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New Insights on Neurobiological Mechanisms underlying Alcohol Addiction

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Abstract

Alcohol dependence/addiction is mediated by complex neural mechanisms that involve multiple brain circuits and neuroadaptive changes in a variety of neurotransmitter and neuropeptide systems. Although recent studies have provided substantial information on the neurobiological mechanisms that drive alcohol drinking behavior, significant challenges remain in understanding how alcohol-induced neuroadaptations occur and how different neurocircuits and pathways cross-talk. This review article highlights recent progress in understanding neural mechanisms of alcohol addiction from the perspectives of the development and maintenance of alcohol dependence. It provides insights on cross talks of different mechanisms and reviews the latest studies on metaplasticity, structural plasticity, interface of reward and stress pathways, and cross-talk of different neural signaling systems involved in binge-like drinking and alcohol dependence.

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The focus of this article is around the theme presented at a minisymposium at the 2011 Society of Neuroscience annual meeting.

1. Introduction

The development of alcohol dependence progresses from impulsive to compulsive alcohol intake via repeated binging, withdrawal, and craving. It is characterized by alcohol consumption despite negative consequences and recurring episodes of abstinence and relapse (Koob, 2013). Recent studies have provided substantial information on the brain circuits that mediate various aspects of alcohol dependence. In particular, studies have shown that alcohol has profound impacts on multiple brain pathways and circuits related to reward, stress, habit formation, and decision-making, which work in concert leading to alcohol dependence/addiction. However, significant challenges remain in understanding, at the molecular and cellular level, how alcohol-induced neuroplasticity and neuroadaptation occur and how different neuropathways cross talk. In this article, we will discuss several neurobiological mechanisms and provide insights on interactions of different mechanisms in the vulnerability, development and maintenance of alcohol dependence. This article is not intended to be comprehensive but rather to focus on several areas that were discussed at a minisymposium at the 2011 Society for Neuroscience annual meeting. We will discuss metaplasticity of dopaminergic neurons, reward and stress pathways in mediating binge-like drinking, interaction of corticotropin-releasing factor (CRF) and GABAergic systems, and structural and functional changes of dendritic spines.

2. Mechanisms Mediating the Development of Alcohol Dependence

Excessive alcohol exposure or binge-like drinking impacts neuroplasticity and signaling associated with reward and stress pathways, as well as their interface. Here, we highlight the role of metaplasticity of the dopaminergic neurons in the ventral tegmental area (VTA), glutamate signaling in the Nucleus Accumbens (NAC), the CRF system in the central amygdala (CeA) in excessive or binge like alcohol exposure, and discuss the potential role of the BNST, the interface of between stress circuits and classical reward centers, in the development of alcohol dependence.

2.1 Metaplasticity in mesolimbic dopamine neurons and addiction vulnerability

Development of addiction involves a maladaptive form of learning and memory in which drug-related experiences are remembered powerfully, resulting in persistent and uncontrollable drug seeking behavior (Hyman et al., 2006). Synaptic plasticity is widely believed to be the key neural substrate underlying the formation and storage of memory in the brain (Kim and Linden, 2007; Malenka and Bear, 2004). Here, activity-dependent alterations in the efficacy of synaptic transmission are typically induced in a manner in which only those subset of synapses that are active in certain temporal proximity to the time of activity of postsynaptic neurons eventually become potentiated (long-term potentiation: LTP) or depressed (long-term depression: LTD). There is another form of plasticity, termed metaplasticity, which affects synapses of postsynaptic neurons in a global manner (Abraham, 2008; Abraham and Bear, 1996; Mockett and Hulme, 2008). This represents higher-order plasticity (i.e., plasticity of synaptic plasticity) in which previous life experiences, such as exposure to certain environmental stimuli (stress, addictive drugs, etc.), or even prior learning experience alter the “susceptibility” of synapses to undergo activity-dependent LTP/LTD, and thus the ability of animals/humans to learn new information in the future.

The mesolimbic dopaminergic system that originates in the VTA is critically involved in the learning of information related to rewards, including addictive drugs (Morikawa and Paladini, 2011; Schultz, 1998). A growing body of evidence indicates that plasticity and metaplasticity of synapses on dopamine neurons play important roles in reward-based learning and the development of addiction (Hyman et al., 2006; Kauer and Malenka, 2007).

It is well established that *in vivo* exposure to different classes of addictive drugs or to stress produces rather global potentiation of AMPA receptor (AMPAR)-mediated glutamatergic transmission onto VTA dopamine neurons (Argilli et al., 2008; Bellone and Luscher, 2006; Conrad et al., 2008; Faleiro et al., 2004; Saal et al., 2003; Ungless et al., 2001). This is thought to saturate AMPAR potentiation and occlude subsequent LTP induction. However, it has recently been proposed that this metaplasticity is a consequence of down-regulation of synaptic NMDA receptors (NMDARs), resulting in suppression of LTP induction (Mameli et al., 2011). This study further demonstrated the emergence of an anti-Hebbian form of AMPAR LTP, confirming that AMPAR potentiation is not saturated. It has also been reported that Hebbian AMPAR LTP may actually be enhanced because of a global reduction in GABAergic inhibition after *in vivo* cocaine exposure (Liu et al., 2005; Pan et al., 2011). Therefore, glutamatergic synapses at dopamine neurons appear to exhibit multiple forms of metaplasticity of AMPAR-mediated transmission. Furthermore, *in vivo* exposure to addictive drugs suppresses LTP of $GABA_A$-mediated transmission via disruption of the LTP induction mechanism (Guan and Ye, 2010; Niehaus et al., 2010; Nugent et al., 2007), indicating that metaplasticity can be induced at GABAergic synapses as well. In principle, various forms of metaplasticity in dopamine neurons, and also in dopamine projection areas [reviewed by (Lee and Dong, 2011)], should regulate how rapidly and efficiently drug-related events and actions are remembered and, possibly, how long those memories persist, thus affecting the vulnerability to develop addiction. Therefore, establishing the roles of metaplasticity would be an important area of addiction research, which requires manipulating neuroadaptive mechanisms underlying metaplasticity in behaving animals without interfering with synaptic transmission or synaptic plasticity per se.

