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Epigenetics and Addiction

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Abstract

As an individual becomes addicted to a drug of abuse, nerve cells within the brain's reward circuitry adapt at the epigenetic level during the course of repeated drug exposure. These drug-induced epigenetic adaptations mediate enduring changes in brain function which contribute to life-long, drug-related behavioral abnormalities that define addiction. Targeting these epigenetic alterations will enhance our understanding of the biological basis of addiction and might even yield more effective anti-addiction therapies. However, the complexity of the neuroepigenetic landscape makes it difficult to determine which drug-induced epigenetic changes causally contribute to the pathogenic mechanisms of drug addiction. In this review, we highlight the evidence that epigenetic modifications, specifically histone modifications, within key brain reward regions are correlated with addiction. We then discuss the emerging field of locus-specific neuroepigenetic editing, which is a promising method for determining the causal epigenetic molecular mechanisms that drive an addicted state. Such approaches will substantially increase the field's ability to establish the precise epigenetic mechanisms underlying drug addiction, and could lead to novel treatments for addictive disorders.

Epigenetic modifications in addiction

The neuroepigenetic landscape

The term 'epigenetics' is commonly used to describe the dynamic molecular modifications deposited upon chromatin within a cell's nucleus, which has the functional consequence of regulating DNA-related processes, such as DNA repair, chromatin organization, and RNA transcription and splicing, among other functions. Drug addiction researchers have become interested in studying epigenetics due to the fact that an individual's experience, specifically volitional, repeated drug consumption, alters the chromatin landscape within the brain in a region- and cell type-specific manner¹. It is widely hypothesized that, by regulating DNA-related processes, these drug-induced epigenetic alterations contribute to aberrant cellular function that drives drug addiction pathogenesis. There may, therefore, be therapeutic potential in targeting key drug-induced epigenetic modifications within the brain as a way of

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combating an individual's spiral into an addictive state. However, to achieve this goal, it is vital to first untangle the remarkable complexity of addiction-related epigenetics to determine which specific, drug-induced epigenetic modifications casually contribute to distinct aspects of the pathophysiological maladaptations underlying drug addiction. The burgeoning field of locus-specific neuroepigenetic editing is uniquely well suited to pursue this goal and is the focus of this review.

To contextualize the complexity of the neuroepigenetic state, it is important to understand the diversity of epigenetic modifications. For simplicity, we concentrate here on histone post-translational modifications (PTMs), but other epigenetic regulatory events include DNA modifications and the actions of non-coding RNAs². Chromatin is the macromolecular complex consisting of DNA wrapped tightly around histone protein octamers to form nucleosomes. Chromatin enables the dense packing of nucleosomes to fit within the cell's nucleus, and provides an instructive scaffold that is responsive to external cues. Histones are highly basic and enriched for lysine and arginine residues. PTMs of these and other residues on histone N-terminal tails, which protrude from the nucleosome core, alter the steric properties and charge distribution of chromatin, controlling DNA-related processes. Histone subunits can be modified by numerous PTMs including acetylation, methylation, phosphorylation, ADP ribosylation, ubiquitylation, and sumoylation, among a growing list of newly discovered modifications, which occur on >50 distinct sites²⁻⁴. The diversity of modifications, and the innumerable sites multiplied across the ~2 meters of linear DNA within a given cell, underscore the vast complexity of the epigenetic landscape.

Histone PTMs are reversible: they are dynamically deposited by "writer" enzymes, recognized by "reader" proteins which mediate the cellular response, and removed by "eraser" enzymes². It is the delicate balance between the function of writers and erasers that dictates the global epigenetic state and downstream functions within a given brain cell. The expression and function of numerous writers, erasers, and readers have been observed to be altered both in addicted humans and in animal models of addiction^{1,5,6}. Restoring normal function to these proteins through the use of small molecules represents a novel area for anti-addiction pharmacotherapies⁷, yet understanding the proteins to target, and how their regulation would affect the broader addictive phenotype, remains inadequately explored.

