ACS Chemical Neuroscience

Review

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Neurochemical and Neurostructural Plasticity in Alcoholism

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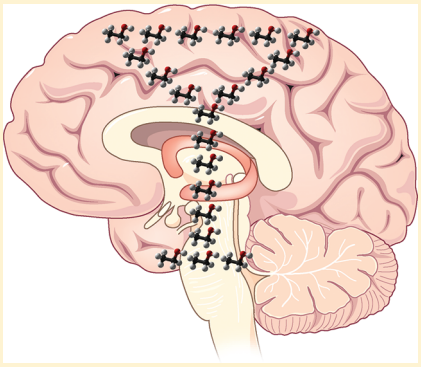
ABSTRACT: The behavioral manifestations of alcoholism are primarily attributable to the numerous and lasting adaptations that occur in the brain as a result of chronic heavy alcohol consumption. As will be reviewed here, these adaptations include alcohol-induced plasticity in chemical neurotransmission, density and morphology of dendritic spines, as well as neurodegeneration and cerebral atrophy. Within the context of these neuroadaptations that have been observed in both human and animal studies, we will discuss how these changes potentially contribute to the cognitive and behavioral dysfunctions that are hallmark features of alcoholism, as well as how they reveal novel potential pharmacological targets for the treatment of this disorder.



KEYWORDS: *Alcoholism, neurotransmission, neurochemistry, neural plasticity, dendritic spine, neurodegeneration, cerebral atrophy*

Alcoholism, alternatively referred to as alcohol addiction, is characterized by the development of (1) tolerance and physical dependence, (2) withdrawal symptoms upon discontinuation of drinking, (3) persistent cravings for alcohol and unsuccessful attempts at reducing its use, (5) excessive drinking despite adverse financial, social, medical, or legal impacts, and (6) a propensity to relapse during attempts at abstinence. Alcoholism inflicts a significant socioeconomic burden on society in terms of lost productivity, legal issues, and treatment costs. Despite alcoholism having persisted as a prevalent neurobehavioral disorder for several millennia, the efficacy of various psychological, cognitive−behavioral, and pharmacological treatment approaches to date is modest at best. Regardless of the treatment modality, the chronic nature of alcoholism and its resistance to successful treatment are often a result of inadequate access to treatment, noncompliance with treatment, poor coping skills, and as will be discussed in the present review, neuroadaptive changes in the brain that occur as a result of chronic alcohol consumption.[1,2]

Owing in large part to inadequate knowledge of the precise neurochemical circuitry mediating alcohol reinforcement and craving, as well as the vast array of neuroadaptations produced by chronic heavy alcohol consumption, only three different pharmacological medications with different mechanisms of action have been developed and approved by the U.S. Food and Drug Administration specifically for the treatment of alcoholism. The first medication to be developed and approved was disulfiram, which causes an aversive accumulation of acetaldehyde during alcohol metabolism by inhibiting the activity of aldehyde dehydrogenase (ALDH). The second medication to be approved was naltrexone, a broad spectrum opioid receptor antagonist with highest affinity for the μ opioid receptor subtype. The modest efficacy of naltrexone suggested an involvement of the endogenous opioid system in mediating alcohol craving, reinforcement, and relapse. In more recent years, extended release formulations of naltrexone have been developed and approved. The third medication to be approved for the treatment of alcoholism was acamprosate (calcium acetylhomotaurinate). Both the clinical efficacy and neurochemical mechanism of action of acamprosate continue to be subjects of debate, but most evidence suggests that acamprosate restores the imbalance between excitatory and inhibitory amino acid neurotransmission induced by chronic alcohol consumption.[3]

Despite numerous gaps in our understanding of the neurochemical and neurostructural plasticity induced by chronic alcohol consumption, our current knowledge base has been bolstered by the development of animal models of alcoholism.[4] Models that have the greatest face validity for human alcoholism include paradigms that involve voluntary alcohol consumption. These include the *two-bottle choice* and *drinking-in-the-dark* paradigms conducted in the home cage, and *operant alcohol self-administration* in specialized test chambers. While some of these paradigms can produce voluntary alcohol consumption to the point of intoxication in both rodents and nonhuman primates, they are limited by the fact that animals will not typically consume enough alcohol on a daily basis to produce physical dependence, despite the development of

Received: January 24, 2012
Accepted: April 16, 2012
Published: April 16, 2012

genetically inbred strains of rats and mice that exhibit higher levels of voluntary alcohol consumption.[4,5] Since dependence is a defining characteristic of alcoholism, this problem has been circumvented by the use of *alcohol vapor inhalation* methods, where animals are exposed to repeated cycles of intoxicating levels of alcohol vapors for prolonged periods of time (~16 h/day), interspersed with periods of withdrawal (~8 h/day). While the primary drawback of this method is the fact that prolonged intoxication is forced rather than voluntary, it produces elevated levels of alcohol consumption paralleled with overt symptoms of alcohol withdrawal following the removal of the alcohol vapors.[4] Relapse has typically been modeled with the *alcohol deprivation effect* or *reinstatement* paradigms, which possess some degree of predictive validity when tested with antirelapse compounds such as acamprosate.[6] The *conditioned place preference* model is widely used due to its simplicity and short duration, but interspecies differences and the acute bolus dosing of alcohol by the experimenter limit the interpretations of findings using this model. Aspects of impulsivity related to alcoholism have been typically investigated using methods such as *delayed discounting* and the *five-choice serial* reaction *time task*. Lastly, negative affective states in alcoholism such as anhedonia have been modeled with paradigms such as *intracranial self-stimulation* and *reduced preference for sweetened solutions*. For more details on animal models of alcoholism, see several excellent reviews on this topic that have recently been published[4,5,7]

In this review, we will summarize different forms of plasticity (neurochemical, dendritic spine, and macrostructural) that occur in the brain as a result of chronic alcohol exposure or consumption. Findings from both animal and human studies will be reviewed, and we will focus our summary largely on studies in which repeated and/or long-term effects of alcohol exposure and consumption have been investigated.

