HHS Public Access

Author manuscript

Neurosci Lett. Author manuscript; available in PMC 2016 August 05.

Published in final edited form as:

Neurosci Lett. 2015 August 5; 601: 11–19. doi:10.1016/j.neulet.2015.01.051.

Molecular mechanisms of synaptic remodeling in alcoholism

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Abstract

Alcohol use and alcohol addiction represent dysfunctional brain circuits resulting from neuroadaptive changes during protracted alcohol exposure and its withdrawal. Alcohol exerts a potent effect on synaptic plasticity and dendritic spine formation in specific brain regions, providing a neuroanatomical substrate for the pathophysiology of alcoholism. Epigenetics has recently emerged as a critical regulator of gene expression and synaptic plasticity-related events in the brain. Alcohol exposure and withdrawal induce changes in crucial epigenetic processes in the emotional brain circuitry (amygdala) that may be relevant to the negative affective state defined as the "dark side" of addiction. Here, we review the literature concerning synaptic plasticity and epigenetics, with a particular focus on molecular events related to dendritic remodeling during alcohol abuse and alcoholism. Targeting epigenetic processes that modulate synaptic plasticity may yield novel treatments for alcoholism.

Keywords

alcoholism; epigenetics; synaptic plasticity; dendritic spines; histone deacetylases; histone acetylation; amygdala

1. Introduction

Alcohol abuse and alcoholism represent significant public health problems that impact both the individual and society as a whole [1]. Alcoholism is defined as compulsive drug seeking behavior that interferes with normal functioning and is related to various psychiatric states such as stress and anxiety [2-7]. Additionally, alcoholism and alcohol abuse are characterized by both positive and negative emotional states [4,5]. The behavioral switch between positive reinforcement (i.e., seeking a drug for its rewarding effects) and negative reinforcement (i.e., seeking a drug in order to remove the negative emotional state

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Other author reports no potential conflicts of interest.

associated with withdrawal) is an important aspect of the cycle of alcohol addiction [4,5]. The negative affective states (e.g., anxiety, stress, and dysphoria) that drive the negative reinforcement of addiction, along with the physical manifestations of drug withdrawal, are referred to as the “dark side” of addiction [4-7].

The dark side of addiction represents a dysfunction of brain reward and emotion systems [5-7], and therefore, targeting aberrant molecular pathways in these circuitries may yield better therapeutic interventions for alcoholism and other addictive behaviors. In particular, the extended amygdala (consisting of the central nucleus of the amygdala [CeA], the bed nucleus of the stria terminalis [BNST] and a transitional zone of the nucleus accumbens [NAc] shell) [7,8] is posited to integrate stress signals in the brain with reward processing through dynamic molecular regulation leading to changes in synaptic plasticity and dendritic arborization [4,9,10]. Several molecular and cellular substrates in key brain regions play a crucial role in the development and maintenance of drug abuse and addictive behaviors [3,9-14].

Uncovering the precise molecular machinery responsible for abnormal dendritic branching and synaptic remodeling remains an important research area in the field of alcoholism. Recent evidence has implicated both genetic and epigenetic mechanisms in the control of synaptic plasticity, particularly as it relates to alcohol consumption and addiction [3,11]. In this review, we will summarize the current knowledge of molecular and epigenetic mechanisms underlying synaptic remodeling and maintenance of the “dark side” or negative affective state of alcohol addiction.

2. Synaptic plasticity and alcohol

2.1. Plasticity at the individual synapse

Drug and alcohol addiction are often characterized as a dysfunction of normal learning and memory circuits within the brain [4,11,12]. These complex processes are governed by the rapid forming and reforming of synaptic structures in particular brain regions that occur concomitantly with changes in signaling at the level of individual synapses, collectively known as synaptic plasticity [13]. At the single synapse level, synaptic transmission at a particular synapse enhances the efficacy of subsequent signaling at that same synapse in a process known as long-term potentiation (LTP) [14]. Conversely, the dampening of future synaptic transmission is called long-term depression (LTD) [15]. In addition to LTP and LTD, changes to the ultrastructure of synapses play a role in the pathogenesis of addiction through the action of several different neurotransmitter systems, particularly the mesolimbic dopamine system [16,17]. Glutamatergic and GABAergic systems also are implicated in synaptic remodeling and are affected by exposure to alcohol and drugs of abuse [17,18].