NMDAR activation in the VTA is necessary for dopamine neuron burst firing and phasic dopamine release in projection areas that occur in response to rewards or reward-predicting stimuli (Sombers et al., 2009; Zweifel et al., 2009). A previous study has reported LTP of NMDAR-mediated transmission that is induced by pairing sustained glutamatergic input stimulation with postsynaptic bursts of action potentials (APs) (Harnett et al., 2009). LTP induction requires amplification of AP-evoked Ca^{2+} signals by preceding synaptic activation of metabotropic glutamate receptors (mGluRs) coupled to the generation of inositol 1,4,5-trisphosphate (IP_3) (Cui et al., 2007). The synaptic stimulation-burst pairing protocol used for LTP induction may resemble the neural activity experienced during cue-reward conditioning in behaving animals, in that cue presentation would give rise to working memory-type sustained glutamatergic input activity, while the reward would elicit dopamine neuron burst firing (Brown et al., 1999; Funahashi et al., 1989). Therefore, this form of Hebbian NMDAR plasticity might contribute to the acquisition of burst responses to environmental stimuli paired with rewards during conditioning (Schultz, 1998).

Recent studies show that repeated *in vivo* exposure to amphetamine or ethanol causes enhancement of NMDAR LTP induction in VTA dopamine neurons (Ahn et al., 2010; Bernier et al., 2011). This form of NMDAR metaplasticity results from an increase in the potency of IP_3 in producing amplification of AP-evoked Ca^{2+} signals, most likely via increased protein kinase A (PKA)-mediated phosphorylation of IP3 receptors (IP3Rs) causing enhanced IP3 sensitivity (Wagner et al., 2008). Importantly, intra-VTA infusion of a PKA inhibitor attenuates amphetamine-induced contextual learning assessed using a conditioned place preference (CPP) paradigm and previous ethanol experience facilitates subsequent acquisition of cocaine-induced CPP (Bernier et al., 2011). Interestingly, CRF, which is increased in the VTA by stressful stimuli or acute withdrawal from addictive drugs (Koob and Zorrilla, 2010; Wise and Morales, 2009), is capable of further amplifying the PKA-mediated increase in IP_3 in ethanol-treated animals. These data suggest that PKA-dependent regulation of IP_3R sensitivity, which gates the “inducibility” of NMDAR plasticity in VTA dopamine neurons, may represent a common neural substrate by which

ethanol, other addictive drugs, and stress influence the capacity of animals to learn reward- and drug-associated environmental stimuli. Given that CRF neurons from the BNST and the CeA project to the VTA (Rodaros et al., 2007; Swanson et al., 1983), the action of CRF on NMDAR metaplasticity in the VTA represents a possible feed forward mechanism mediating the cross-talk between stress and reward pathways. Moreover, as discussed in a later section, PKA plays an essential role in regulating CRF-induced GABA release in the CeA (Ameri, 1999; Cruz et al., 2011a). Thus, PKA regulates two important mechanisms, metaplasticity and CRF-induced GABA release, which contribute to the vulnerability and the maintenance of alcohol dependence.

2.2 A substrate for binge alcohol drinking in the nucleus accumbens

As a key component of the brain reward circuitry, the nucleus accumbens (NAC) receives robust glutamatergic innervations from frontal cortex, hippocampus, amygdala, and the thalamus. Considerable evidence supports that this glutamatergic component is critical in the development of addiction (Gass and Olive, 2008; Kalivas and Volkow, 2011). Glutamatergic signaling in the NAC interacts with dopaminergic signaling and plays a role in reward, reinforcement, and relapse. The Homer protein family, which is known to regulate both pre- and post-synaptic aspects of glutamate transmission, regulates behavior and neurochemical sensitivity to alcohol (Szumlinski et al., 2008b; Szumlinski et al., 2005). Homer2 expression was found to be up-regulated within the NAC by alcohol intake under continuous access conditions, as well as by repeated alcohol injections, and the changes in Homer2 expression coincided with increases in the expression of the NR2a/b subunit of the NMDA receptor, mGluR1/5, as well as indices of phosphatidylionsitol 3-kinase (PI3K) and protein kinase C epsilon (PKCε) activity (Goulding et al., 2011; Obara et al., 2009; Szumlinski et al., 2008b). Recent studies have further established the interactions of Homer and PI3K signaling with binge drinking (Cozzoli et al., 2012; Cozzoli et al., 2009; Neasta et al., 2010; Neasta et al., 2011). The contribution of Homer2-dependent signaling through Group 1 mGluRs to certain downstream effectors to the maintenance of excessive alcohol intake has been demonstrated under Scheduled High alcohol Consumption (SHAC) and Drinking-in-the-Dark (DID) procedures (Finn et al., 2005; Rhodes et al., 2005).