NEUROCHEMICAL ALTERATIONS IN ALCOHOLISM

The primary molecular protein targets of alcohol in the brain are either ligand- or voltage-gated ion channels.[8] Ligand-gated ion channels affected by alcohol include the γ-aminobutyric acid type A ($GABA_A$) receptor, the *N*-methyl-D-aspartate (NMDA) receptor for glutamate, the 5-hydroxytryptamine type 3 ($5\text{-}HT_3$) receptor for serotonin, nicotinic receptors for acetylcholine (nAChRs), and glycine receptors. Voltage-gated ion channels that are affected by alcohol include L-type Ca^{2+} channels and G-protein activated inwardly rectifying potassium (GIRK) channels. Despite this broad array of ion channels affected by alcohol, $GABA_A$ and NMDA receptors often receive the most scientific attention, as they show the most adaptive changes as a result of chronic alcohol exposure or consumption, and are affected by low to moderate concentrations of alcohol in the brain.[8] However, as outlined below, not all neurochemical systems affected by chronic alcohol are direct molecular targets of alcohol itself.

Dopamine. The mesolimbic dopamine system, comprising dopamine-producing cell bodies in the ventral tegmental area (VTA) of the midbrain that project rostrally to regions such as the frontal cortex and nucleus accumbens (NAc), is well established in mediating the rewarding and reinforcing effects of alcohol and other drugs of abuse.[9,10] Numerous neurotransmitter systems, including glutamate, GABA, opioid peptides, acetylcholine, endocannabinoids, and other neuromodulators regulate basal and alcohol-induced activation of the mesolimbic reward system at various anatomical sites (i.e., at the level of cell bodies in the midbrain or rostral forebrain terminal regions; see Figure 1). This system is also activated

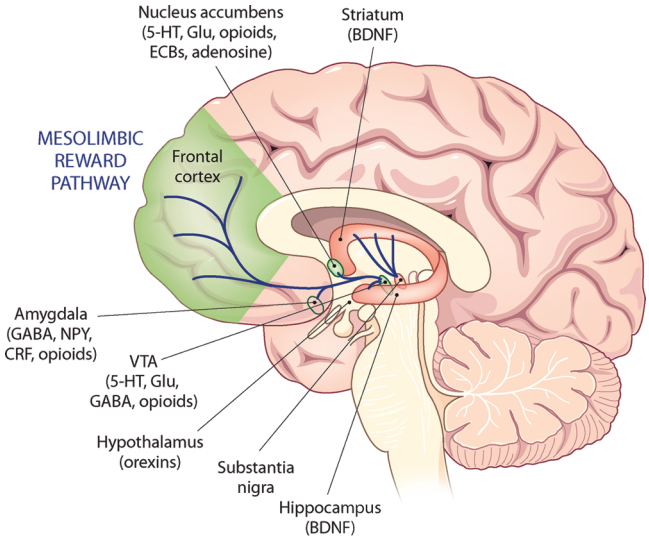


Figure 1. Schematic of the mesolimbic reward pathway in the human brain and neurochemical influences on this pathway that are modulated by alcohol. The reinforcing effects of alcohol are believed to be mediated by the activation of dopamine-containing cell bodies in the ventral tegmental area (VTA) that project rostrally to the nucleus accumbens and frontal cortex. This activation occurs primarily at the level of the VTA, where alcohol promotes the release of endogenous opioid peptides which hyperpolarize local inhibitory GABAergic neurons, thereby disinhibiting mesolimbic dopamine neurons. In addition, alcohol promotes the release of the excitatory amino acid glutamate in the VTA and also affects various ionic currents intrinsic to VTA dopamine neurons that regulate the excitability of these cells. Dopaminoceptive terminal field regions targeted by mesolimbic dopamine projections are modulated by various neurotransmitters and neuromodulators as detailed in this review. Abbreviations: Glu, glutamate; 5-HT, 5-hydroxytrypamine (serotonin), ECBs, endocannabinoids; NPY, neuropeptide Y; CRF, corticotropin releasing factor, BDNF, brain-derived neurotrophic factor.

following the presentation of alcohol-associated cues to alcoholics or alcohol-exposed animals,[11,12] supporting the notion that this system also mediates maladaptive reward-related learning.[13]

Following long-term alcohol exposure, various neurochemical adaptations in the mesolimbic dopamine system occur. Numerous lines of evidence suggest that the mesolimbic reward pathway becomes hypofunctional in the addicted brain.[14] In alcohol-dependent rats, the spontaneous firing rate of midbrain dopaminergic neurons is significantly reduced,[15] resulting in reduced dopaminergic transmission in the ventral striatum. Similar deficits in striatal dopamine output have been observed in human alcoholics.[16,17] Perhaps as a compensatory mechanisms in response to reduced dopaminergic output, the expression of the D_3 dopamine receptor in the striatum has been shown to be up-regulated following chronic alcohol consumption (~1 year) in alcohol-preferring rats.[18] Such findings were accompanied by the demonstration that both a D_3 antagonist and a D_3 partial agonist suppressed the alcohol deprivation effect as well as cue-induced reinstatement of alcohol-seeking behavior.[18] These findings suggest that alcohol-induced alterations in dopaminergic signaling subunits such as the D_3 receptor may represent promising new candidates for

