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A review on genetic and epigenetic editing for substance use disorders

Abstract

Chronic alcohol and drug abuse and dependence, commonly known as alcohol and substance use disorders (AUD and SUDs), are a major burden on global health, taking thousands of lives annually. Existing pharmacological and behavioral interventions are not entirely effective and remission is difficult to achieve in chronic cases. The complexity of these disorders and the limitations of commonplace treatments highlights the dire need for novel strategies to target the neurobiological causes and consequences of long-term drug exposure. There is expansive evidence showing that drugs of abuse and alcohol can induce persistent epigenetic modifications, such as altered acetylation and methylation patterns in histones, dysregulated miRNA networks, and transcriptional shifts, all of which increase vulnerability to relapse in the brain's striatal and limbic circuits that regulate pleasure and dopamine release. Advances in gene editing technologies, namely CRISPR, offer the ability to erase the "molecular memory" of chronic drug abuse. This review synthesizes emerging and recent preclinical work showing how the activation or repression of genes relevant to addiction and pleasure pathways can reduce drug seeking behavior and withdrawal symptoms in mice. Overall, these findings demonstrate the potential of genetic and epigenetic editing to mitigate the neurobiological consequences of substance abuse and enable personalized or even preventative interventions.

Introduction

Substance use disorders are one of the leading causes of death in the world. In the US alone, there are an estimated 48.7 million individuals living with some type of substance abuse disorder (Substance Abuse and Mental Health Services Administration, 2023). These disorders are often chronic, and individuals are highly prone to relapse even after extended periods of treatment. The high overdose burden of these diseases, as well as the long-term health consequences of substance abuse, result in the high mortality associated with these conditions. Alcohol and drug use among young adults and adolescents is a particularly pressing issue, as early exposure increases lifetime risk of dependence and developing other psychiatric disorders (Hingson, Heeren & Winter, 2006). Existing treatments include pharmacotherapies and psychosocial interventions, yet long-term remission is difficult to achieve (McLellan et al., 2000). The existing therapeutic gap has been a strong motivator in the search for other interventions that can target the central neurobiological mechanisms of drug abuse.

Understanding the neurobiological processes and circuitry underlying addiction has shown that substance abuse disorders are characterized by maladaptive learning and plasticity in the cortico-striatal-limbic circuits, which include the ventral tegmental area, nucleus accumbens, amygdala, and prefrontal cortex. Repeated and long-term exposure to addictive substances can cause lasting changes in synaptic strength and excitability (Luscher & Malenka, 2011). At the molecular level, these changes are associated with epigenetic changes that shift gene-expression. GWAS and locus-specific studies have shown that drugs can alter acetylation and methylation patterns and modify non-coding RNA networks in key reward regions (Nestler, 2014). This epigenetic view of addiction shows that the long-term behavioral susceptibility to addiction is encoded in DNA and is not merely behavioral (Nestler, 2014; Robison & Nestler, 2011).

Recent work utilizing novel gene editing technologies such as CRISPR-Cas9 systems have been able to highlight the possibility of reversing the epigenetic changes associated with substance use exposure. These preclinical studies highlight the possibility of interventions that can "rewrite" the molecular remnants of addiction, opening the door for a new variety of treatments. In this review, I synthesize emerging evidence on potential gene and epigenome editing for substance use disorders, and potential for future research.

Review

The epigenetic changes associated with drug abuse

First, it is important to establish the effects of substance abuse on the genome and the epigenome. All drugs of abuse, including alcohol, result in changes in miRNA expression, and subsequently, the downstream targets and processes (Smith & Kenny, 2018). Substance abuse mostly results in regulatory changes; chronic alcohol and cocaine use has been shown to induce changes in the regulatory regions of the *FosB*, *Cdk6*, and *Bdnf* genes (Nielsen et al., 2012). Addiction has also been shown to induce epigenetic alternations, and epigenetic alterations can themselves induce addiction (Hamilton & Nestler, 2019).