Drug experience alters the neuroepigenetic landscape

Drug addiction is characterized by the compulsive seeking and taking of drug despite adverse consequences⁸. Vulnerability to addiction emerges through approximately equal parts of genetic predisposition and environmental risk, strongly suggesting an important role for epigenetic mechanisms. All abused drugs target the mesolimbic dopamine circuitry⁹, which serves the evolutionary purpose of reinforcing activities important for an individual's survival and reproduction, such as seeking palatable food and sex¹⁰. The mesolimbic circuitry is composed of dopaminergic neurons in the midbrain ventral tegmental area (VTA) and their innervation of medium spiny neurons (MSNs), the predominant cell type within the nucleus accumbens (NAc)¹¹. All natural rewards and abused drugs share the property of acutely increasing dopaminergic neurotransmission in NAc¹². Chronic drug use induces long-lasting structural, electrophysiological, and transcriptional changes in this region,

which are considered the sustained biological substrates of addiction (Figure 1). For this reason, researchers are examining epigenetic maladaptations within NAc cell types as driving addiction pathogenesis¹³.

The functional consequence of histone PTMs within NAc is well illustrated by histone acetylation. This PTM occurs on all core histone subunits, is catalyzed by histone acetyltransferases (HATs), and is reversed by histone deacetylases (HDACs). Histone acetylation is typically associated with an ‘opening’ of higher order chromatin structure¹⁴—that is, increased spacing between nucleosomes—which facilitates transcription activation by making the surrounding DNA more accessible to transcription factors and the transcriptional machinery. In support of the importance of histone acetylation in drug addiction, acute or chronic exposure to psychostimulants, opiates, alcohol, or nicotine all serve to increase total cellular levels of acetylation of histones H3 and H4 in NAc^{15–20}. This increase in total acetylation levels is the net result of drug-induced alterations to the balance of HAT and HDAC function. cAMP response element-binding protein (CREB)-binding protein (CBP), a HAT critical to learning and memory²¹, is required for cocaine-induced increases in histone acetylation in the NAc²². Conversely, chronic cocaine²³ or alcohol²⁴ use reduces HDAC enzymatic activity and disrupts sub-cellular HDAC localization²⁵. Further, while chronic cocaine exposure promotes an increase in NAc expression of sirtuins²⁶, class III HDACs, the genomic accessibility, particularly within gene promoters, is reduced²⁷. This limits the capacity of this enzyme to reduce histone acetylation, specifically at key gene sites.

Indeed, the deposition of histone acetylation that occurs in NAc in addiction models is highly locus specific. For instance, in response to acute psychostimulant exposure, H4 acetylation occurs within the promoters of immediate early genes like *c-Fos* and *Fosb*, which associates with their rapid expression in response to drug consumption^{15,28}. For *Fosb*, this increase in histone acetylation requires CBP²⁹, indicating gene-specific actions of these chromatin modifiers upon drug-experience.

The biological relevance of chromatin modifiers to drug-related behavior is further strengthened by NAc-targeted viral or genetic manipulations. Viral over-expression of the transcription factor CREB reduces the rewarding properties of cocaine³⁰ and morphine³¹, indicating that CREB activation may be a general mechanism for reward tolerance. Conversely, viral over-expression of FosB promotes the rewarding properties of cocaine³² and morphine³³, revealing how transcriptional regulators can oppositely impact drug-related behaviors. Further, genetic deletion of the HAT CBP impairs cocaine sensitivity and cocaine-related memory²², and a sizeable body of work demonstrates that direct manipulations to HDACs alter behaviors related to multiple classes of abused substances^{23,28,34,35}. Manipulation of enzymes that control histone or DNA methylation also control behavioral responses to drugs of abuse^{36,37}. As well, viral delivery of accessory subunits to chromatin remodeling proteins alters drug-related behaviors³⁸. Taken together, these direct manipulations to chromatin modifiers indicate that the actions of these factors contribute to addiction pathogenesis. However, it remains unclear whether these and many other chromatin modifications are required, causal events in driving key transcriptional adaptations, or if they are simply correlated with such regulation. This has remained a

challenging question, since all pharmacological, viral, and genetic tools manipulate histone PTMs genome-wide. The advent of locus-specific neuroepigenetic editing within specific brain cell types has enabled researchers to make crucial advances in establishing the casual consequences of gene-specific PTMs within the context of drug addiction.