dx.doi.org/10.1021/cn300013p | ACS Chem. Neurosci. 2012, 3, 494–504

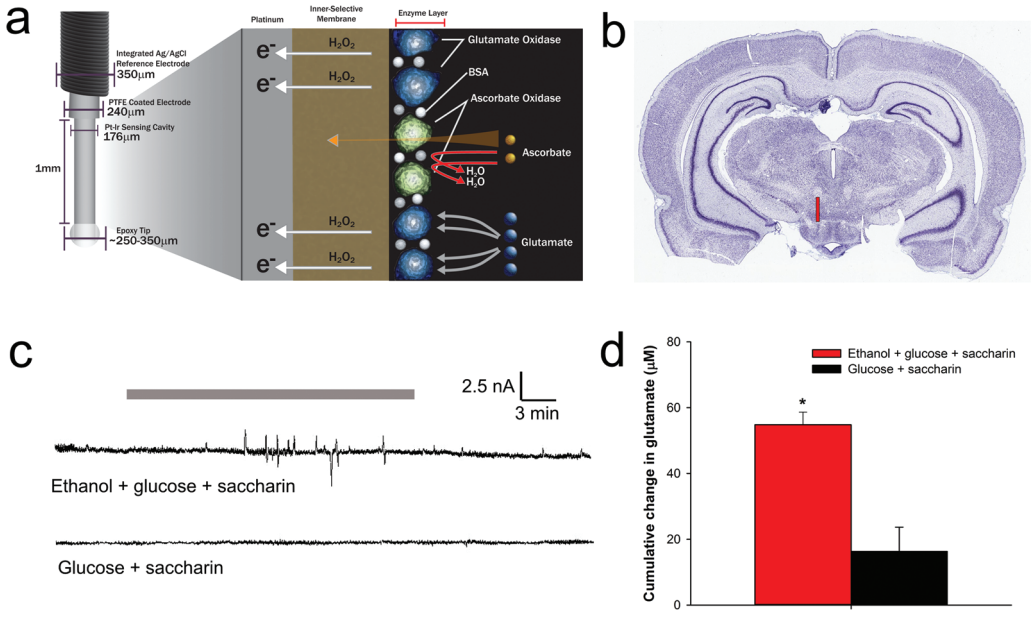


Figure 2. Voluntary ethanol consumption increases extracellular levels of glutamate in the VTA as measured by enzyme-coated glutamate biosensors. See text for details of experimental procedures. (a) Principles of amperometric detection of changes in extracellular glutamate in near real-time by glutamate biosensors (Pinnacle Technologies, Lawrence, KS).[133] Biosensors are equipped with a 1 mm sensor cavity (176 μm O.D.) consisting of a Pt−Ir electrode coated with Nafion (to repel anionic interferants) and an enzyme layer containing immobilized glutamate oxidase (GluOx, which catalyzes the breakdown of glutamate to α-ketoglutarate and electroactive H_2O_2) and ascorbate oxidase (AscOx, which catalyzes the breakdown of ascorbate to neutral dehydroascorbate and H_2O). An amperometric current of +600 mV applied to the electrode oxidizes H_2O_2 to form electrons (e−) that provide a near real-time (~1 s resolution) indirect measurement of changes in extracellular levels of glutamate. Image courtesy of Jim Urich (Pinnacle Technologies). (b) Histological photograph of a rat brain showing the location of glutamate biosensor placement in the VTA. (c) Sample biosensor current tracings obtained during the consumption of sweetened alcohol (10% v/v ethanol + 3% w/v glucose + 0.125% w/v saccharin, n = 5) or control (3% w/v glucose + 0.125% w/v saccharin, n = 5) solutions. Consumption of the solution, as determined from video recordings, occurred during the time indicated by the shaded gray bar. In animals consuming the sweetened alcohol solution, the amount of alcohol consumed during the 30 min session was 0.45 ± 0.07 g/kg (mean ± SEM), resulting in blood alcohol levels assessed immediately following the session of 25.4 ± 2.5 mg/dL (mean ± SEM). (d) Cumulative changes in extracellular glutamate during the 30 min consumption session as determined by cumulative changes in GluOx-mediated currents (in nA) converted to changes in extracellular glutamate (in μM) based on preimplantation calibration curves. Animals consuming the sweetened alcohol solution exhibited significantly greater changes in glutamate in the VTA as compared to animals consuming the control solution ($p < 0.05$).

the treatment for alcoholism. However, elevated levels of D_2 receptor in the striatum have been shown to play a protective role against alcoholism in familial incidences of the disorder.[19] Thus, dopaminergic changes in the alcoholic brain appear to be rather complex. Unfortunately, the role of dopamine neurotransmission in basal ganglia function has limited the feasibility of targeting dysregulated dopamine signaling for the treatment of alcoholism.[10]

Serotonin. The serotonergic system is a potent regulator of the mesolimbic dopamine system, and numerous studies have shown that serotonergic ligands such as selective serotonin reuptake inhibitors (SSRIs) reduce alcohol intake in animals. However, the efficacy of SSRIs in reducing alcohol intake in human alcoholics is less consistent, likely due to the high comorbidity of affective disorders in alcoholics and allelic variations in the serotonin transporter (SERT) gene.[20] More consistent reductions in alcohol intake and relapse have been demonstrated with the 5-HT_3 antagonist ondansetron,[20] which acts on 5-HT_3 receptors that are molecular targets of alcohol. Animal studies have shown that chronic alcohol produces a supersensitivity of 5-HT_3 receptor function,[21] which is in agreement with the efficacy of ondansetron in reducing alcohol intake and relapse. 5-HT_3 receptors in the VTA appear to be important for the ability of serotonin to modulate alcohol intake.[20] Recently, polymorphisms in the gene encoding the 5-HT transporter have been shown to potentially serve as novel biomarkers for treatment response to ondansetron in alcoholism.[22]