As observed in the earlier studies of rodents with a chronic history (3-6 months) of alcohol drinking (Obara et al., 2009; Szumlinski et al., 2008b), animals with a 6-day history of binge drinking alcohol under SHAC procedures exhibited elevated Homer2 protein expression, that was accompanied by increases in NR2a/b expression (Cozzoli et al., 2009). The SHAC-induced rise in NAC Homer2 levels co-occurred with increases in indices of both PI3K and PKCε activity, but no change in the levels of either mGluR1 or mGluR5 (Cozzoli et al., 2009). The lack of an effect of binge drinking upon NAC Group 1 mGluR expression might relate to the relatively short SHAC drinking history of the mice (6 presentations) as a parallel immunoblotting study conducted on tissue from mice with a 30-day history of binge drinking under DID procedures revealed parallel increases in NAC levels of Homer2, mGluR1/5 and NR2a/b, that coincided with increases in both PI3K and PKCε activity (Cozzoli et al., 2012; Cozzoli et al., 2009). These immunoblotting data were consistent with other results demonstrating the engagement of PI3K and mGluR in binge drinking. For example, a meta-analysis indicats a positive association between striatal levels of PI3K mRNA and binge drinking under DID procedures (Mulligan et al., 2011); the AKT pathway, which is at the down stream of PI3K signaling, is activated by acute ethanol challenge (Bjork et al., 2010); inhibition of AKT or PI3K within the NAC attenuates binge drinking (Neasta et al., 2011); both non-selective and selective mGluR5 antagonists exhibit the “anti-binge” efficacy in behavioral pharmacological assessments (Blednov and Harris, 2008; Gupta et al., 2008).

The functional relevance of mGluR/Homer2-mediated signaling for the maintenance of binge drinking has been revealed using a combination of neuropharmacological and transgenic approaches. An infusion of small hairpin RNAs against Homer2b into the shell subregion of the NAC significantly reduced alcohol drinking under both SHAC and DID procedures (Cozzoli et al., 2012; Cozzoli et al., 2009). Similarly, intra-NAC infusion of the selective mGluR5 antagonists MPEP and MTEP and the PI3K inhibitors wortmannin and/or LY 294002 lowered alcohol intake in both paradigms, as did the local infusion of a Tat-eV1-2 peptide inhibitor of PKCε (Cozzoli et al., 2012; Cozzoli et al., 2009). Interestingly, the "anti-binge" effects of inhibiting mGluR5 and PI3K or mGluR5 and PKCε were not additive, nor were they apparent in *Homer2* knock-out mice or in mice with a point mutation in mGluR5 that disrupts Homer binding (Cozzoli et al., 2012; Cozzoli et al., 2009). Such data point to a signaling pathway involving mGluR5-Homer2-PI3K/PKCε in the continued propensity to consume excessive amounts of alcohol under limited access conditions. It is possible that mGluR5-mediated stimulation of PI3K (via βγ activation) and PKCε (via αq activation) have independent roles in the regulation of binge drinking. However, co-infusion of PI3K and PKCε inhibitors into the NAC failed to reduce alcohol intake under DID procedures to a greater degree than that produced by either kinase inhibitor alone. The apparent inter-dependency between PI3K and PKCε is consistent with an earlier indication that mGluR5-mediated activation of PKCε is dependent upon PI3K activity (Olive et al., 2005) and suggests that binge drinking-induced increases in mGluR5/Homer2-mediated signaling through PI3K to PKCε within the NAC is an imporant intracellular pathway underpinning excessive alcohol consumption.

Evidence also points out that mGluR1/5-Homer2 signaling may also play an important role in genetic predisposition to binge drinking. The basal protein expression of Homer2 and mGluR1 is elevated within the NAC of mice selectively bred for high binge drinking under either SHAC procedures (SHAC) or DID procedures (HDID-1), yet neuropharmacological studies using the selective mGluR1 antagonist CPCCOEt have revealed only modest reductions in binge alcohol drinking under either procedure (Cozzoli et al., 2012; Cozzoli et al., 2009). However, more recent attempts to target mGluR1 within the NAC involved JNJ 16259685, an mGluR1 antagonist with greater potency and higher solubility than CPCCOEt, and revealed a significant reduction in alcohol intake under DID procedures that was not additive with that produced by PKCε inhibition. The data for JNJ 16259685 provide novel evidence to support the relevance of mGluR1/Homer2-mediated intracellular signaling pathways within the NAC in not only the manifestation of binge drinking, but also in the genetic predisposition to binge drinking.

It is noteworthy that, as scalfolding proteins, Homer proteins not only interact with various signaling molecules at the post synaptic density but also regulate dendritic spine morphology (Sala et al., 2001; Shiraishi-Yamaguchi et al., 2009a). This property of Homer proteins may contribute to the dendritic spine remodeling associated with chronic alcohol exposure.

2.3 Effects of binge-like ethanol exposure on extended amygdala stress systems

In addition to the brain reward system, recent studies using the DID model of binge-like drinking suggest that binge-like ethanol exposure engages central stress systems, specifically CRF signaling in the extended amygdala, which is similar to dependence-induced alcohol drinking in the vapor exposure model. However, despite the common pharmacology of alcohol drinking between vapor exposure and DID, there appear to be different cellular mechanisms engaged by alcohol experience in these distinct exposure paradigms.