Studies of the superior prefrontal gyrus in the human postmortem brain tissue shows an upregulation of 48 miRNAs in patients with chronic alcohol abuse disorder (Lewohl et al., 2011). One well-characterized example is the downstream effects of alcohol use disorder on the regulation of miRNA activity. Chronic alcohol exposure increases miR-9 levels in central neurons, but only one of three BK channel splice variants contains an miR-9 recognition element (MRE). This results in the degeneration of the remaining miRNA, causing the reorganization of the BK splice variant. This brings about a shift of the BK channel isoforms to variants that are resistant to miR-9, and thus less sensitive to alcohol. The adaptive reorganization of BK splice variants in response to higher alcohol exposure contributes to the molecular pathways of alcohol tolerance (Pietryzkowski et al., 2008).

Similarly, studies on cancer patients who receive long-term opioid treatments for pain management have shown higher *OPRM1* promoter methylation, reducing *OPMR1* (mu-opioid receptor) expression, contributing to higher opioid tolerance (Viet et al., 2017). Experimental evidence by Liu al. (2021) shows that the targeted re-expression of the mu-opioid receptor on cancer cells using viral delivery of *OPRM1* can inhibit thermal and mechanical hypersensitivity and prevent opioid tolerance in the treated mice. This has profound implications for cancer and other chronic pain management; if reversing the hypermethylation caused repression of *OPRM1* is made possible using gene editing technologies, the need for administering higher doses of opioids is reduced.

The idea of inducing epigenetic changes for the treatment of neuropsychiatrist disorders is not new to gene editing technologies. FDA approved drugs for substance abuse and withdrawal treatment have been shown to induce epigenetic effects, demonstrating the possibility of direct epigenetic and genetic modification for the treatment of SUDs. For instance, Sulpiride and Clozapine have been reported to promote DNA demethylation in loci involved in dopaminergic and glutamatergic signaling, both of which are central to reward and pleasure pathways. Clozapine also induces acetylation of histone H3 at lysine 9 (H3K9ac) in PFC. This modification is associated with the elevated transcriptional activation of genes involved in neuroplasticity and antipsychotic response (Koijam et al., 2024).

CRISPR-based epigenetic and transcriptional modulation

Carpenter et al. (2020) used CRISPR-Cas9 to activate and repress Nr4a1 in vivo in mice. sgRNA-Nr4a1 and dCas9-CP64 were contralaterally injected in the Nucleus Accumbens. They used KRAB, a transcriptional repressor, and VP64, a transcriptional activation domain to either suppress or activate Nr4a1 activity. Co-transfection of sgRNA-*Nr4a1* alongside dCas9-KRAB repressed Nr4a1 activity at 4-days post-transfection. CRISPR-induced activation of this gene using dCas9-VP64 depleted H3K27me3 levels and increased H3K27ac levels. sgRNA-*Nr4a1* and dCas9-VP64 treated mice with higher Nr4a1 activation levels showed a lower preference for cocaine compared to the treatment. Another cohort of cocaine-conditioned treated mice also showed a reduced conditioned place preference activity and higher *Cartpt (cocaine and amphetamine regulated transcript peptide)* activation, which is activated with Nr4a1 binding and is associated with reduced reward-seeking and is expressed at lower levels during abstinence. Conversely, CRISPR-treated mice with attenuated levels of Nr4a1 activation showed increased preference for the cocaine chamber. Overall, the results showed that CRISPR-induced activation or repression of the Nr4a1 gene can suppress or increase affinity for cocaine in treated mice, respectively. This has profound implications for the future of using CRISPR-Cas9 and single-gene targeting to reverse or reduce cocaine cravings for those in abstinence.

Bohnsack et al. (2017) used CRISPR dCas9-300 to target the histone acetylation of the *Gabra1* promoter to prevent the decrease of *Gabra1* expression. Ethanol exposure has been shown to decrease *α1* expression and *Gabra1* transcription in neuronal cultures. Similar patterns have been observed in vivo (Devaud et al., 1995). *α1* protein expression is believed to be the underlying mechanism for GABA_a-R hypofunction and alcohol withdrawal in rodents (Devaud et al., 1995). p300 is an acetyltransferase that catalyzes the transfer of acetyl groups to histone H3 and H4 lysine residues. This removal of acetyl groups “opens” the chromatin structure and elevates transcriptional activation. Thus, using specific sgRNAs alongside p300 would increase acetylation levels in *Gabra1*, or prevent the deacetylation that alcohol exposure induces. The researchers used dCas9-P300 to target the *Gabra1* promoter using lentiviruses, and observed a 350% increase in histone acetylation in the promoter region and saw marked reductions in the alcohol-induced deacetylation in *Gabra1*. These findings indicate that locus-specific restoration of histone acetylation patterns can result in reversions to original expression patterns, offering support for targeted epigenetic editing to treat alcohol dependence and tolerance.