Changes in other aspects of serotonergic transmission have also recently been identified as either treatment targets for or biomarkers of alcoholism. For example, a recent study showed that in Brazilian subjects, there was a significant association of the rs11568817 variant of the 5-HT_{1B} receptor and development of alcoholism.[23] In another study, the CC variant of the T102C polymorphism in the 5-HT_{2A} receptor was found to be associated with impulsivity in alcoholism. Behavioral pharmacology studies in rodents have identified the 5-HT_{2C} receptor as a mediator of alcohol consumption.[24] However, studies examining changes in the expression of various 5-HT receptor subtypes, of which more than a dozen have been identified, following chronic alcohol exposure are clearly needed, as are examinations of the effects of more recently developed serotonergic receptor ligands on alcohol consumption in dependent subjects.[20]

GABA. Acute exposure to alcohol potentiates both pre- and postsynaptic components of GABAergic transmission, including increasing presynaptic GABA release and potentiation of postsynaptic $GABA_A$ receptor function. Following chronic alcohol exposure, however, neuroadaptations in these components of GABAergic transmission emerge, including diminished alcohol-evoked presynaptic GABA release[8] and alterations in the expression levels, subunit configurations, and surface

dx.doi.org/10.1021/cn300013p | ACS Chem. Neurosci. 2012, 3, 494−504

expression of $GABA_A$ receptor proteins in various regions such as the cerebellum, cerebral cortex, and hippocampus.[25] For example, chronic alcoholo exposure induces decreases in α_1 and α_2 expression and increases in α_4, α_6, and γ_2 expression, and many of these changes either persist or reverse directionality into withdrawal.[25] Alcohol-induced changes in GABAergic transmission are believed to contribute to the phenomenon of tolerance to numerous psychological and physiological effects of alcohol.[8]

Alcohol has also been shown to diminish functioning of the metabotropic $GABA_B$ receptor, which is primarily localized to presynaptic elements and serves as an autoreceptor at GABAergic synapses. In rodents chronically exposed to alcohol, the functionality of presynaptic $GABA_B$ receptors in the hippocampus is diminished.[26,27] In agreement with this, postmortem analyses of hippocampi from alcoholic patients reveal decreased expression levels of the $GABA_B$ R1 receptor subunit.[28] Taken together with the aforementioned alterations in $GABA_A$ receptor function, these findings indicate significant perturbations in GABAergic transmission in alcoholism. These findings are of clinical importance to the treatment of alcoholism since benzodiazepines, which activate $GABA_A$ receptors, are frequently used to treat symptoms of alcohol withdrawal, and recent evidence suggests that the $GABA_B$ agonist baclofen may be of potential use in the treatment of alcoholism.[29] Thus, alcohol-induced disruptions in the functioning of these receptor systems may influence treatment outcomes and efficacy.

Glutamate. Glutamate is considered to be the major excitatory neurotransmitter in the brain. One of its primary receptors, the NMDA receptor, is a known molecular target for alcohol where it exerts inhibitory actions.[8,30] Glutamate acting at NMDA receptors is also a primary mediator of various aspects of synaptic plasticity.[8] As a result of alcohol-induced inhibition of NMDA receptor function, chronic alcohol consumption causes an up-regulation of NMDA receptor subunit expression and receptor functioning, leading to a state of CNS hyperexcitability during withdrawal, which can manifest as severe seizures and excitotoxicity. Alcohol-induced inhibition of the functioning of other ionotropic glutamate receptors such as the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainic acid (KA) receptor subtypes, along with elevated extracellular levels of glutamate[31] as well as hypofunctionality of inhibitory GABAergic transmission[8,31] may also contribute to subsequent CNS hyperexcitability during withdrawal.

As mentioned previously, alcohol activates the mesolimbic reward circuitry. One puzzling aspect of this phenomenon is precisely how alcohol, a predominantly inhibitory molecule, activates this pathway. In addition to hyperpolarization of local GABAergic interneurons by promoting the release of opioid peptides in the VTA, there is recent in vitro evidence to suggest that alcohol also activates glutamatergic afferents to this midbrain region,[32] providing a mechanism for excitation of the mesolimbic reward pathway. In order to determine if this phenomenon occurs during active alcohol consumption, we recently conducted a study utilizing glutamate biosensors to detect near real-time changes in extracellular glutamate in the VTA during alcohol consumption (Figure 2). In this experiment, male Wistar rats were implanted with guide cannula aimed at the VTA, allowed to recover for at least 3 days, and then allowed to consume a sweetened alcohol solution (10% v/v ethanol + 3% glucose + 0.125% saccharin, $n = 5$) or a control solution (3% glucose + 0.125% saccharin, $n = 5$) for 30 min per day, 3 days per week. Glutamate biosensors were then calibrated in vitro in phosphate buffered saline with four 10 μM increments of L-glutamate and verified to be nonresponsive to 50 μM DOPAC, 12.5 nM dopamine, and 200 μM ascorbate.[33] Biosensors were then implanted into the VTA, and on the following morning, changes in extracellular glutamate were then assessed during a 30 min consumption session. As can be seen in Figure 2, consumption of a sweetened alcohol solution produced significant increases in extracellular glutamate, as compared with consumption of a alcohol-free sweetened solution. These findings provide further evidence that alcohol may activate the mesolimbic dopamine pathway by elevating extracellular levels of glutamate in the VTA. A recent microdialysis study confirmed low dose stimulatory effects of alcohol on extracellular glutamate levels in the VTA.[34] Of course, for these findings to be relevant to alcoholism, additional studies are needed to determine if such changes exist or are altered in alcohol-dependent animals.