2.2. The effect of alcohol on dendritic spine formation

Modulation of signaling events at the single synapse are accompanied by the structural reorganization of neuronal dendritic spines [19], sometimes referred to as metaplasticity [16]. The observation of certain mRNA and protein transcripts, such as activity-regulated cytoskeleton-associated protein (Arc), found in recently activated synapses led to the hypothesis that specific molecular players were involved in structural synaptic remodeling

[20,21]. Arc is transported to dendritic spines by actin-based motor proteins, where it modulates glutamatergic neurotransmission and structural organization [22–24]. By this process, dendrites form and re-form connections to other neurons in the surrounding area. Dendritic branching and arborization are robustly affected by many drugs of abuse, including cocaine, heroin, and alcohol [17,24], and several cellular mechanisms [1-4] have been implicated in long-lasting behavioral changes associated with drug addiction such as compulsive drug-seeking and negative affective states [4,7,12].

Alcohol exerts effects on the morphology of dendritic spines. In particular, acute alcohol administration is associated with an increase in dendritic spines in the CeA and medial nucleus of the amygdala (MeA) of rats [24]. These structural effects coincide with alcohol-induced anxiolysis and an increase in expression of brain-derived neurotrophic factor (BDNF) and Arc, gene products known to play a role in synaptic plasticity [24]. Interestingly, withdrawal from chronic alcohol exposure decreases dendritic spines and Arc and BDNF expression in the CeA and MeA, leading to anxiogenesis in rats [24,25]. Withdrawal from chronic alcohol exposure also decreases dendritic arborization in the hippocampus and the NAc [26,27]. This data mimics results from postmortem studies of human alcoholics showing decreased dendritic spine density in cortical pyramidal neurons [28]. These studies support the notion of a synaptic plasticity-driven model of alcoholism.

Acute alcohol exposure provokes anxiolysis and increased dendritic spines in the amygdala, whereas continued exposure normalizes dendritic arborization and attenuates anxiolytic-like behavior. However, withdrawal from chronic or binge ethanol consumption, and especially repeated withdrawal, causes a stark decrease in the number of dendritic spines in the amygdala and other important brain regions, accompanied by increased anxiety-like behaviors. Relapse to drinking possibly normalizes dendritic spine density (Figure 1). Notably, changes in structural plasticity are accompanied by changes in BDNF and Arc gene expression in these studies, providing a molecular framework for the structural changes seen with altered synaptic plasticity. Several studies in the field have shown that Arc directly regulates dendritic spines both in the hippocampus and amygdala [24,29]. Interestingly, increased BDNF and Arc expression leads to increased dendritic spine density in the CeA and decreased anxiety-related and drinking behavior, while decreased BDNF and Arc expression in the CeA leads to decreased dendritic spine density and increased anxiety and drinking behavior (Figure 2) [24,25,30]. This cellular mechanism provides a possible explanation for many of the behavioral consequences of alcoholism, including compulsive drug-seeking and negative affective states seen during withdrawal.

2.3. NMDA receptors and alcohol-dependent synaptic reorganization

The glutamatergic N-methyl-D-aspartate (NMDA) receptor is known to play an essential role in both short- and long-term activity-dependent synaptic plasticity [31–33]. These receptors act as modulators for the transmission of excitatory impulses to the postsynaptic nerve terminal. Interestingly, acute alcohol exposure potently inhibits NMDA-mediated currents, resulting in less depolarization in postsynaptic neurons [34,35]. Chronic ethanol exposure, on the other hand, increases the sensitivity of NMDA receptors to glutamate, potentiating neuronal depolarization and subsequent activation [35,36]. These effects are