Determining causality with locus-specific neuroepigenetic editing

The introduction of easy-to-use, artificial fusion proteins has launched the new field of *in vivo* neuroepigenetic editing in brain, making it possible for the first time to explore the causal epigenetic mechanisms driving drug addiction. The long-term goal of this research is to distill which drug-induced epigenetic marks contribute most prominently to the deleterious pathogenic mechanisms of addiction, and to use this knowledge to guide the design of novel therapies to correct these maladaptations with minimal off-target effects. In theory, this approach could yield superior addiction treatments with the capacity to halt or even reverse the pathogenesis of an addicted state.

Neuroepigenetic editing refers to the targeted rewriting of the epigenome at a single genomic locus within a given neuronal or other cell type in a given brain region. The ability to accomplish this novel approach is built upon the fields of gene editing and epigenome engineering, which have enjoyed a renaissance within the past eight years, but have been applied almost exclusively *in vitro*^{39–43}. The much more recent ability to adapt these technologies in awake, behaving animals has allowed for key advances in studying the causal epigenetic mechanisms underlying neuropsychiatric syndromes^{44–46}.

DNA-binding domains

Neuroepigenetic editing is accomplished through the expression of bi-functional constructs consisting of a programmable, DNA-binding domain that binds a desired genomic locus with high affinity and specificity, and an effector moiety, which mediates an epigenetic modification confined exclusively to the chromatin landscape proximal to DNA-binding (Figure 2). The first used DNA-binding domains were zinc finger proteins (ZFPs)^{47,48}, derived from eukaryotic transcription factors, or transcription activation-like effectors (TALEs)^{49,50}, derived from plant pathogenic prokaryotes. Fusing either of these DNA-binding domains to effector moieties allowed researchers to demonstrate the feasibility and utility of epigenetic editing for a variety of neuroscience research-related approaches^{51–56}. However, as ZFPs and TALEs utilize protein-DNA interactions to mediate DNA-targeting, the synthesis and validation of these tools are time-consuming, expensive, and technically challenging, which has limited their application.

The more recent development of CRISPR/dCas9-based eukaryotic genome targeting has radically simplified the design and synthesis of DNA-targeting domains⁵⁷. In contrast to ZFP- and TALE-based platforms, CRISPR/dCas9 DNA-targeting is mediated by programming a 20 bp sequence within a single guide RNA (sgRNA) (see Figure 2). The sgRNA complexes with nuclease-deficient dCas9 and targets the complex to the genomic region where the guide sequence binds its complementary DNA sequence⁵⁸. Tethering an effector moiety to the sgRNA or, more commonly, to dCas9 allows for DNA-targeted localization of a desired effector. The ease and cost-effectiveness of design and synthesis of

the CRISPR/dCas9 platform has helped usher in a new era of epigenome engineering research.

Effector moieties

Shortly after applying programmable DNA-binding domains for genome editing, researchers began fusing to transcriptional activator domains as a means of inducing expression of targeted endogenous genes. One of the first, and most effective, effector moieties was VP64, derived from herpes simplex virus (HSV), which directly recruits RNA polymerase II for gene-targeted transcriptional activation⁵⁹. VP64 does not directly mediate epigenetic reprogramming, although permissive chromatin remodeling is associated with its function^{60,61}; rather, VP64 is a scaffold for recruiting gene-activating transcriptional machinery⁶². By targeting VP64 to the promoter region of a single gene with ZFP-⁶³, TALE-^{60,64}, or CRISPR/dCas9-^{60,65} DNA-binding domains, laboratories have consistently reported the ability to induce transcription of targeted genes *in vitro* and even *in vivo*^{61,66}.

Many laboratories have pursued improvements to the magnitude of gene activation, which is accomplished by concentrating effector moieties at a single genomic region to induce a proportional increase in gene activation. One approach was termed SunTag, a peptide scaffold containing repeating binding sites for effector domains like VP64⁶⁷. Another strategy was termed CRISPR-SAM, wherein the sgRNA is re-engineered at protruding RNA loops with RNA aptamers to serve as a scaffold for recruitment of activation domains⁴². A third strategy to concentrate effector domains is to target multiple DNA-binding sites within a given promoter region simultaneously⁵⁷. These approaches are useful for *in vitro* assays where large magnitude effects are often desired, yet can be prohibitively complex when attempting to deliver the multiple components to targeted cells within the brain.