Glutamate also acts on G-protein coupled metabotropic glutamate receptors (mGluRs) to produce modulatory effects on synaptic transmission. A wealth of studies have shown that antagonism of Group I mGluRs (mGluR1 or mGluR5) reduces voluntary ethanol consumption in animal subjects.[35,36] A recent study of single nucleotide polymorphisms in glutamate-related genes in over 1,000 alcoholic subjects revealed a moderate association of alcoholism with polymorphisms in the mGluR5 receptor;[37] however, these authors also reported results of a replication study in which no association between mGluR5 receptor and alcoholism was found. Activation of Group II mGluRs (mGluR2 and mGluR3) has also been shown to reduce alcohol consumption or relapse-like behaviors in rodents.[35,36] Recently, several studies have shown that the ability of mGluR2/3 or mGluR5 receptor modulation to inhibit relapse-like behaviors in rodents are altered in rodents with a history of alcohol dependence, such that ligands acting on mGluR2/3 receptors appear to exhibit increased efficacy, whereas ligands acting on mGluR5 receptors display decreased efficacy in alcohol dependent rodents.[38,39] Thus, alcohol dependence may produce functional alterations in mGluR receptor expression, cell surface expression, sensitivity, or signal transduction efficacy, and these dependence-induced changes may have implications for the development of mGluR-based ligands for the treatment of alcoholism.

Endocannabinoids. Numerous preclinical pharmacological experiments with antagonists of the type 1 cannabinoid (CB_1) receptor have revealed a role for endogenous cannabinoids such as anandamide (*N*-arachidonoylethanolamide, AEA) and 2-arachidonoylglycerol (2-AG) in mediating alcohol reinforcement and relapse.[40] Acute forced alcohol exposure inhibits endocannabinoid formation,[41] yet there is in vivo evidence that extracellular levels of 2-AG, but not AEA, are increased in the NAc during active alcohol consumption[42] and that these increases are further potentiated by a history of chronic alcohol exposure.[43] Alcohol also modulates endocannabinoid signaling in the central nucleus of the amygdala (CeA), another region implicated in alcohol dependence.[44] Consistent with the notion of alcohol-induced increases in endocannabinoid transmission, various studies have shown that chronic alcohol exposure down-regulates levels of CB_1 receptors well as desensitizes cannabinoid-activated signal transduction various brain regions.[45,46]

Pharmacological evidence also suggests that the endocannabinoid system regulates alcohol intake, as numerous studies have shown that CB_1 receptor activation increases alcohol consumption in rodents, whereas CB_1 receptor antagonism or genetic deletion decreases alcohol consumption and/or preference.[40,47] As a result, CB_1 antagonism has been purported to be a novel pharmacological mechanism for the treatment of alcoholism.[48] However, to our knowledge, no large scale clinical trials on the efficacy of CB_1 antagonists in reducing alcohol craving or relapse have been conducted.

Opioid Peptides. The efficacy of the opioid antagonist naltrexone in reducing alcohol consumption and relapse in both animals and humans suggests that endogenous opioid peptides (endorphins, enkephalins, and dynorphins) mediate alcohol reinforcement and/or craving.[49−51] Alcohol promotes the release of opioid peptides in various reward-related regions of the brain,[52,53] and following chronic alcohol intake, the expression and normal functioning of various opioid peptides and their cognate receptors become dysregulated.[49,54] A host of studies have shown that pharmacological manipulation of opioid receptors, primarily μ and/or δ receptor antagonism, suppresses alcohol consumption.[55] In addition, pharmacogenetic studies on the use of naltrexone in the treatment of alcoholism have focused on the A118G point mutation in the μ opioid receptor that alters its sensitivity to opioid ligands.[56] However, precisely how allelic variations in μ opioid receptor function as well as alcohol-induced adaptations in the endogenous opioid system function contribute to problematic drinking and alcoholism are yet to be determined.

Although the μ and δ opioid receptor subtypes and their endogenous ligands have historically been the subject of a majority of scientific investigations into the role of endogenous opioid regulation of alcohol consumption, recently there has been a surge in interest in exploring the contributions of the κ opioid receptor and its endogenous dynorphin ligands. κ receptor stimulation by dynorphins or exogenous κ agonists generally produces aversive effects, and it has been hypothesized that, similar to brain stress systems discussed below, chronic alcohol consumptions produces an allostatic shift toward increased dynorphin/κ receptor activation, which leads to a negative affective state that drives further alcohol consumption in order to alleviate this affect via negative reinforcement processes.[57,58] Recent findings that κ receptor antagonists suppress alcohol consumption preferentially in dependent rats lends support to this hypothesis.[54] Familial alcohol dependence has also been shown to be associated with specific alleles of the κ opioid receptor,[58] suggesting that pharmacogenomic approaches to the treatment of alcoholism with ligands targeting κ opioid receptors in specific individuals warrants further exploration.

A final member of the endogenous opioid system that has recently received increasing attention with regards to its role in excessive alcohol intake is nociceptin/orphanin FQ (N/OPQ) and its cognate receptor opioid-receptor-like 1 (ORL_1, also referred to as NOP).[59] In general, stimulation of NOP receptors tends to suppress alcohol intake,[60] and recent studies have identified the CeA as a potential site of action.[61] In addition, activation of NOP receptors also suppresses withdrawal symptoms following chronic alcohol exposure.[62] Chronic alcohol produces transient decreases in tissue content of N/OFQ in various brain regions.[63] In the human alcoholic brain, it has been demonstrated that prepronociceptin as well as NOP receptors are down-regulated in the hippocampus and amygdala,[64] and two studies have identified single nucleotide polymorphisms and allelic variants in the NOP gene that are associated with alcoholism.[65,66] Future studies will hopefully solidify a definitive role of the N/OFQ system as a viable novel target for the treatment of alcoholism.