specific to NMDA receptors targeted to the synapse, rather than extrasynaptic receptors [37] and are localized to the amygdala in rodent models [35,36,38]. Withdrawal from alcohol produces robust anxiety-like behavior which is reversed upon administration of a glutamatergic antagonist in the amygdala [39]. This data suggests that the glutamatergic system and NMDA receptors are at least partly responsible for the anxiety-like behavior seen during alcohol withdrawal. Additionally, exposure to chronic ethanol caused a decrease in dendritic spine density in medium spiny neurons in the nucleus accumbens (NAc), which was associated with an increase in NMDA receptor NR1 subunit expression (discussed further below) [26]. Other studies showed the decrease in NAc spine density, particularly in "long thin" spines, was accompanied by a decrease in post-synaptic density-95 (PSD95)-positive and tyrosine hydroxylase (TH)-positive immunostaining in rats undergoing ethanol withdrawal [40]. This led to decreased NMDA flux and decreased LTD induction in NAc slices [40].

NMDA receptors are heteromeric molecules composed of different combinations of the subunits NR1, NR2, and NR3 [33]. During early development, immature synapses are enriched with NMDA receptors composed of NR1-NR2B heteromers. Conversely, mature synapses undergo a developmental switch to express higher levels of NR1-NR2A heteromer receptors [41–44]. NR2B-containing receptors are thought to be important for the formation of strong synaptic connections during a critical developmental period based on the observation that overexpression of this subunit increases the density of dendritic spines [45]. Activation of NMDA receptors causes downstream activation of calcium (Ca^{2+})/calmodulin-dependent protein kinase II (CaMKII) signaling pathways, and NR2B-containing receptors show a higher affinity for CaMKII than NR2A-containing receptors [46]. Activation of CaMKII regulates activity-dependent synaptic changes, thus providing a signaling mechanism for the developmental switch underlying synaptic maturation [47]. Hippocampal slices exposed to alcohol show selective internalization of NR2A subunits, changing the composition of NMDA receptors at the synapse to essentially pure NR2B-containing heteromers [48]. Enhanced NR1 and NR2B expression and increased mushroom-type dendritic spines were observed *in vivo* in postsynaptic density fractions taken from the mouse prefrontal cortex in animals exposed to ethanol chronically [49]. Chronic intermittent ethanol exposure increases NR2B without increasing NR2B phosphorylation or PSD95 [50]. Interestingly, the prefrontal cortex has been implicated in long-term learning mechanisms that lead to relapse even a longer period of abstinence [7,51]. Similar increases in NR2B-containing receptors were observed in the hippocampus of ethanol-exposed rat brain slices [52]. These receptors were associated with clusters of F-actin and PSD95, indicating an increase in dendritic spine density [52]. The increased expression of NR2B subunits also is observed in response to other drugs of abuse, such as cocaine, heroin, and nicotine [53–55].

NR2B subunits likely mediate the enhanced LTP seen in animals chronically exposed to drugs of abuse due to the higher affinity for downstream activating mechanisms. The increased responsivity of these receptors, when compared to NR2A subunits, forms a critical period for learning and synaptic plasticity in the brain, and the loss of NR2B receptor subunits may play an important role in the decreased plasticity seen in the aging brain [56,57]. The re-enrichment of synapses with NR2B subunits may hijack normal dendritic

spine formation to reopen a critical period-like state. This process is hypothesized to contribute to the aberrant learning mechanisms responsible for the compulsive drug seeking and negative affective states associated with the dark side of addiction [7,12,18]. Understanding the molecular processes that cause these effects will likely lead to new approaches to treat alcohol and drug addiction.

3. Epigenetic basis of the dark side of addiction

3.1. Epigenetic modifications

Recently, epigenetics has emerged as a novel mechanism underlying the processes of synapse formation and maintenance. The term “epigenetics” defines modifications of the genome that modulate transcription and/or translation without affecting the underlying DNA sequence [58,59]. Epigenetic mechanisms include the covalent modifications of histone proteins around which DNA is wrapped (i.e., the chromatin complex) [59] and the addition of methyl groups directly to DNA, known as DNA methylation [60]. These chemical modifications to chromatin can alter the accessibility of DNA to transcriptional machinery, leading to changes in gene expression without any change to the DNA sequence [61,62].