To achieve bi-directional control of gene expression, repression of an endogenous gene is most universally accomplished with Kruppel-associated box (KRAB) effector moieties. KRAB, a transcriptional repression domain found in human zinc finger transcription factors, recruits heterochromatin-forming complexes, which in turn deposit repressive H3K9me3 marks and represses transcription⁶⁸. Like VP64, the KRAB domain does not possess enzymatic activity, but instead recruits secondary factors to edit the epigenome. Promoter- or enhancer-targeted KRAB has been utilized in cell culture and in brain^{69,70}.

While these methods of bi-directional regulation are more physiologically-relevant than conventional transgenic overexpression or knockout approaches, directly regulating genes in this manner lacks the construct validity of replicating the actual aberrant molecular mechanisms present within the addicted state. Indeed, decades of research have revealed the specific transcription factors and epigenetic writers, readers, and erasers implicated in the pathogenesis of drug addiction. In the past few years, there has been an explosion in the use of effector moieties for neuroepigenetic editing. These include: DNA-modifying enzymes like DNMT3a (which methylates CpG)^{71,72} and TET1 (which catalyzes hydroxymethyl-CpG)^{73,74}; proteins that control histone PTMs including: NFκB subunit p65 (which recruits HATs to acetylate core histones)⁵²⁻⁵⁴, histone methyltransferase G9a (which catalyzes H3K9me2)⁵²⁻⁵⁴, p300 HAT (which acetylates all four core histones)⁴³, SID (which recruits HDACs)^{75,76}, LSD1 (which demethylates H3K4 and H3K9)⁷⁷, PRDM9 (which methylates

H3K4 and H3K36)⁷⁸, and DOT1L (which methylates H3K79)^{79,80}, and even transcription factors like CREB—thus mimicking a known mechanism of transcriptional regulation⁸¹. The modularity of designing neuroepigenetic editing constructs presents the ability to apply a wide range of effector domains for epigenome editing purposes that more closely models the molecular mechanisms underlying drug addiction.

Utility in studying drug addiction

Neuroepigenetic editing approaches present several advantages over conventional transgene overexpression or knockout approaches, including controlling the expression of the targeted gene from its endogenous promoter (potentially via physiologically relevant mechanisms depending on the effector moiety utilized), as well as controlling the magnitude of gene expression levels within physiological ranges. These advantages justify the more widespread use of these approaches, particularly in researching neuropsychiatric syndromes like drug addiction. However, the major hurdle for applying neuroepigenetic editing to studies of addiction is the challenge in delivering these tools to the brain of awake, behaving animals. Delivery of such constructs to intact tissues is an active area of investigation for both research and therapeutic purposes⁸². To date, scientists have applied several approaches for *in vivo* brain delivery, most involving injection of viral vectors via stereotaxic surgery (Figure 3).

Viruses like adeno-associated virus (AAV) are challenging to use for neuroepigenetic editing owing to their limited packaging capacity (~4.5 kb). The most commonly utilized Cas9, derived from *Streptococcus pyogenes*, is ~4.1 kb⁸³. Fusing even the smallest effector moiety pushes it above the AAV packaging limit. While other Cas9 species are ~1 kb shorter (e.g., *Staphylococcus aureus*)⁸⁴, most researchers have elected to utilize viruses with larger packaging capacities. The most widely applied viruses are HSVs, which mediate short-lived transgene expression (up to 7 days) with a large packaging capacity (~14 kb), or lentivirus, which mediates long-term transgene expression of >6 months with a packaging capacity of 8-10 kb. Figure 3 compares the features of these viral delivery methods. Importantly, incorporation of loxP sites within these vectors, combined with the use of Cre-driver lines, makes it possible to target neuroepigenetic editing constructs to specific types of neurons or other cell types within a brain region of interest^{54,55}.

