), a glial-specific cell marker, has been reported after brain injury which suggests activation of glial proliferation in response to damage (Eng et al., 1992; Norton et al., 1992). Studies have demonstrated reduced glial cell proliferation and reduced expression of GFAP by ethanol exposure in astrocyte cultures (Crews et al., 2004; Guerri & Renau-Piqueras, 1997). In addition, acute alcohol exposure or acute alcohol-induced brain damage results in gliosis (enlargement and increased proliferation of astrocytes) and increases in GFAP levels (Crews

et al., 2004; Evrard et al., 2006). In contrast, chronic ethanol exposure results in decreased levels of GFAP (Duvernoy et al., 1981; Franke et al., 1997; Miguel-Hidalgo, 2005; Udomuksorn et al., 2011).

Postmortem studies in patients with alcohol dependence showed low glial densities in the Pre-Frontal Cortex (PFC). However, in these patients glutamine sythetase (GS) levels as well as GFAP levels were significantly higher (Miguel-Hidalgo et al., 2010). One hypothesis for this activation of astrocytes in alcoholism involving increased GS/GFAP expression may be due to the repeated acute exposure to alcohol or to periods of withdrawal that defines alcoholism. This augmentation of GS expression in astrocytes of alcoholics is supported by augmented GS immunoreactivity detected in the PFC of alcohol-consuming rats three days after withdrawal from alcohol (Miguel-Hidalgo, 2005). In this animal model, the GS immunoreactivity was significantly correlated with the amount of ethanol ingested in the days before withdrawal. It has been suggested that astrocytes play a critical role in controlling glutamatergic activity and take up most of the synaptically released glutamate that terminates neurotransmitter activity. Glutamate can then be delivered to neurons via the glutamate–glutamine cycle (Danbolt, 2001). Therefore, changes in the glial expression of GS/GFAP suggest an impairment of certain aspects of glutamatergic processing during alcohol exposure and withdrawal. Further research should determine whether the morphological plasticity and GS/GFAP expression are induced more readily in chronic alcoholics despite a paradoxical association of chronic alcohol intake with low glial or astrocyte density (Korbo, 1999; Miguel-Hidalgo et al., 2002; Miguel-Hidalgo et al., 2006).

3.4 Clinical studies: The role of NMDA receptor antagonists ketamine and memantine

Several studies have suggested that NMDA receptor antagonists are an effective method of treatment for alcohol disorders. Ketamine, a NMDA receptor antagonist, has been evaluated in subjects with a strong family history of ethanol dependence versus subjects with no such family history (Petrakis, 2004). This study demonstrated that during ketamine infusion individuals with a family history of ethanol dependence showed an attenuated response in terms of perceptual alterations and dysphoric mood relative to those without such a family history. This study reaffirms NMDAR dysfunction as an important contributing factor of alcohol dependence. Another study by Phelps et al. investigated whether a family history of alcohol dependence influences ketamine's initial antidepressant effect. The study reported that subjects with a family history of alcohol dependence showed significantly greater improvement in MADRS (Montgomery-Asberg Depression Rating Scale) scores compared with subjects who had no family history of alcohol dependence. The study concluded that a family history of alcohol dependence appears to predict a rapid initial anti-depressant response to NMDA receptor antagonists (Phelps, 2009). The precise reasons underlying the better response of the family history of alcohol dependence (FHP) group to ketamine remains unknown but reaffirms NMDARs association with LTD. Another study compared the ethanol-related effects of ketamine and thiophental on both NMDA and $GABA_A$ receptor activity. This study reported that the ethanol-like effects of ketamine were greater than that of thiopental (Dickerson, 2008). The results obtained are important because ketamine (a NMDAR antagonist) produced alcohol alterations in perception that were not produced by thiopental (a $GABA_A$ receptor agonist). This also reaffirms the role of the NMDAR in alcohol dependence.

Memantine, another NMDAR antagonist, also has been evaluated in clinical trials for the treatment of alcoholism. The first study was conducted by Bisagra et al. by evaluating the acute effects of memantine on the subjective, physiological, and performance effects of alcohol in moderate (10–30 drinks per week) alcohol drinkers. This study reported that pre-treatment with memantine attenuated the craving for alcohol before alcohol administration but not after alcohol was given. It demonstrated that memantine increased the dissociative effects of alcohol without altering its sedative, stimulant, and overall intoxicating effects. It reported that memantine had no effect on alcohol-induced impairment in performance, physiological changes, or pharmacokinetics. This study also showed that memantine increased subjective reports of dissociation, confusion, stimulation, and impaired motor coordination on the balance task (Bisaga & Evans, 2004). Due to the high comorbidity shared between alcoholism and depression Muhonen et al. also evaluated memantine as well as escitalopram, a selective serotonin reuptake inhibitor (SSRI), for the treatment of comorbid with alcohol dependence. This study reported that both treatments significantly reduced the baseline level of depression and anxiety according to Montgomery-Asberg Depression Rating Scale (MADRS) and Hamilton Rating Scale for Anxiety (HAM-A). This evidence provides safety and potential efficacy of memantine and escitalopram for major depressive disorder in patients with comorbid alcohol dependence (Muhonen, 2008).

4. Conclusion

Approximately 2 billion people world-wide consume alcoholic beverages. Alcohol use is related to a wide range of physical, mental, and social detriments. Additionally, alcohol affects almost every organ in the human body as well as the central nervous system. The protein theory is the generally accepted theory on how alcohol affects the CNS. This theory proposes that alcohol acts specifically on membrane protein receptors such as the iGluR-NMDA. The iGluR-NMDA is one of the most active molecules in the central nervous system and has been shown to be directly inhibited in a non-competitive manner by alcohol. It is a post-synaptic receptor critical in most neural activities such as learning and memory.

NMDARs are hetermeric complexes composed of three major types of subunits NR1, NR2, and NR3. Alcohol effects on the NMDAR are dependent on the NMDAR subunit composition as well as alcohol concentration. The major effects of alcohol on the NMDAR activity are thought to be conferred by alcohol's direct interaction with the NR2 subunits of the NMDA receptor. The effects of alcohol can be categorized into several stages. These stages are referred to as initiation of alcohol consumption, maintenance of alcohol consumption, craving and reinstating of alcohol seeking, and relapse to alcohol use. A combination of ionotropic receptors such as NMDAR, non-ionotropic receptors, and other receptors of the glutamatergic system are intimately involved in the acquisition of alcohol dependence. The NMDAR has a critical role in the stages of initiation of alcohol consumption and relapse to alcohol use. In response to the NMDA receptors role in alcohol addiction several NMDAR antagonists have been used in clinical trials to alleviate alcohol dependence. These antagonists include ketamine and memantine. Both have been shown to be successful in alleviating some of the symptoms of alcohol dependence. Future research should focus on the continued characterization of the NMDAR structure as well as its structural variation in different tissue compartments within the brain. Also, further studies are needed to elucidate the interactions of alcohol on specific NMDAR subunits and

characterize their effects on NMDAR activity. This will be crucial in developing novel therapeutic targets against alcohol addiction.

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