Several histone chemical modifications [59,63] can either promote or inhibit transcription of the underlying genetic information, depending on the type of epigenetic mark and the specific residue on which it is located. For example, acetylation, mainly occurring at lysine residues, is a marker of active gene transcription [59]. Methylation of histone proteins, particularly methylation of lysine and arginine, can either promote or repress transcription, depending on the context of the modified residue. Namely, methylation of H3K4 (histone H3 lysine residue K4), H3K36, and H3K79 are associated with active transcription, while methylation of H3K9, H3K27, and H4K20 are associated with decreased transcriptional states [59,63]. These covalent modifications are regulated by a group of enzymes that function to add and/or remove specific epigenetic markers from specific histone residues. This group includes, for example, histone acetyltransferases (HATs; responsible for adding acetyl groups to histone lysine residues) and histone deacetylases (HDACs; responsible for removing acetyl groups from histone lysine residues). The language created by the dynamic interaction of these enzymes and their respective chromatin marks is enormously complex, but understanding these interactions is crucial to determining the precise regulation of gene transcription [64]. In the context of this review, it is important to note that covalent histone modifications are important for ongoing synaptic plasticity and are involved in various neurological and psychiatric disease processes [65,66].

In addition to covalent histone modifications, methyl groups can be added directly to cytosine residues within DNA (i.e., DNA methylation), usually in genomic regions rich in CpG (cytosine-phosphate-guanine) residues known as CpG islands [67,68]. DNA methylation most often causes transcriptional repression of the underlying DNA sequence via steric hindrance of the binding of transcriptional machinery and, additionally, via the recruitment of methyl-CpG-binding proteins (MeCPs) and HDACs [69,70]. DNA methylation plays a vital role in brain development, neuronal differentiation, synaptogenesis and ongoing synaptic plasticity in differentiated neurons [71–73]. Much like HATs and HDACs, the DNA methylation process is catalyzed by particular enzymes that add or

remove methyl groups to DNA [74]. The DNA methyltransferase (DNMT) family is responsible for the catalytic addition of methyl groups. After this process occurs, newly methylated CpG islands are bound by members of the MeCP family of proteins which influence transcription [75,76]. The MeCP2 protein is particularly important for the regulation of synaptic plasticity and dendritic spine maturation [72,77,78].

Methylated DNA in the form of methylcytosine can be further converted to hydroxymethylcytosine by a family of enzymes known as the ten eleven translocation (TET) group [79]. The presence of hydroxymethylcytosine is enriched in the brain and appears to be associated with actively transcribed genes [80,81]. Another family of enzymes, known as growth arrest and DNA damage repair (Gadd45), participate in active demethylation of DNA and are responsible for epigenetic activation [82,83]. Notably, TET1 was recently shown to directly influence active DNA demethylation of essential synaptic plasticity genes in two different memory extinction studies [84,85]. Genetic knockout of Gadd45b in a rodent model causes lasting changes in memory formation and synaptic plasticity [86]. Taken together, these studies implicate DNA methylation and demethylation as critically important regulators of neuronal function and dendritic spine formation. In the subsequent sections, we will outline the epigenetic contribution to synaptic regulation in response to alcohol abuse and addiction.

3.2. Epigenetic regulation of the κ-opioid system by alcohol

The action of drugs of abuse is likely mediated through multiple signaling molecules and molecular cascades, many of which involve epigenetic processes. The endogenous dynorphin system is of particular importance, as binding of dynorphin to κ-opioid receptors likely mediates negative affective states seen in alcohol withdrawal [87–89]. Prodynorphin mRNA, the molecular precursor to dynorphin, is induced in the NAc following dopamine release (and, consequently, drug use) [90]. This increase is postulated to mediate feedback inhibition of dopamine release, contributing to the dysphoria and anxiety seen in the latter stages of addiction and other psychiatric disorders [91,92]. Acute ethanol administration in rats causes an increase in H3K9 acetylation and a decrease in repressive H3K27 methylation at the prodynorphin gene promoter in the amygdala, providing an epigenetic mechanism for the acute increase of prodynorphin expression [93]. Antagonism of κ-opioid receptors, the major postsynaptic target of dynorphin, curbs ethanol self-administration in alcohol-dependent rodents [94]. Interestingly, a study of human alcoholics showed an increase in DNA methylation in the prefrontal cortex at the prodynorphin gene associated with a single nucleotide polymorphism (SNP) [95].