There are now several published examples of utilizing locus-specific neuroepigenetic editing to demonstrate that a single type of histone PTM (i.e., histone acetylation or methylation) at a single gene promoter in a single brain region or even cell type within that region alters expression levels of that gene and induces downstream behavioral effects in drug addiction and related models⁵²⁻⁵⁶. The first demonstration came in 2014 when Heller *et al.*⁵² designed a suite of ZFPs that bound the promoter region of the *FosB* gene and were fused to either G9a or p65. These constructs were packaged in HSVs and delivered to the NAc of mice via stereotaxic surgery. Depending on promoter localization and the effector moiety, these *FosB*-ZFPs deposited the specific histone PTM (methylation with G9a, acetylation with p65) proximal to their DNA-binding site and consequently bi-directionally regulated NAc levels of mRNA and protein expression of FosB, and the splice variant FosB—whose accumulation in this brain region promotes behavioral responses to cocaine and other drugs

of abuse, as mentioned earlier. The authors demonstrated further that such deposition of PTMs and regulation of *FosB* expression occurred solely at the *FosB* locus, and that *FosB*-G9a decreased, whereas *FosB*-p65 increased, the locomotor behaviors associated with repeated cocaine exposure. This provided an authoritative demonstration that the epigenetic state at the *FosB* locus and subsequent NAc accumulation of FosB is necessary and sufficient to modulate cocaine-evoked behaviors. Heller, Hamilton *et al.* 2016⁵³ built on these approaches by directing ZFP tools to *Cdk5*, a locus epigenetically regulated by chronic cocaine exposure, and demonstrating that both the rewarding and locomotor properties of cocaine were dependent on the epigenetic state of *Cdk5*. These studies reveal that epigenetic regulation at the *FosB* and *Cdk5* loci are not merely correlative, but actually causal molecular adaptations that drive the pathogenesis of addictive behaviors, and establish more generally that manipulation of a single type of PTM at a single locus in a single brain region controls the expression level of the targeted gene and downstream behavioral outcomes. While not yet applied to study addiction, CRISPR-based neuroepigenetic editing approaches have been used in targeted brain regions and have proved fruitful for exploring other brain illnesses^{73,74,81,85}, supporting their utility to study addiction. These findings establish that epigenetic modifications at a given locus can in and of themselves mediate transcriptional regulation in fully differentiated adult neurons *in vivo*, and these approaches enable a form of *in vivo* manipulation that was previously unattainable.

Conclusions and future directions

The ability to deposit or remove specific epigenetic marks at restricted gene sites within targeted cell types in key brain regions provides researchers with the unparalleled ability to experimentally interrogate the causal contribution of these epigenetic molecular mechanisms to neuropsychiatric syndromes, like addiction. The modularity and flexibility of neuroepigenetic editing approaches make them ideal and adaptable tools to untangle *in vivo* epigenetic complexity. It is theoretically possible, for example, to use CRISPR/dCas9 to target multiple genes within the same cells and thereby experimentally manipulate networks of drug-regulated genes. By combining these approaches with cutting-edge animal models of addiction, like drug self-administration, researchers will be able to isolate the specific epigenetic maladaptations that drive the most damaging elements of drug addiction, like bingeing, craving, and relapse. These novel insights will guide the development of targeted, and likely more effective, pharmacotherapies in an effort to alleviate the human suffering associated with drug addiction.

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References

References of interest:

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1. Robison AJ & Nestler EJ Transcriptional and epigenetic mechanisms of addiction. *Nature reviews Neuroscience* 12, 623–637, doi:10.1038/nrn3111 (2011). [PubMed: 21989194]
2. Allis CD & Jenuwein T The molecular hallmarks of epigenetic control. *Nature reviews. Genetics* 17, 487–500, doi:10.1038/nrg.2016.59 (2016).
3. Bowman GD & Poirier MG Post-translational modifications of histones that influence nucleosome dynamics. *Chemical reviews* 115, 2274–2295, doi:10.1021/cr500350x (2015). [PubMed: 25424540]
4. Bannister AJ & Kouzarides T Regulation of chromatin by histone modifications. *Cell research* 21, 381–395, doi:10.1038/cr.2011.22 (2011). [PubMed: 21321607]
5. Rogge GA & Wood MA The role of histone acetylation in cocaine-induced neural plasticity and behavior. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 38, 94–110, doi:10.1038/npp.2012.154 (2013). [PubMed: 22910457]
6. Egervari G, Ciccocioppo R, Jentsch JD & Hurd YL Shaping vulnerability to addiction - the contribution of behavior, neural circuits and molecular mechanisms. *Neuroscience and biobehavioral reviews* 85, 117–125, doi:10.1016/j.neubiorev.2017.05.019 (2018). [PubMed: 28571877]
7. Kennedy PJ & Harvey E Histone Deacetylases as Potential Targets for Cocaine Addiction. *CNS & neurological disorders drug targets* 14, 764–772 (2015). [PubMed: 26022264]
8. Koob GF & Volkow ND Neurobiology of addiction: a neurocircuitry analysis. *The lancet. Psychiatry* 3, 760–773, doi:10.1016/S2215-0366(16)00104-8 (2016). [PubMed: 27475769]
9. Koob G & Kreek MJ Stress, dysregulation of drug reward pathways, and the transition to drug dependence. *The American journal of psychiatry* 164, 1149–1159, doi:10.1176/appi.ajp.2007.05030503 (2007). [PubMed: 17671276]
10. Kelley AE & Berridge KC The neuroscience of natural rewards: relevance to addictive drugs. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 22, 3306–3311, doi: 20026361 (2002). [PubMed: 11978804]
11. Hyman SE, Malenka RC & Nestler EJ Neural mechanisms of addiction: the role of reward-related learning and memory. *Annual review of neuroscience* 29, 565–598, doi:10.1146/annurev.neuro.29.051605.113009 (2006).
12. Diana M The dopamine hypothesis of drug addiction and its potential therapeutic value. *Frontiers in psychiatry* 2, 64, doi:10.3389/fpsy.2011.00064 (2011). [PubMed: 22144966]
- 13*. Feng J et al. Chronic cocaine-regulated epigenomic changes in mouse nucleus accumbens. *Genome biology* 15, R65, doi:10.1186/gb-2014-15-4-r65 (2014). [PubMed: 24758366]
14. Shogren-Knaak M et al. Histone H4-K16 acetylation controls chromatin structure and protein interactions. *Science* 311, 844–847, doi:10.1126/science.1124000 (2006). [PubMed: 16469925]
15. Kumar A et al. Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. *Neuron* 48, 303–314, doi:10.1016/j.neuron.2005.09.023 (2005). [PubMed: 16242410]
16. Shen HY et al. Additive effects of histone deacetylase inhibitors and amphetamine on histone H4 acetylation, cAMP responsive element binding protein phosphorylation and DeltaFosB expression in the striatum and locomotor sensitization in mice. *Neuroscience* 157, 644–655, doi:10.1016/j.neuroscience.2008.09.019 (2008). [PubMed: 18848971]
17. Schroeder FA et al. Drug-induced activation of dopamine D(1) receptor signaling and inhibition of class I/II histone deacetylase induce chromatin remodeling in reward circuitry and modulate cocaine-related behaviors. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 33, 2981–2992, doi:10.1038/npp.2008.15 (2008). [PubMed: 18288092]
18. Renthal W et al. Genome-wide analysis of chromatin regulation by cocaine reveals a role for sirtuins. *Neuron* 62, 335–348, doi:10.1016/j.neuron.2009.03.026 (2009). [PubMed: 19447090]
19. Levine A et al. Molecular mechanism for a gateway drug: epigenetic changes initiated by nicotine prime gene expression by cocaine. *Science translational medicine* 3, 107ra109, doi:10.1126/scitranslmed.3003062 (2011).