In addition to its critical role in anxiety and relapse, numerous reports have suggested that the extended amygdala is selectively involved in increased drinking behavior associated

with chronic alcohol exposure and withdrawal but not in basal drinking (Koob, 2008). Consistent with this, pharmacological manipulations in both the BNST and CeA can reduce alcohol-drinking behavior (Eiler et al., 2003; Finn et al., 2007; Funk et al., 2006; Hyytia and Koob, 1995; Roberts et al., 1996). However, recent results from several groups have suggested that the extended amygdala is not limited to 'dependence-induced drinking', but can also gate excessive or binge-like drinking behavior in rodents (Lowery-Gionta et al., 2012). Recent studies found that CRFR1 antagonists also protect against excessive ethanol intake in non-dependent animal models of binge-like ethanol drinking but fail to reduce non-binge-like ethanol intake (Argilli et al., 2008; Cippitelli et al., 2012; Lowery et al., 2010; Sparta et al., 2008), observations similar to results obtained with models of dependence. A study using gene knockout mice demonstrated that mice with CRFR1 deletion exhibited significant lower alcohol intake in the DID model of binge-like drinking (Kaur et al., 2012). In addition, binge-like drinking during adolescence significantly reduced the number of CRF expressing neurons in the CeA in rats (Gilpin et al., 2012). Furthermore, a recent study indicated the CeA as the site of action for these anti-binge effects of CRF antagonists in C57BL/6J mice (Lowery-Gionta et al., 2012). Specifically, binge-like ethanol drinking increased CRFR1 in the CeA and VTA, consistent with the idea that CRF signaling is upregulated during a binge-like drinking episode. Importantly, administration of the selective CRF receptor 1 (CRFR1) antagonist Antalarmin into the CeA blunted binge-like ethanol drinking, but failed to alter sucrose drinking. Injection of the same dose of Antalarmin into the nearby basolateral amygdala (BLA) failed to alter binge-like ethanol drinking, showing that the effects of CRFR1 blockade on binge-like ethanol drinking are specific to the CeA. Thus, as with dependence-induced ethanol drinking, binge-like ethanol drinking is modulated by CRFR1 signaling in the CeA. Further, a history of binge-like ethanol drinking functionally altered CRF receptor signaling as CRF failed to augment GABAergic transmission in slice preparations from the CeA, an effect evident in slice preparations from water drinking control mice. These results obtained from animals with a history of binge-like ethanol drinking are different from the results seen in animals following alcohol vapor exposure procedure. In a series of elegant studies, it was demonstrated that alcohol vapor exposure lead to a CRFR1 dependent increase in GABAergic transmission in the CeA (Roberto et al., 2010) *(see the discussion in the next section)*. The lack of effect of CRF on GABAergic transmission seen following DID procedures may be due to a functional downregulation or desensitization of CRFR1 receptors, as seen in the dorsal raphe following stress (Waselus et al., 2009). Future experiments that vary the length of withdrawal time should shed light on the nature of these differences.

Thus, similar to the dependence models, repeated cycles of binge-like ethanol exposure lead to enhanced activity of the CRF neurons in the CeA, which underlies increased alcohol preference. Despite the potentially critical importance of this CRF pathway, to date there has been no work investigating either pathway or cell-specific plasticity or the contribution of this specific pathway to binge drinking behavior. This lack of direct evaluation of this pathway and cell type specific neuroplasticity is likely due to the complicated heterogeneous neurochemical nature of the extended amygdala and the difficulty of examining long range GABA projections. Future studies using genetic methods to manipulate activity in *vivo* and ex *vivo* will shed light on this critical question.

2.4 Extended amygdala connectivity with ventral tegmental area: the interface of stress and reward pathways

Development of alcohol dependence engages both brain reward and stress systems. However, how these two systems interact in mediating the transition to alcohol addiction remains largely unknown. As part of extended amygdala, the BNST is an important

anatomical region that connects stress and reward neuropathways. It interconnects with the CeA to form a structure crucial for neural encoding of affective states related to stress, anxiety, and reward (Davis et al., 2010; Koob, 2008). The BNST also projects to the ventral midbrain, including the ventral tegmental area (VTA) and substantia nigra pars compacta (SN) (Georges and Aston-Jones, 2001, 2002; Jalabert et al., 2009; Lee and Dong, 2011; Phillipson, 1979) which are important components of the brain reward circuit. Studies suggest that one of the possible mechanisms that the BNST mediates the interaction between stress and reward pathways involves CRF and dopamine signaling. CRF originating from the BNST modulates dopaminergic neuronal function in the VTA (Rodaros et al., 2007; Ungless et al., 2003). As discussed earlier, CRF amplifies the PKA-mediated increase in IP_3 signaling in the VTA, which suggests that CRF regulates the metaplasticity of NMDARs in dopaminergic neurons. Conversely, dopamine enhances glutamatergic transmission in the BNST via the CRF signaling (Kash et al., 2008; McElligott and Winder, 2009). Taken together, this suggests that the VTA and BNST may form a feed-forward loop that leads to persistent changes in behavior.