Dynorphin is known to activate corticotrophin releasing factor (CRF), an important mediator of stress responses in the brain and the rest of the body [96,97]. Of note, CRF is a well-known modulator of synaptic plasticity and dendritic branching in the amygdala and other brain regions such as the hippocampus and the ventral tegmental area (VTA) [97,98]. CRF antagonists injected into the brain reverse the anxiogenic effect of ethanol withdrawal [99,100], and this reversal is mainly mediated by CRF neurons in the CeA [100]. CRF receptor antagonism suppresses the response to stress which is normally increased during ethanol withdrawal [101].

3.3. Epigenetic regulation of synaptic plasticity by alcohol

Recently, chromatin remodeling has emerged as a novel mechanism which could explain the effects of alcohol on anxiety [102,103]. Alcohol exposure exerts potent effects on cyclic AMP response-element binding protein (CREB), a transcription factor implicated in neuronal plasticity and cognition [2,104,105]. CREB is crucial for synaptogenesis, axonal growth, dendritic spine density, and the fine-tuning of synaptic connectivity [106–108]. Rats exposed to acute ethanol displayed anxiolysis that paralleled increases in phosphorylated CREB, CREB binding protein (CBP), acetylated histone H3 and H4, neuropeptide Y (NPY) expression in the CeA and MeA, and, perhaps most importantly, inhibition of HDAC activity in the amygdala [102]. These results indicate an open chromatin conformation in response to acute alcohol exposure. It is important to note that CBP itself acts as a histone acetyltransferase, or HAT [109]. Ethanol withdrawal after chronic ethanol treatment produces a significant decrease in phosphorylated CREB, CBP, acetylated H3 and H4, and NPY expression in the amygdala, with a parallel increase in HDAC activity and development of anxiety-like behaviors. Opposite to the acute alcohol effects, these results indicate a condensed chromatin conformation upon repeated alcohol exposure. These effects were rescued by treatment with trichostatin A (TSA), a pan HDAC inhibitor, which prevented the alcohol withdrawal related anxiety [102].

As mentioned earlier, BDNF and Arc are gene products important for synaptic plasticity, and activation of these genes lies downstream of CREB phosphorylation [110,111]. Both BDNF and Arc are induced upon acute ethanol exposure, but these signaling molecules show decreased amygdaloid function during chronic ethanol exposure and/or withdrawal [24,30]. Decreased BDNF and Arc expression are linked to increased HDAC activity, as well as decreased histone H3 acetylation and dendritic spine density in the CeA and MeA. TSA treatment returned BDNF and Arc expression to normal levels and restored dendritic spine density to control amounts [25,102]. In a recent study, TSA treatment was effective in reversing deficits in NPY expression and deficits in global and NPY-specific H3K9 acetylation in the amygdala of alcohol-preferring (P) as compared to non-preferring (NP) rats [112]. Additional research by our group found the reversal of rapid tolerance to the anxiolytic effects of ethanol, including NPY expression in the CeA and MeA, upon treatment with TSA [113]. In an ethanol-induced behavioral sensitization paradigm, treatment with sodium butyrate (NaB), an HDAC inhibitor, increased BDNF expression in the striatum [114]. These studies cleary indicate that histone modifications regulate important synaptic plasticity-associated genes in the amygdala and other brain regions, thereby modulating alcohol-related behaviors.

3.4. Direct HDAC2 regulation of dendritic spines in alcoholism and cognition

In recent studies conducted in our lab, increased innate HDAC2 isoform expression and decreased H3K9 acetylation were observed in the CeA and MeA of P versus NP rats, a well-established model used to study the genetic predisposition to alcoholism [103]. P rats displayed decreased BDNF and Arc expression in the CeA and MeA and markedly decreased dendritic spine density in those same brain regions as compared to NP rats [30,103]. BDNF and Arc expression were normalized by an infusion of small interfering RNA (siRNA) against HDAC2 into the CeA. HDAC2 siRNA infusion subsequently

increased dendritic spine density which were innately lower compared to NP rats [30,103]. HDAC2 siRNA infusion into the CeA also corrected the deficits in histone H3 acetylation of BDNF and Arc genes and expression of these genes. HDAC2 siRNA infusion into the CeA attenuated the anxiety-like and alcohol-drinking behaviors of P rats. These data firmly established the regulation of synaptic plasticity and dendritic arborization in alcoholism by direct HDAC2 regulation of BDNF and Arc [30,103].