20. Botia B, Legastelois R, Alaux-Cantin S & Naassila M Expression of ethanol-induced behavioral sensitization is associated with alteration of chromatin remodeling in mice. *PloS one* 7, e47527, doi:10.1371/journal.pone.0047527 (2012). [PubMed: 23110077]
21. Barrett RM & Wood MA Beyond transcription factors: the role of chromatin modifying enzymes in regulating transcription required for memory. *Learning & memory* 15, 460–467, doi: 10.1101/lm.917508 (2008). [PubMed: 18583646]
22. Malvaez M, Mhillaj E, Matheos DP, Palmery M & Wood MA CBP in the nucleus accumbens regulates cocaine-induced histone acetylation and is critical for cocaine-associated behaviors. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 31, 16941–16948, doi:10.1523/JNEUROSCI.2747-11.2011 (2011). [PubMed: 22114264]
23. Renthall W et al. Histone deacetylase 5 epigenetically controls behavioral adaptations to chronic emotional stimuli. *Neuron* 56, 517–529, doi:10.1016/j.neuron.2007.09.032 (2007). [PubMed: 17988634]
24. Pandey SC, Ugale R, Zhang H, Tang L & Prakash A Brain chromatin remodeling: a novel mechanism of alcoholism. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 28, 3729–3737, doi:10.1523/JNEUROSCI.5731-07.2008 (2008). [PubMed: 18385331]
25. Taniguchi M et al. Histone deacetylase 5 limits cocaine reward through cAMP-induced nuclear import. *Neuron* 73, 108–120, doi:10.1016/j.neuron.2011.10.032 (2012). [PubMed: 22243750]
26. Ferguson D et al. Essential role of SIRT1 signaling in the nucleus accumbens in cocaine and morphine action. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 33, 16088–16098, doi:10.1523/JNEUROSCI.1284-13.2013 (2013). [PubMed: 24107942]
27. Ferguson D et al. SIRT1-FOXO3a regulate cocaine actions in the nucleus accumbens. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 35, 3100–3111, doi:10.1523/JNEUROSCI.4012-14.2015 (2015). [PubMed: 25698746]
28. Renthall W et al. Delta FosB mediates epigenetic desensitization of the c-fos gene after chronic amphetamine exposure. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 28, 7344–7349, doi:10.1523/JNEUROSCI.1043-08.2008 (2008). [PubMed: 18632938]
29. Levine AA et al. CREB-binding protein controls response to cocaine by acetylating histones at the fosB promoter in the mouse striatum. *Proceedings of the National Academy of Sciences of the United States of America* 102, 19186–19191, doi:10.1073/pnas.0509735102 (2005). [PubMed: 16380431]
30. Carlezon WA Jr. et al. Regulation of cocaine reward by CREB. *Science* 282, 2272–2275 (1998). [PubMed: 9856954]
31. Barrot M et al. CREB activity in the nucleus accumbens shell controls gating of behavioral responses to emotional stimuli. *Proceedings of the National Academy of Sciences of the United States of America* 99, 11435–11440, doi:10.1073/pnas.172091899 (2002). [PubMed: 12165570]
32. Grueter BA, Robison AJ, Neve RL, Nestler EJ & Malenka RC FosB differentially modulates nucleus accumbens direct and indirect pathway function. *Proceedings of the National Academy of Sciences of the United States of America* 110, 1923–1928, doi:10.1073/pnas.1221742110 (2013). [PubMed: 23319622]
33. Zachariou V et al. An essential role for DeltaFosB in the nucleus accumbens in morphine action. *Nature neuroscience* 9, 205–211, doi:10.1038/nn1636 (2006). [PubMed: 16415864]
34. Li X et al. Role of Dorsal Striatum Histone Deacetylase 5 in Incubation of Methamphetamine Craving. *Biological psychiatry* 84, 213–222, doi:10.1016/j.biopsych.2017.12.008 (2018). [PubMed: 29397902]
35. Wang L et al. Chronic cocaine-induced H3 acetylation and transcriptional activation of CaMKIIalpha in the nucleus accumbens is critical for motivation for drug reinforcement. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 35, 913–928, doi:10.1038/npp.2009.193 (2010). [PubMed: 20010550]
36. Maze I et al. Essential role of the histone methyltransferase G9a in cocaine-induced plasticity. *Science* 327, 213–216, doi:10.1126/science.1179438 (2010). [PubMed: 20056891]