Evidence suggests that the BNST is a key neuroanatomical substrate underlying drug and alcohol abuse (Dumont et al., 2005; Grueter et al., 2006; Grueter et al., 2008; Wills et al., 2012). Ethanol exposure can directly alter the neurophysiological properties of BNST neurons. Chronic, intermittent ethanol leads to an increase in postsynaptic NMDAR currents in BNST neurons (Kash et al., 2009; Wills et al., 2012). Additionally, acute administration of ethanol alters NMDAR-dependent long-term potentiation (Weitlauf et al., 2004). These studies demonstrate that both acute and long-term ethanol exposure can promote transient or long-lasting neuroadaptations in postsynaptic excitatory synaptic transmission in the BNST. It is also worth noting that GABA neurons in the juxtacapsular BNST show decreased intrinsic excitability following withdrawal from ethanol, although these cells are thought to project to the amygdala, which may act to increase the negative affective state during ethanol withdrawal (Francesconi et al., 2009). Because the BNST is composed of a heterogeneous mix of different neuronal types (as well as being made of up of many sub-nuclei), it remains unclear how acute and repeated ethanol exposure alters the neurophysiological properties of genetically defined and/or anatomically-specific subpopulations of BNST neurons. In addition, it is not known if there are input-specific alterations in presynaptic function within the BNST following ethanol exposure. Studies addressing these questions will shed light on the cross talk of reward and stress pathways in developing alcohol addiction.

The BNST sends a dense projection to the VTA as observed in retrograde tracing studies (Georges and Aston-Jones, 2001, 2002; Jalabert et al., 2009; Phillipson, 1979). Recently, BNST projection neurons to the VTA were shown to exhibit increased c-fos activation following stress exposure (Briand et al., 2010). VTA-projecting BNST neurons exhibit higher input resistance, lower capacitance, as well as inward rectifying potassium currents when compared to other non-VTA projecting BNST neurons (Dumont and Williams, 2004; Kash et al., 2008). These data indicate that these neurons may be easily excited by synaptic input that may promote burst firing (Egli and Winder, 2003). Interestingly, electrical stimulation of the BNST results in heterogeneous firing patterns of VTA neurons (Georges and Aston-Jones, 2001), which is consistent with the heterogeneous nature of the BNST. Given that genetically targeted control of neural activity within these neural circuits is now possible (Nichols and Roth, 2009; Stuber et al., 2011; Yizhar et al., 2011), it will be of great interest to determine how selective activation or inaction of genetically defined BNST neurons or their efferent projections to the midbrain alter reward-related behaviors. To accomplish this, optogenetic strategies, where light-gated channels and pumps are expressed in a genetically and anatomically specific fashion, will likely provide powerful tools to further elucidate the neural circuitry that underlie both stress and reward seeking. The

implementation and utility of these strategies has recently been reviewed elsewhere (Yizhar et al., 2011). It is important to point out that these techniques are rapidly evolving and already allow for activation and inactivation of neural circuitry function with subsecond temporal precision. Given that genetic targeting strategies are also becoming more precise, and that the implementation of these techniques with behavioral and electrophysiological methods is now possible, a combined *in vivo* optogenetic and electrophysiological approach to study the interface of stress and reward circuitry in alcohol drinking will likely produce exciting results in the coming years.

3. Mechanisms Underlying the Maintenance of Alcohol Dependence

The chronic consumption of large quantities of drugs, including alcohol, promotes a transition from casual drug use to drug dependence that is defined by the downregulation of dopamine signaling in the mesocorticolimbic reward system, hyperactivity of glutamate signaling, and dysregulation of brain stress systems (Koob and Volkow, 2010). An important element in the development of drug addiction is the brain's attempt to chemically counteract the influence of the repeated drug exposure (i.e., neuroadaptation). Here, we will discuss neuroadaptation of the CRF stress system and remodeling of dendritic spines, which play a critical role in the maintenance of alcohol dependence and contribute to the long lasting behavior changes associated with addiction.

3.1 Cellular mechanisms of CRF at the GABAergic synapses in the central amygdala: role in ethanol dependence

The activation of brain stress systems is hypothesized to be a key element of the negative emotional state produced by dependence that drives drug seeking through negative reinforcement mechanisms (Koob, 2013). To understand cellular mechanisms underlying neuroadaptive changes of the brain stress system in this process, ample studies have been conducted on the brain CRF system (Koob, 2008; Martin-Fardon et al., 2010; Sillaber et al., 2002). Recent research has highlighted the role of the GABAergic and the CRF system in the CeA in anxiety associated with ethanol dependence (Gilpin and Roberto, 2012). CRF release in the CeA is increased during withdrawal in alcohol-dependent animals and contributes to withdrawal-related anxiety and to increased alcohol consumption in dependent animals (Merlo Pich et al., 1995; Zorrilla et al., 2001). Importantly CRFR1 antagonists and CRFR1 deletion both reduced the increased ethanol self-administration in dependent but not nondependent animals and blocked the anxiogenic effects produced by stressors and alcohol withdrawal (Funk et al., 2007; Gehlert et al., 2007; Hansson et al., 2006; Lowery et al., 2008; Marinelli et al., 2007; Muller et al., 2003; Richardson et al., 2008). In vivo microdialysis studies have further revealed a four-fold increase of baseline dialysate GABA concentrations in the CeA of alcohol-dependent rats relative to alcohol-naïve controls, as well as lack of tolerance for alcohol-induced increases in dialysate GABA levels in alcohol-dependent rats (Roberto et al., 2010). These results strongly suggest that chronic alcohol alter pre-synaptic components of GABAergic synapses in the CeA.