Notably, HDAC2 is crucial for other neurocognitive domains, such as learning and memory [115,116]. A recent study showed that increases in HDAC2 were responsible for decreased gene expression seen in Alzheimer's disease-like neurological deficits. These deficits were reversed by injection of a short hairpin RNA (shRNA) targeted knockdown of HDAC2, which restored normal levels of synaptic plasticity [117]. HDAC2 overexpression in neurons decreases dendritic spine density, synapse number and memory formation. Conversely, *HDAC2*-deficient mice show increased dendritic spine density and synapse number, which is paralleled by increased memory formation [115]. This study was the first to show the direct regulation of dendritic spine density by the HDAC2 isoform and its involvement in cognitive processes.

As demonstrated above, HDACs, and in particular the HDAC2 isoform, have emerged as significant players in synaptic plasticity-related chromatin remodeling and subsequent dendritic spine alteration. Increased HDAC2 expression decreases the expression of genes important for the maintenance of dendritic spine density such as BDNF, Arc, and NPY, leading to increased anxiety and alcohol-seeking behavior. Decreasing HDAC2 reverses both the molecular and behavioral consequences of alcohol addiction, thus implicating this enzyme as a potential treatment target (Figure 3). HDAC2 is also crucial for the induction and maintenance of structural synaptic plasticity in other neurological domains such as memory formation [115]. Taken together, these findings underscore the potential usefulness of HDAC inhibition in treating alcohol use disorders and other neurocognitive ailments.

4. HDAC inhibitors as novel treatments for addiction

Given the ability of HDAC inhibitors to potently modulate the synaptic plasticity of learning and memory [118], these drugs hold potential as treatment for substance abuse-related disorders. The HDAC2 isoform has emerged as the principal effector of epigenetic remodeling in both cognitive decline and alcohol addiction (Figure 3) [103,112,117,119]. All aforementioned HDAC inhibitors may be acting via inhibition of HDAC2 in the brain. The lack of specificity often leads to concerns about side effects and unintended consequences of HDAC inhibitor administration [120,121]. These drugs have been utilized as cancer therapy agents [121,122]. The clinical side effects observed during HDAC inhibitor cancer therapy include benign problems, such as gastrointestinal disturbances and fatigue, and more serious issues including thrombocytopenia, anemia, hypotension, inflammation, infection, electrolyte disturbances, and cardiovascular complications [123–125]. This evidence, combined with the knowledge that HDAC inhibitors are toxic to neurons in culture at high concentrations [126,127], has tempered optimism regarding the use of these drugs to treat brain diseases. Nonetheless, it must be noted that HDAC inhibitors are exceedingly well tolerated in most cases [118,128]. Additionally, it is possible

that the dose needed to modify brain chromatin conformation in addictive and other neurological disorders may be significantly lower than the doses used as adjuvants in cancer chemotherapy.

HDAC inhibitors have shown promising results in the treatment of other neurological disorders, particularly with regard to rescuing aberrant synaptic plasticity. For example, HDAC inhibitors have successfully ameliorated the symptoms of neurocognitive decline and the decreased dendritic arborization seen in a variety of animal models of Alzheimer disease (AD) [129–131]. This data is further corroborated by lower BDNF expression and higher levels of repressive H3K9 methylation (a chromatin marker which prohibits H3K9 acetylation) in neuronal cultures obtained from a mouse model of AD [132].