Corticotropin Releasing factor (CRF) and Neuropeptide Y (NPY). Koob and Le Moal[67,68] hypothesized that the transition to alcohol dependence involves maladaptive changes in the neural circuits underlying alcohol reward and reinforcement as well as brain regions that mediate stress responses. These investigators posited that chronic alcohol consumption engages the brain's stress and antistress systems to produce a negative affective state in alcohol-dependent subjects. The primary stress neuropeptide believed to underlie this phenomenon is corticotropin releasing factor (CRF), which binds to CRF_1 and CRF_2 receptors. Studies have repeatedly shown that CRF_1 antagonists preferentially reduce excessive alcohol consumption in alcohol-dependent animals and are relatively ineffective in nondependent animals.[69] For instance, blockade of the CRF type 1 (CRF_1) receptor attenuates binge-like alcohol consumption in mice.[70] Additionally, administration of a CRF_1 antagonist and CRF_2 agonist also decreased binge-like alcohol consumption.[71] Regions of the extended amygdala as well as the lateral septum appear to mediate CRF involvement in alcohol intake.[67,72] Alcohol consumption is also attenuated in CRF_1 knockout mice when compared to that of littermate controls.[73] These findings suggest that alcohol dependence engages the brain's stress systems by either altering CRF release or up-regulating CRF_1 receptors in limbic regions such as the amygdala.[74,75] This engagement of CRF systems in limbic brain regions appears to be related to the neuroadaptations that result in alcohol dependence, and this notion has been supported by recent findings that antagonism of CRF_1 receptors in the CeA attenuates alcohol self-administration in dependent but not nondependent rodents [76]

Conversely, the activity of neuropeptide Y (NPY) transmission, which produces anti-stress and anxiolytic effects, is also altered as a result of alcohol dependence. For example, alcohol dependence results in the reduction in NPY content in the amygdala,[77] and activation of NPY transmission in the amygdala counteracts the ability of alcohol dependence to increase alcohol consumption in rodents.[78] Intracerebroventricular infusion of NPY has been shown to significantly reduce binge-like alcohol consumption in mice.[79] Within the CeA, binge-like alcohol consumption significantly alters NPY immunoreactivity and augments the ability of NPY to inhibit GABAergic transmission.[79] It has been shown, however, that centrally administered NPY fails to affect low-level voluntary alcohol consumption in mice.[80] Additionally, microinjection of NPY into the CeA failed to affect alcohol consumption in binge-drinking nondependent rats.[81] While these studies suggest a role for NPY in the neurobiological effects of alcohol, the influence of NPY may strongly depend on the history of alcohol intake.

Together, both CRF "stress" and NPY "anti-stress" systems in the brain are recruited as a result of alcohol dependence, and further characterization of these changes may provide novel pharmacological targets for the treatment of alcoholism.

Substance P. Substance P is a member of the neurokinin/tachykinin neuropeptide family that displays preferential affinity for the type 1 neurokinin (NK_1) receptor. Substance P and NK_1 receptors are found in high concentrations in addiction-

related brain regions such as the extended amygdala, mesolimbic reward pathway, and other regions of the basal forebrain. Numerous lines of evidence point to a role for this neuropeptide system in mediating addictive behaviors including alcoholism. Perhaps the most convincing line of evidence comes from a landmark study by George and colleagues,[82] who showed that genetic ablation of the NK_1 receptor gene in mice reduces alcohol consumption. In this same study, a small clinical trial revealed that the NK_1 receptor antagonist LY686017 suppressed alcohol cravings as well as activation of the insula produced by negative affective images. Recently, a SNP in the NK_1 gene has been associated with increased risk for alcoholism,[83] and thus, it appears that NK_1 receptors may be promising new pharmacological targets for the treatment of alcoholism.[84,85]

Orexins. The orexin neuropeptides, also known as hypocretins, are primarily expressed by neurons in hypothalamic subregions but send projections widely throughout the brain. Orexins have been shown to be involved in numerous physiological processes including narcolepsy, feeding, and addictive behaviors, and thus, a number of orexin receptor ligands have recently been developed for a variety of CNS disorders. Orexin receptor antagonists reduce alcohol-seeking in nondependent rats,[86] but recently, it has been demonstrated that chronic alcohol consumption produces a down-regulation of orexin production in the hypothalamus.[87] Thus, there is a need to determine whether orexin receptors up-regulate as a result of chronic alcohol consumption or dependence, and whether such changes result in altered efficacy of orexin receptor antagonists to suppress alcohol consumption and relapse.

Adenosine. Adenosine is an endogenous nucleotide neuromodulator derived from various sources including adenosine monophosphate (AMP), cyclic AMP, and adenosine di- and triphosphate (ADP and ATP, respectively). Adenosine binds to one of 4 adenosine receptor subtypes: A_1, A_{2A}, A_{2B}, and A_3, and extracellular levels of adenosine are actively regulated by equilibrative nucleoside transporters ENT1 and ENT2. Recently, Butler and Prendergast[88] provided a comprehensive review of the effects of long-term alcohol exposure on adenosinergic signaling. In rodents, prolonged ethanol exposure up-regulates A_1 receptor densities in various brain regions including the cerebral cortex, striatum, and cerebellum. A_{2A} receptor levels are largely unaffected by chronic alcohol exposure, although there is evidence that their responses to pharmacological ligands become down-regulated.[89] Alcohol increases extracellular adenosine levels by inhibiting ENT1,[90] and mice lacking ENT1 have been shown to exhibit increased levels of alcohol consumption.[91] The ability of adenosine to mediate alcohol consumption has recently been demonstrated to be mediated by NMDA receptor signaling,[92] supporting the notion that adenosine interacts with glutamatergic transmission to regulate behavioral responses to alcohol as well as alcohol consumption.[93] Given the well-known ability of adenosinergic transmission to regulate neural excitability and neuroprotection, ligands acting on adenosine signaling may be of clinical use in the treatment of excessive alcohol consumption or alcohol-induced CNS hyperexcitability.