Further studies have shown that CRF produces robust increases in GABAergic transmission in the CeA of rats and mice via CRFR1 activation at presynaptic level (Nie et al., 2004; Roberto et al., 2010). GABA release is increased by CRF and decreased by antagonism of CRFR1s. Moreover, alcohol-dependent rats exhibit heightened sensitivity to the effects of CRF and CRFR1 antagonists on CeA GABA release, suggesting an upregulation of the CRF-CRFR1 system. These electrophysiological findings are further corroborated by increased CRF and CRFR1 mRNA levels in the CeA of alcohol-dependent rats, indicating that neuroadaptation occurs in those systems during the development of ethanol dependence. In addition, *in vivo* intra-CeA administration of a CRFR1 antagonist via retro-microdialysis reverses dependence-related elevations in extracellular GABA and blocks ethanol-induced

increases in GABA in both dependent and nondependent rats (Roberto et al., 2010). Importantly, chronic treatment with CRFR1 antagonist protects against the development of dependence-induced increases of ethanol drinking (Roberto et al., 2010).

Although the precise mechanism(s) by which alcohol enhances GABA release have yet to be identified, past studies have examined the role of intracellular signaling pathways such as adenylyl cylase (AC) or protein kinase C (PKC) in the facilitatory effect of acute alcohol on GABAergic transmission. The ability of CRF and acute alcohol to augment GABAergic transmission in the CeA is contingent on the integrity of PKCε intracellular signaling pathways (Bajo et al., 2008). The ethanol- and CRF-induced increase of GABA release is abolished in the CeA of mice that lack PKCε (Bajo et al., 2008), suggesting that PKCε facilitates vesicular GABA release. The role of PKCε in regulating GABA release from CeA neurons was also confirmed by using a PKCε inhibitor peptide, Tat-εV1–2 (Qi et al., 2007). PKA, which is activated by CRFR1 activation (via Gs and Gq proteins), also play an important role in ethanol and CRF modulation of presynaptic CeA GABA release (Cruz et al., 2011a; Cruz et al., 2011b). A PKA antagonist blocked CRF from regulating spontaneous GABA release, whereas a PKA antagonist limited to the postsynaptic neuron did not alter CRF action on GABA release, suggesting that the presynaptic PKA pathway plays an essential role in the CRF-induced GABA release (Ameri, 1999; Cruz et al., 2011a). Further studies are needed to shed light on a possible crosstalk between PKA and PKCε in regulating the CeA GABA release. Interestingly, PKA and PKCε mediated signaling pathways have also been implicated in the susceptibility of alcohol addiction and binge-like drinking. As discussed earlier, PKA-mediated phosphorylation of IP3Rs plays a critical role in NMDAR metaplasticity in the VTA, and increase in indices of PKCε activity coincides with increased Homer2 expression in the NAC in binge drinking animal models, and inhibition of PKCε activity within the NAC attenuates binge alcohol intake. Thus, PKA and PKCε mediated signaling pathways regulate both reward and stress pathways during development and maintenance of alcohol dependence.

Given the CRF system in the CeA is recruited during both repetitive binge-like drinking and ethanol dependence, it suggests that the CRF signaling may play a key role the development and maintenance of ethanol dependence. CRFR1 antagonists may have the potential in treating alcoholism by reversing a key cellular process that drives transition to ethanol dependence.

3.2 Structural and functional plasticity of dendritic spines in alcohol dependence

Alcohol-induced changes in molecular signaling and synaptic activity are associated with alterations in the network connectivity, which produce long lasting changes in behaviors. Dendritic spines, as the structural and functional units of excitatory synapses (Yuste and Denk, 1995), hold most of the crucial postsynaptic components of the synapse. Spines are dynamically influenced by environmental enrichment, stress, neuronal activity, and they are altered in pathological states (Christoffel et al., 2011; Fiala et al., 2002; Irwin et al., 2000; van Praag et al., 2000). Thus, plasticity of spines reflects the neuroadaptive changes of the network connectivity at the functional and structural level. Studies have demonstrated that drugs of abuse affect dendrite and spine morphology (Robinson and Kolb, 2004; Carpenter-Hyland and Chandler, 2007; Russo et al., 2010). Chronic alcohol exposure regulates morphology and/or densities of dendritic spines at brain regions that are implicated in reward, learning, stress, executive function, and habit formation (Carpenter-Hyland and Chandler, 2006; Lescaudron et al., 1989; Pandey et al., 2008; Tarelo-Acuña et al., 2000; Zhou et al., 2007) (See Table 1). Here, we discuss dendritic spine remodeling associated with chronic alcohol exposure and highlight recent advances in understanding structural and functional plasticity of dendritic spines associated with alcohol dependence.