Additionally, older mice with lower levels of acetylated H4K12 displayed increased associative memory formation, gene expression and histone acetylation when administered HDAC inhibitors [133]. HDAC inhibitors also have been effective in reducing the motor symptoms of Huntington disease (HD) in animal models of the disease [134,135]. In particular, genes involved in HD pathology show hypo-acetylation in the promoter region that is corrected with HDAC inhibitor treatment [136]. Hypo-acetylation also was observed in dying motor neurons in a rodent model of amyotrophic lateral sclerosis (ALS) [137]. The HDAC inhibitor sodium valproate provided neuroprotective effects in this same model in an effect mediated through CBP [138]. HDAC inhibitors have been effective in a rare neurodevelopmental disease known as Rubinstein-Taybi syndrome (RTS), which is caused by mutations in the CBP gene [139]. CBP activates gene promoters by acting as a HAT at critical synaptic plasticity genes [140]. The histone acetylation deficits and the observed neurodevelopmental defects of RTS are reversed by the administration of the HDAC inhibitor TSA [140,141]. These drugs have also proven effective in improving symptoms of depression, schizophrenia, and cocaine addiction in animal models [142–144].

Our lab and others have published extensively on the ability of HDAC inhibitors to reverse the gene expression deficits caused by multiple models of alcoholism and alcohol abuse, the results of which were discussed above [25,112,113]. This data supports further examination of histone modifying agents as potential therapeutic drugs in the treatment of alcohol addiction. Treatment with TSA and HDAC2 siRNA was able to normalize the deficits in dendritic spines in the CeA under various conditions of alcoholism, suggesting that HDACs are important targets within the epigenome regulating synaptic remodeling.

5. Conclusions

Here, we have summarized the current knowledge regarding alcohol-induced modulations of dendritic branching and the epigenetic and molecular mechanisms underlying these processes (Figs 1-3). As discussed above, alcohol is an important regulator of synaptic organization and function, and that these processes are controlled, at least in part, by specific epigenetic regulators. In particular, alcohol exposure induces near-immediate changes to chromatin and synaptic plasticity-associated gene expression. These alterations could induce a change in neuronal phenotype marked by re-enrichment of immature synapses, aberrant synaptic plasticity and dysfunctional dendritic spine formation. These structural changes are

posited to underlie the compulsive drug seeking, negative affective states, and behavioral changes seen in alcohol addiction (Figure 3). We also have highlighted the potential usefulness of HDAC inhibitors as a novel treatment for alcohol use disorders (25,102,103,112). Future studies should continue to elucidate the specific epigenetic mechanisms underlying compulsive alcohol use and alcoholism, as this is likely to provide new molecular targets for clinical intervention.

Acknowledgements

This work was supported by National Institute on Alcohol Abuse and Alcoholism Grants AA-019971 (NADIA project), AA-010005, and AA-013341, and by the Department of Veterans Affairs (Merit Review Grant, I01BX000143; Research Career Scientist award) to S.C.P. SCP reports that a US patent application entitled "Histone acetyl transferase activators and histone deacetylase inhibitors in the treatment of alcoholism" (serial number 60/848237 filed on *September 29th, 2006) is currently pending.*

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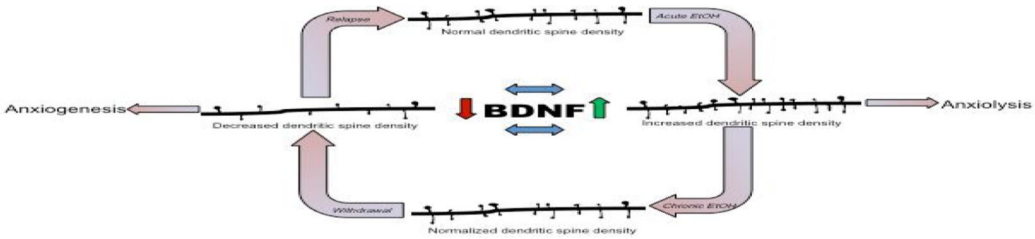

Figure 1. Effect of alcohol exposure on dendritic spines

This diagram illustrates the modulation of dendritic spine density in the amygdala during various stages of alcohol (EtOH) exposure. Acute alcohol increases the density of dendritic spines, which is accompanied by marked anxiolysis. Continued alcohol exposure will normalize spine density, while withdrawal from alcohol intake causes decreased dendritic arborization and this is correlated with decreased BDNF system in the amygdala. This decrease in BDNF causes a subsequent increase in anxiety-like behaviors during alcohol withdrawal. Relapse to alcohol use possibly attenuates both the decreased spine density and anxiety-like behaviors. Diagram is based on published data from our laboratory [24,25,30].