■ ALCOHOL-INDUCED STRUCTURAL PLASTICITY OF DENDRITIC SPINES

Several theories of addiction suggest that repeated drug use results in alterations in neuronal plasticity in the same brain areas that mediate normal learning and memory processes.[94,95] Excitatory neurotransmission in the central nervous system is thought to take place mainly at dendritic spines,[96] and these structures are considered to be the primary location of synaptic plasticity in the brain.[97,98] The contribution of these structures to alcohol addiction, however, has yet to be fully understood.

Dendritic spines are small protrusions found on the shaft of dendrites that specialize in the reception and modulation of synaptic input from neighboring neurons. First described by Santiago Ramon y Cajal in the late 1800s, changes to and within these structures are now widely thought to provide the cellular basis for learning and memory.[96] Therefore, on the basis of the increasingly supported view that drug addiction results from changes in synaptic connectivity and efficiency, it is logical to assume that this disease is associated with alterations in the morphology of dendritic spines.

An increasing number of studies suggest that the morphology of spines play an important role in controlling the function of individual synapses.[98] Dendritic spines consist of a narrow "neck" ending in a wider, spherical spine "head." Spines vary greatly in their length and shape and have generally been morphologically classified into the following categories: (1) stubby, which have a wide neck and less pronounced head; (2) mushroom, which have a large head and short neck; (3) long/thin, which have a small head and long, narrow neck; and (4) filopodia, which have a long neck without a head (see Figure 3).[99] It has been proposed that, due to their stability, mushroom spines are "memory" spines while other, less stable and smaller spines, are thought to be "learning" spines.[100]

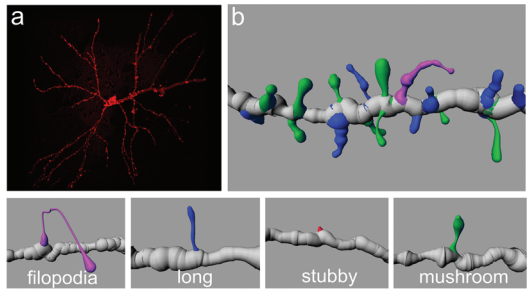


Figure 3. Analysis of structural plasticity of dendritic spines. (a) Diolistically labeled neuron in the prefrontal cortex. (b) 3-D reconstruction of a labeled dendrite showing spine morphology based on the classification of spine shape as filopodia, long, stubby, or mushroom (bottom row).

The exact functional role of these different spine classes has yet to be fully established, but there is substantial evidence suggesting that synaptic strength is regulated in part by changes spine morphology. The mature, stable mushroom spines tend to maintain their form and have strong synaptic connections.[100] Conversely, less stable spines, such as stubby spines, are more likely to undergo structural plasticity but have relatively weaker synaptic connections. The shape of individual spines also affects their sensitivity to glutamate. For instance, in CA1 pyramidal neurons, glutamate sensitivity was highest at spines with the largest heads (e.g., mushroom), while less stable spine (e.g., thin, filopodia) were less sensitive to the effects of glutamate.[101] Glutamate receptors are also involved in various connective properties of dendritic spines. For example, actin-dependent changes in spine morphology are thought to regulate both the initiation of connections with the presynaptic terminal as well as the stabilization of newly formed spines. Glutamate receptors

dx.doi.org/10.1021/cn300013p | ACS Chem. Neurosci. 2012, 3, 494−504

interact with the spine's actin cytoskeleton to alter calcium influx. AMPA receptors can indirectly control calcium through their regulation of voltage-dependent activation of NMDA receptors and voltage sensitive calcium channels, while NMDA receptors can directly gate calcium influx.[97,102] Therefore, it has been suggested that that spine formation initiated by NMDA receptor activation is stabilized by postsynaptic AMPA receptor insertion and activation.[97,98,103,104] The functional consequence of these mechanisms may be the induction of LTP since it has been shown to correlate with altered spine size as well as the emergence of new spines.[98,105−107]

Brain derived neurotrophic factor (BDNF) is also involved in various forms of synaptic plasticity. In the hippocampus, BDNF has been shown to facilitate the induction[108,109] and expression[110] of LTP. One mechanism by which BDNF may affect synaptic plasticity is through the alteration of NMDA receptor activity. For instance, BDNF enhanced the magnitude of synaptically evoked NMDA (but not AMPA) receptor-mediated responses in both hippocampal and neocortical pyramidal neurons.[111] Thus, BDNF in the hippocampus, as well as other regions such as the striatum,[124] may play a role in alcohol-induced neuroplasticity and maladaptive patterns of alcohol consumption.

Influence of Alcohol Exposure on Dendritic Spines. The size and shape of dendritic spines can be influenced by many different external factors including age, temperature, environmental conditions, neuronal pathology, and other disorders. Alterations in spines number and/or morphology has been observed in both developmental (Down's syndrome, inherited metabolic diseases, fetal alcohol syndrome, and fragile X syndrome) and late-stage disorders (e.g., dementia, stroke).[112]

Several studies have shown alterations in spine characteristics after repeated drug exposure. For instance, in animals, changes in dendritic spines have been observed in areas of the prefrontal cortex (PFC) and nucleus accumbens (NAc) after repeated exposure to amphetamine.[113] In these studies, amphetamine-exposed animals showed a significant increase in spine length, spine density, and the number of branched spines in both medium spiny neurons in the NAc and in the apical dendrites of neurons in the medial PFC (mPFC). Similar results have been found with other stimulants such as cocaine[114,115] and nicotine.[116]