The brain stress systems, particularly the CRF system in the CeA, play a crucial role in the maintenance of alcohol dependence. Studies have shown that Long-term alcohol exposure alters the density of dendritic spines in the amygdala (Moonat et al., 2011; Pandey et al., 2008). Alcohol withdrawal after long-term exposure reduced dendritic spine densities in the CeA and medial amygdala (MeA), while acute alcohol exposure had opposite effects. BDNF-Arc signaling pathway was proposed to mediate the distinct spine density changes associated with acute alcohol exposure and withdrawal (Pandey et al., 2008). Furthermore, a study on comparing P and NP rats suggests that the heightened innate anxiety of P-rats, which also exhibit greater ethanol intake, is associated with reduced dendritic spine density in the CeA and the MeA (Moonat et al., 2011). Thus, these studies reveal the link of long-term alcohol consumption and dendritic spine remodeling in the brain region that is important for the maintenance of alcohol dependence. Similarly, significant lines of evidence indicate that the CRF signaling is also an important mediator for spine remodeling associated with stress (Chen et al., 2008; Pittenger and Duman, 2008; Radley et al., 2008; Shansky et al., 2009; Treweek et al., 2009). CRF receptors were detected in dendritic spines in the amygdala (Treweek et al., 2009) and mice lacking the CRFR1 receptor showed augmented spine density (Chen et al., 2008). Further evidence suggests that CRF-CRFR1 signaling induces spine remodeling through destabilizing spine F-actin (Chen et al., 2008). Altogether, these pieces of evidence support the role of CRF mediated signaling in regulating dendritic spine changes associated with stress. It remains to be found how CRF signaling changes associated with alcohol exposure contribute to spine density changes in the amygdala.

Chronic alcohol exposure also alters structure and density of spines of medium spiny neurons in the NAC (Zhou et al., 2007). Specifically, 14 weeks of alcohol exposure decreased spine density, enlarged spine head size, and caused a variety of distinct morphological changes (Zhou et al., 2007). These dendritic spine changes in the NAC by chronic ethanol exposure may be associated with changes in Homer proteins, as these proteins are not only capable of dynamically interacting with glutamate-related signaling molecules to regulate function of mGluRs and NMDARs at spines, but this protein family also interacts with F-actin and other cytoskeletal protein to regulate the size and shape of dendritic spines at excitatory synapses (e.g., (Sala et al., 2001; Shiraishi-Yamaguchi et al., 2009b; Szumlinski et al., 2008a). As discussed earlier, studies have shown that Homer2, group 1 mGluR and NMDAR expression are in the NAC by repeated, binge alcohol intake (Cozzoli et al., 2012; Cozzoli et al., 2009) and chronic alcohol consumption (Obara et al., 2009; Szumlinski et al., 2008b). Thus, changes in Homer2 and NMDAR signaling by alcohol may be associated with the structural and functional plasticity of spines, which in turn may impact the network connectivity. Given Homer2/mGluR/NMDAR signaling is altered by both binge and chronic ethanol exposure, it may serve as another pathway that gates the transition to alcohol dependence.

A recent study further revealed that chronic intermittent ethanol exposure (CIE) selectively increased the density of mature spines in the medial prefrontal cortex (mPFC) (Kroener et al., 2012), a brain area that is implicated in the executive control of behaviors. The change in dendritic spines was associated with the enhanced NMDA receptor mediated plasticity and deficit in the cognitive flexibility (Kroener et al., 2012). These results suggest that CIE-induced changes of glutamatergic transmission in the mPFC may contribute to the impairment of loss of behavior control associated with alcohol dependence. Moreover, chronic alcohol induces changes of dendritic spines in brain regions that is important for the habit formation. After more than two years of intermittent alcohol exposure, male cynomolgus monkeys showed increased spine density and enhanced glutamatergic transmission in the putamen, but no change in the caudate, regions equivalent to dorsolateral and dorsomedial striatum in the rodent, respectively (Cuzon Carlson et al., 2011). These

structural and functional changes of spines may contribute to the alcohol consumption associated habit learning, which is believed to play an important role in the maintenance of drug use (Gerdeman et al., 2003; Tiffany and Conklin, 2000; Tricomi et al., 2009).

Taken together, these results point out effects of chronic alcohol exposure on the plasticity of dendritic spine in various brain regions that are known to be important in alcohol dependence. We are just beginning to understand the extent of the dendritic spine changes induced by alcohol exposure. Future studies are needed to uncover how the spine remodeling by the long-term alcohol exposure may contribute to changes of neuronal network connectivity.

4. Conclusion

In this article, we have focused on several exciting research areas targeting mechanisms mediating susceptibility to alcohol addiction, stress and reward pathways in binge-like drinking, activation of the extrahypothalamic stress system, and structural plasticity. Importantly, these seemingly independent mechanisms exhibit significant interactions to drive the development and maintenance of alcohol dependence. New insights presented here also raise several challenging issues. Although metaplasticity discussed here focuses on synaptic activity mainly, it may also extend to structural plasticity of dendritic spines, which has been demonstrated to be true for other drugs of abuse (Shen et al., 2009). Given the heterogeneity of neuronal cell types in a particular brain region, significant challenges exist in understanding cell type or pathway specific changes associated with alcohol exposure. Rapidly developing optogenetic techniques may offer an effective strategy to overcome these challenges. It is clear that studies addressing the crosstalk of neural circuits involved in reward, stress, habit formation, and decision-making will be critical for better understanding of neurobiological mechanisms driving the development and maintenance of alcohol addiction. Furthermore, the role of CRF, PKA, Homer2, and PKCε mediated signaling in binge-like drinking and alcohol dependence suggest critical roles of these signaling pathways in the transition to compulsive alcohol consumption. It remains to be determined whether or not these signaling molecules function in some universal manner to gate synaptic plasticity and morphology within the neural circuitry subserving the transition to and maintenance of alcohol dependence. Finally, studies using different animal models often reveal distinct neurochemical and neurophysiological changes. This could be due, in part, to intrinsic differences in neuroplasticity associated with different alcohol drinking paradigms. Therefore, cross-comparison of neuroadaptive alterations associated with different ethanol exposure paradigms may shed light on mechanisms underlying different types of alcohol drinking behaviors.