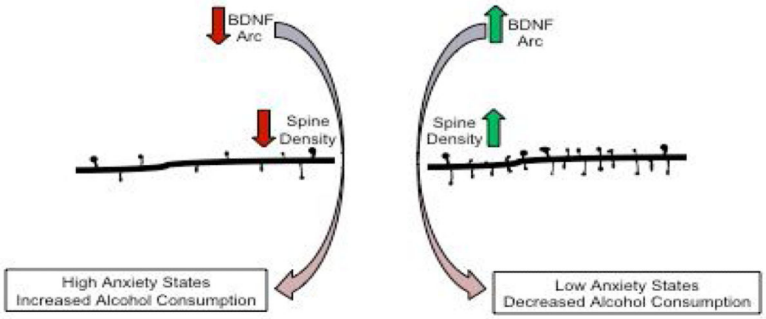

Figure 2. Molecular players underlying alcohol-related alterations to dendritic spine density

Brain-derived neurotrophic factor (BDNF) and activity-regulated cytoskeleton-associated protein (Arc) play a critical role in the induction and maintenance of structural synaptic plasticity in the amygdala. In particular, decreased BDNF and Arc expression are associated with decreased spine density, leading to an increase in anxiety and alcohol consumption. Conversely, increased expression of BDNF and Arc due to acute ethanol exposure induces spinogenesis in the amygdala and produces anxiolytic-like effects. Interestingly, decreases in dendritic spines in the central nucleus of amygdala (CeA) due to knock down of Arc is associated with increased consumption of alcohol and heightened anxiety states. Diagram derived from data published from our laboratory [24,25,30].

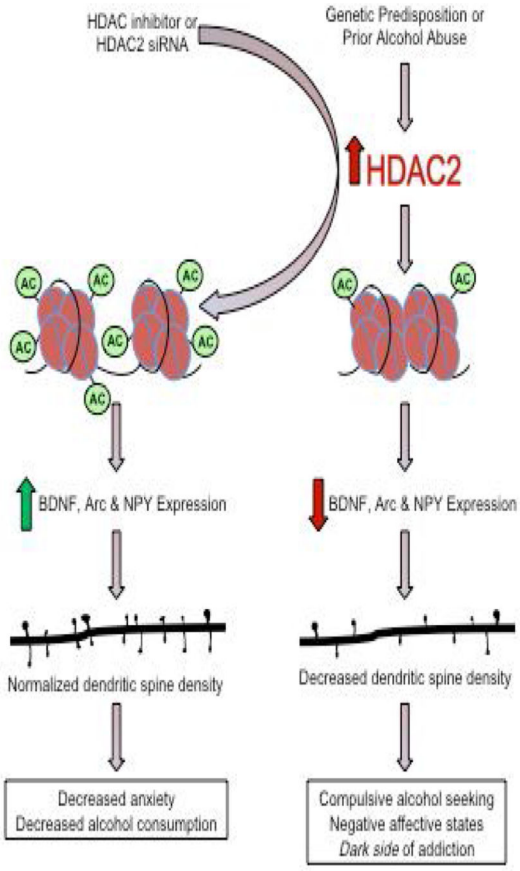

Figure 3. Reversal of the *dark side* of addiction by inhibition of HDAC2

Increased expression of histone deacetylase isoform 2 (HDAC2) and/or higher HDAC activity can be caused either by a genetic predisposition (exemplified by alcohol-preferring P rats) or by prior alcohol abuse. Higher HDAC activity and subsequent deacetylation of histone H3 induces a condensed chromatin conformation around crucial synaptic plasticity genes such as BDNF, Arc and neuropeptide Y (NPY), leading to decreased expression in the amygdala. The resultant decrease in dendritic spine density causes the expression of negative affective states (anxiety) and compulsive drug-seeking behavior, collectively referred to as the “dark side” of addiction [5,6]. This process can be reversed by the inhibition of HDAC activity by HDAC2 siRNA infusion into central nucleus of amygdala (CeA) or by direct pharmacological inhibition (HDAC inhibitors), thereby increasing gene expression and dendritic spine density. Diagram is derived from published data from our laboratory [25,102,103,112].