Studies have also shown an influence of alcohol use on dendritic spine characteristics. For instance, in CA1 hippocampal pyramidal neurons, chronic alcohol exposure resulted in a significant transient loss of dendritic spines and also reduced the length of remaining spines, which returned to normal after a 2-month period of withdrawal.[117] Adolescent alcohol exposure has been shown to increase spine density in the somatosensory cortex.[118] Conversely, chronic alcohol has been shown to increase the number of stubby and wide spines[119] in CA1 hippocampal pyramidal neurons. In cerebellar Purkinje neurons, chronic alcohol exposure is associated with a significant elongation of dendritic spines.[120] Additionally, in hippocampal cell cultures, chronic alcohol exposure was correlated with an increase in dendritic spine size, which was likely due to increased NR2B receptor subunit expression and increased NMDA receptor clustering with postsynaptic density 95 (PSD-95) proteins.[121] Another recent study found that chronic alcohol exposure was associated with an increase in the size of dendritic spines in medium spiny neurons of the NAc.[122]

Several studies have examined alcohol-induced changes in BDNF and neuronal plasticity. For instance, it has been shown that polymorphisms in the BDNF gene, such as the Val66Met single nucleotide polymorphism, are associated with a higher risk of relapse in alcohol-dependent patients.[123] In animal studies, BDNF-deficient mice display a higher preference for alcohol as compared to wild-type controls.[124] Acute ethanol has been shown to increase spine density in the central and medial nuclei of the amygdala, which has been linked to increased BDNF signaling.[125] Additionally, withdrawal from chronic alcohol decreased BDNF signaling and spine density (in addition to other neurochemical changes including CREB activation and activity-related gene 3.1 expression), and these changes were associated with anxiety-like behavior.[125] Interestingly, these behavioral changes were reversed when BDNF was infused into the CeA.[125]

The results of these studies should be interpreted with caution since extraneous factors such as alcohol-induced nutritional insufficiency and neuronal cell death can influence dendritic spine morphology.[97,98] Nonetheless, it appears that several factors, such as the timing of alcohol exposure as well as the brain region involved, are critical in determining the specific influence of alcohol exposure on dendritic spines.

Diolistic Labeling of Dendritic Spines. While Golgi staining has been used for years to image neuronal tissue (including dendritic spines), the use of lipophilic carbocyanine dyes that exhibit fluorescence when incorporated into membranes is becoming increasingly popular. Benefits of this method include nontoxicity to cells and noninterference in cell growth. Another major benefit of this type of labeling is that these dyes can be used in either live or fixed tissue. Two commonly used carbocyanine dyes include a fluorescent red "DiI" [1,1-dioctadecyl-3,3,3,3-tetramethylindocarbocyanine perchlorate, DiI-C18-(3)] and a fluorescent green "DiO" [3,3-dioctadecyloxacarbocyanine perchlorate, DiO-C18-(3)]. Technical problems associated with the use of microinjections and sprinkling of dye crystals to introduce such dyes into the tissue has led to the use of biolistic delivery of these dyes using a gene gun.[126]

Depending on the type of tissue used, either gold or tungsten particles serve as microcarriers. The dyes are dried onto these microcarriers that are attached to the inside of tubing (known as "bullets.") The bullets are then loaded in a cartridge that is placed inside the gene gun. The gene gun is attached to a helium cylinder that provides pressure to "fire" the gun. The gun is placed an appropriate distance above the tissue and fired. The dye is allowed to perfuse along the membrane for a period of time, and then the tissue is more thoroughly fixed and prepared for confocal imaging. Once in the cell, the dye diffuses and results in labeling of the entire cell surface, which in turn allows for examination of individual neurons and their processes (e.g., dendritic spines).

■ MACROSTRUCTURAL NEURAL PLASTICITY ASSOCIATED WITH ALCOHOLISM

In vivo neuroimaging and postmortem pathological studies have consistently revealed evidence of neurodegeneration and decreased volume of various brain structures including the cerebral cortex (particularly the frontal lobes), cerebellum, and brainstem[127] as well as reduced overall brain volume.[128] There is also evidence of compromised white matter integrity in the brains of alcoholics,[129] as well as evidence of degeneration of deeper structures such as the anterior thalamus and dorsal

hippocampus.[130] Animals studies have shown similar neurodegeneration induced by high dose alcohol, particularly in the suppression of neurogenesis in the adolescent rat hippocampus.[131] These regions of alcohol-induced atrophy and degeneration correlate well with the known cognitive and psychomotor functions associated with alcoholism, including loss of executive function, episodic and nonverbal memory loss, decrements in visuospatial function, and gait and balance abnormalities.[132] The mechanisms underlying alcohol-induced macrostructural damage and plasticity are usually attributed to excitotoxicity during alcohol withdrawal as well as vitamin deficiencies.[132]

CONCLUSIONS

Chronic alcohol exposure or consumption leads to a host of adaptive changes in the brain, ranging from changes in neurotransmitter release or receptor functionality, to changes in the shape and density of dendritic spines, to macrostructural changes in the volume of specific brain regions. Each of these changes potentially contributes to the maladaptive cognition, motivation, affective state, and aberrant learning that are characteristic of alcoholism. Further elucidation of the mechanisms underlying alcohol-induced neurochemical and neurostructural plasticity will hopefully lead to newer and more efficacious treatment options.

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Author Contributions

Both authors contributed to the generation of glutamate biosensor data in Figure 2. M.F.O. contributed to the introduction, Conclusions, and the sections on neurochemical changes and macrostructural neural changes in alcoholism. J.T.G. contributed to the section on dendritic spine plasticity.

Funding

J.T.G. is supported by a grant from the National Institute on Alcohol Abuse and Alcoholism (NIAAA K99AA020537). M.F.O. is supported by a grant from NIAAA (R01AA013852).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We are grateful to Katie Ris-Vicari for her assistance in generating Figure 1 and Jim Urich at Pinnacle Technologies for providing the illustration for Figure 2a and for valuable assistance in conducting the studies utilizing the glutamate biosensors.

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