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- Metaplasticity in mesolimbic dopamine neurons and addiction vulnerability
- A substrate for binge alcohol drinking in the nucleus accumbens
- Effects of binge-like ethanol exposure on extended amygdala stress systems
- Extended amygdala connectivity with ventral tegmental area: the interface of stress and reward pathways
- Cellular mechanisms of CRF at the GABAergic synapses in the central amygdala: role in ethanol dependence.
- Structural and functional plasticity of dendritic spines in alcohol dependence

Table 1

Summary of morphological effects of alcohol

a

	Cell-type	Species	Alcohol Treatment	Age Studied	Method	Main Findings	Ref
Hippocampus	CA1 Pyramidal	Rat	5 months liquid diet (1-6 months old)	6-8 months	Golgi-Cox	↓ basilar dendritic branching ↑ basilar dendritic branching after 2 months withdrawal	1
		Rat	Prenatal and postnatal drinking (E1-P21)	P15 - 3 months	Golgi	↓ thin spines at P15-40 ↑ wider spines (wide, mushroom, or stubby) at P15-40 ≈ spine morphology at P90	2
		Mouse	single i.p. injection	3 WIV	GFP labeling	Altered spine shape and density by 2 alcohol-related genes	3
	Primary cultures	Rat	4 day *in vitro* (50 mM)	15 DIV	F-actin and PSD-95 staining	↑ synaptic PSD-95 and larger F-actin clusters ↑ spine density	4
Cortex	2/3 pyramidal (associative)	Rat	4 days vapor inhalation (P2-P6; 3h/day)	3 months	Biotin retrograde labeling	↓ dendritic length ↓ dendritic branching	5
	2/3 pyramidal (mPFC)	Rat	Prenatal and postnatal intragastric (E1-P10; 3.0- 4.5 g/kg/day)	3 months	Golgi	↓ apical dendritic branching ↓ spine density	6
		Rat	5 days gavage (P4-P9; 5.25 g/kg/day)	1 months	Golgi-Cox	↓ spine density ≈ dendritic length and branching	7
Striatum	MSN (NAc)	Rat	14 weeks continuous vs. intermittent drinking (3-6 months old)	~ 6 months	Fluorescent dye micro-injection	↓ spine density at 3rd order dendrites ↑ spine head size (intermittent) Altered dendritic morphology (thickened, beaded, or curved)	8
		Rat	Prenatal and postnatal intragastric (E1-P10; 3.0- 4.5 g/kg/day)	3 months	Golgi	↑ dendritic branching (females) ≈ spine density and soma size	6
	MSN (dorsal)	Macaque	2.5 years intermittent drinking (8-10 years old)	~ 10 years	DiOlistics	↑ spine density in putamen (dorsolateral striatum) ≈ spine density in caudate (dorsomedial striatum)	9
Subst. Nigra	Fusiform / pyramidal	Rat	Prenatal liquid diet (E0- birth)	P15	Golgi-Cox and TH labeling	↓ size of cell bodies (fusiform neurons) ↓ dendritic branching (2nd, 3 rd, 4 th order) ≈ number of TH-positive neurons	10
Cerebellum	Purkinje	Rat	1, 3, 6, 12 and 18 months drinking	1-18 months	Golgi	↓ spine density after 3 months ↓ branching (1st and 2nd order; progressive after 6 months) ↓ dendritic length	11

a

	Cell-type	Species	Alcohol Treatment	Age Studied	Method	Main Findings	Ref
		Rat	5 months alcohol in sucrose drinking (20% alcohol in 5% suc)	6 months	Golgi	↑ spine length	12
	Primary cultures	Rat	8-48 hours *in vitro* (50-200 mM)	8-48 HIV	Biotin filling	↑ neurite outgrowth (branch numbers and total length) ↑ soma size	13

b

Cell Density			Soma Size			Branching			Dendritic Length			Spine Density			Spine Size			Wide Spines			Thin Spines			Spine Length		
↑	↓	≈	↑	↓	≈	↑	↓	≈	↑	↓	≈	↑	↓	≈	↑	↓	≈	↑	↓	≈	↑	↓	≈	↑	↓	≈
							1 5 6 10 11						6 7 8 11													
		10	13	10	6 7	6 1		7		5 11	7	4 9		6 9	4 8			2		2		2	2	12		

a. Selected studies are categorized by brain region (left-most column). Subsequent columns describe other characteristics and findings for each particular study. The alcohol treatment column is typically presented as: *duration of treatment, type of treatment (age of exposure and/or ethanol concentration).* HIV=hours in vitro; WIV=weeks in vitro; DIV=days in vitro; "↑"=increase; "↓"=decrease; "≈" = no significant difference; E=embryonic day; P=postnatal day.

b. Findings summarized according to specific morphological perturbations. References appear as numbers: 1 – McMullen et al., 1984; 2 - Tarelo-Acuna et al., 2000; 3 - Piechota et al., 2010; 4 - Carpenter-Hyland and Chandler, 2006; 5 - Granato and Van Pelt, 2003; 6 - Lawrence et al., 2012; 7 - Whitcher and Klintsova, 2008; 8 - Zhou et al., 2007; 9 - Cuzon Carlson et al., 2011; 10 - Shetty et al., 1993; 11 - Tavares et al., 1983; 12 - Wenisch et al., 1998; 13 - Zou et al., 1993.