Bohnsack et al. (2022) also investigated targeted epigenomic editing for adult anxiety and excessive drinking in alcohol-exposed mice. Histone 3 lysine 27 acetylation (H3K27ac) has been found to be associated with active enhancers in alcohol exposure and drinking. Alcohol exposure during adolescence lowers *Arc* expression in the amygdala, which is part of the pathway regulating alcohol abuse and anxiety. This likely happens through the deposition of H3K27 trimethylation, removal of H3k27ac, increased EZH2 activity, and reduced levels of histone demethylase KDM6B and CREB-binding protein levels at the *Arc* SARE enhancer (Bohnsack et al., 2022; Pandey et al., 2008). The researchers used 235 bp of the rat *Arc* SARE region as a template to design sgRNA binding sites. They used CRISPR-dCas9 to increase and decrease histone acetylation and methylation at the *Arc* SARE site. They hypothesized that restoring normal acetylation patterns at the *Arc* SARE site using dCas9-P300 would reduce excessive drinking and anxiety in alcohol induced mice. Four sgRNAs were used to target the *Arc* SARE site using lentivirus. They found increased *Arc* mRNA and increased H3K27ac occupancy and no off-target effects at the predicted loci. The treated mice showed lower anxiety and alcohol use.

They found that dCas9-KRAB infusion into the amygdala can increase H3K27me levels at the *Arc* SARE site, reduce *Arc* expression, and increase anxiety symptoms and alcohol consumption in alcohol-treated adult mice. Overall, their results indicate that targeting the methylation levels in the *Arc* SARE site can bidirectionally alter alcohol abuse disorder symptoms in adult mice.

Kong, Wu & Xu (2021) exploit the fact that GLP1 receptors can mediate pleasure pathways to develop skin grafts for attenuating the activation of reward pathways in response to stimulants and alcohol. GLP1s are mainly known for their ability to moderate appetite, satiety, and glucose levels, but they are also present in the brain reward circuits in the VTA and the NAc (Alves et al., 2025). GLP1s or GLP1 analogs can suppress mesolimbic DA transmission or signaling in response to food, but they can also modulate drug reward circuitry that are activated in response to alcohol stimulants such as cocaine. The authors developed a skin stem cell-based gene delivery approach that lengthens the short in-vivo half-life of GLP1s to transplant CRISPR-Cas9 engineered cells using skin grafts. They developed skin grafts to express BChE, which is an enzyme that hydrolyzes acetylcholine and metabolizes cocaine. In mice who received BChe-expressing skin grafts, they found lower DA levels in the NAc and lower cocaine-taking and cocaine-induced drug seeking behaviors. They found similar effects for alcohol intake, observing that GLP1 expression in the grafted skin can reduce alcohol seeking behavior in mice. They also administered a skin graft that expressed both GLP1 and hBChE to degrade cocaine faster and lower alcohol seeking. This gene cocktail showed to be effective in treating polysubstance abuse and resulted in lower toxicity.

Lardner et al. (2021) investigated the possibility of genetically targeting transcription patterns of *FosB* using CRISPR. *FosB* encodes the transcription factor *FosB*, which is mainly present in the nucleus accumbens (NAc) and has been established to control gene transcription and behavior after cocaine and opioid exposure. Both natural (sucrose, drinking) and unnatural rewards (drugs of abuse) induce the accumulation of a truncated highly stable splice variant, *ΔFosB* accumulation in D1 and D2 expressing medium spiny neurons in the NAc. The researchers used a CREB mimicking molecule, which is a transcription factor upstream of *FosB* and induces *ΔFosB* induction in the nucleus accumbens after cocaine exposure, and fused it to dCas9 and used it alongside gRNA-11 (gRNA-*Fosb*) to selectively target the *FosB* promoter. They find that dCas9-CREB can significantly increase *ΔFosB* expression both in vivo and ex vivo. These results imply the potential of using targeted epigenetic editing to modulate long-term plasticity pathways.

Limitations and future research

Furthermore, it is important to note that susceptibility to SUD is not entirely environment dependent and uniform across individuals. Family studies show that addiction is among the most heritable psychiatric diseases, with genetic factors accounting for ~50% of the variance in substance abuse disorders (Deak & Johnson, 2021). GWAS studies have identified specific loci that contribute to this vulnerability, namely alcohol-metabolizing enzymes such as ADH1B and ALDH2, mu receptor gene *OPRM1* for opioid use disorder, as well as other loci that have joint effects for depressive and stimulant substances (Gaddis et al., 2022; Hibberd et al., 2020). Although the majority of these conditions are polygenic and the genes are pleiotropic, these identifications raise the possibility that in the future, preventative strategies could be developed for those with a strong susceptibility for severe addiction.

Nevertheless, in spite of the promising early results of epigenetic and genetic treatments for SUDs in animal models, the execution of these therapies in humans is not without challenge. The blood-brain barrier poses a significant challenge regarding the administration of these therapies. Although there have been significant recent advancements in terms of novel CRISPR delivery methods to selectively permeate the BBB (Zou et al., 2022), this remains a central challenge for targeting the CNS.

Lastly, although beyond the scope of this review paper to be analyzed in depth, the ethical considerations associated with genetic and epigenetic editing for the treatment of substance abuse disorders are paramount. First, many editing techniques can have bidirectional effects, raising concerns about potentially increasing susceptibility to addiction. Moreover, these therapies target the NAc and other central brain pathways that mediate naturally-occurring forms of pleasure, and the genetic and epigenetic editing of these regions could have profound implications for pleasure processing. Lastly, genetic editing raises questions of moral hazard. An argument put forth by Greg Stevens, Professor of Pharmacology at Oklahoma State University, created a discourse on the ethical implications of gene editing for SUDs. He argues that genetic editing of the mu-opioid receptor system to reduce risk of fatal overdose from opioids while preserving the hedonic and analgesic functions could be a reasonable and ethically justified use of genetic editing technologies for harm reduction (Stevens, 2022). However, although such interventions could in theory save millions of lives from opioid overdose, they also open the possibility of taking away perhaps the strongest deterrent of drug abuse, resulting in increased consumption.

Conclusion

The vast and diverse body of literature reviewed in this paper demonstrates the potential to accurately identify the mechanisms of substance use disorders and treat them using epigenetic and transcriptional interventions. Substance abuse disorders are characterized by maladaptive neuroplasticity and drug-induced epigenetic changes to the reward circuitry. This offers the opportunity to reverse the drug-induced changes to mitigate withdrawal symptoms. Gene and epigenome editing technologies, mainly CRISPR, offer a unique tool to target the molecular traces of chronic drug abuse. Locus-specific manipulation of genes established to be relevant for addiction and pleasure, namely *Nr4a1, Gabra1, Arc, FosB*. Moreover, even GLP1/BChE pathways have been shown to induce long-term downstream changes in rodent models that were previously exposed to alcohol and cocaine. Notably, the treated mice showed attenuated cocaine and alcohol preference, lower anxiety-like behaviors associated with alcohol withdrawal, and even lower tolerance in response to continuous opioid treatment. Collectively, these results indicate that CRISPR, and potentially even more precise editing technologies, can be a powerful therapeutic tool in treating a condition that takes thousands of lives annually around the world. Nevertheless, any clinical translations of existing research must take into consideration the limitations of penetrating the BBB, the polygenic and pleiotropic nature of addiction, reward, and mood pathways, as well as the profound and far-reaching implications of any potential off-target effects. Ultimately, although using genetic and epigenetic editing to treat and potentially prevent substance abuse disorders is still a novel idea, preclinical evidence shows promising results for future translational and clinical research and eventual treatments.

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