37. LaPlant Q et al. Dnmt3a regulates emotional behavior and spine plasticity in the nucleus accumbens. *Nature neuroscience* 13, 1137–1143, doi:10.1038/nn.2619 (2010). [PubMed: 20729844]
38. Sun H et al. BAZ1B in Nucleus Accumbens Regulates Reward-Related Behaviors in Response to Distinct Emotional Stimuli. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 36, 3954–3961, doi:10.1523/JNEUROSCI.3254-15.2016 (2016). [PubMed: 27053203]
39. Cong L et al. Multiplex genome engineering using CRISPR/Cas systems. *Science* 339, 819–823, doi:10.1126/science.1231143 (2013). [PubMed: 23287718]
40. Jinek M et al. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 337, 816–821, doi:10.1126/science.1225829 (2012). [PubMed: 22745249]
41. Gilbert LA et al. CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. *Cell* 154, 442–451, doi:10.1016/j.cell.2013.06.044 (2013). [PubMed: 23849981]
42. Konermann S et al. Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex. *Nature* 517, 583–588, doi:10.1038/nature14136 (2015). [PubMed: 25494202]
43. Hilton IB et al. Epigenome editing by a CRISPR-Cas9-based acetyltransferase activates genes from promoters and enhancers. *Nature biotechnology* 33, 510–517, doi:10.1038/nbt.3199 (2015).
44. Hamilton PJ, Lim CJ, Nestler EJ & Heller EA Neuroepigenetic Editing. *Methods in molecular biology* 1767, 113–136, doi:10.1007/978-1-4939-7774-1_5 (2018). [PubMed: 29524131]
45. Yang MG & West AE Editing the Neuronal Genome: a CRISPR View of Chromatin Regulation in Neuronal Development, Function, and Plasticity. *The Yale journal of biology and medicine* 89, 457–470 (2016). [PubMed: 28018138]
46. Savell KE & Day JJ Applications of CRISPR/Cas9 in the Mammalian Central Nervous System. *The Yale journal of biology and medicine* 90, 567–581 (2017). [PubMed: 29259522]
47. Klug A The discovery of zinc fingers and their applications in gene regulation and genome manipulation. *Annual review of biochemistry* 79, 213–231, doi:10.1146/annurev-biochem-010909-095056 (2010).
48. Gersbach CA, Gaj T & Barbas CF 3rd. Synthetic zinc finger proteins: the advent of targeted gene regulation and genome modification technologies. *Accounts of chemical research* 47, 2309–2318, doi:10.1021/ar500039w (2014). [PubMed: 24877793]
49. Boch J et al. Breaking the code of DNA binding specificity of TAL-type III effectors. *Science* 326, 1509–1512, doi:10.1126/science.1178811 (2009). [PubMed: 19933107]
50. Moscou MJ & Bogdanove AJ A simple cipher governs DNA recognition by TAL effectors. *Science* 326, 1501, doi:10.1126/science.1178817 (2009). [PubMed: 19933106]
51. Snowden AW, Gregory PD, Case CC & Pabo CO Gene-specific targeting of H3K9 methylation is sufficient for initiating repression in vivo. *Current biology : CB* 12, 2159–2166 (2002). [PubMed: 12498693]
- 52*. Heller EA et al. Locus-specific epigenetic remodeling controls addiction- and depression-related behaviors. *Nature neuroscience* 17, 1720–1727, doi:10.1038/nn.3871 (2014). [PubMed: 25347353]
53. Heller EA et al. Targeted Epigenetic Remodeling of the Cdk5 Gene in Nucleus Accumbens Regulates Cocaine- and Stress-Evoked Behavior. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 36, 4690–4697, doi:10.1523/JNEUROSCI.0013-16.2016 (2016). [PubMed: 27122028]
- 54**. Hamilton PJ et al. Cell-Type-Specific Epigenetic Editing at the Fosb Gene Controls Susceptibility to Social Defeat Stress. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 43, 272–284, doi:10.1038/npp.2017.88 (2018). [PubMed: 28462942]
55. Aleyasin H et al. Cell-Type-Specific Role of DeltaFosB in Nucleus Accumbens In Modulating Intermale Aggression. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 38, 5913–5924, doi:10.1523/JNEUROSCI.0296-18.2018 (2018). [PubMed: 29891732]
- 56*. Sase AS et al. Sex-Specific Regulation of Fear Memory by Targeted Epigenetic Editing of Cdk5. *Biological psychiatry*, doi:10.1016/j.biopsych.2018.11.022 (2018).

57. Qi LS et al. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell* 152, 1173–1183, doi:10.1016/j.cell.2013.02.022 (2013). [PubMed: 23452860]
58. Sander JD & Joung JK CRISPR-Cas systems for editing, regulating and targeting genomes. *Nature biotechnology* 32, 347–355, doi:10.1038/nbt.2842 (2014).
59. Hall DB & Struhl K The VP16 activation domain interacts with multiple transcriptional components as determined by protein-protein cross-linking in vivo. *The Journal of biological chemistry* 277, 46043–46050, doi:10.1074/jbc.M208911200 (2002). [PubMed: 12297514]
60. Polstein LR et al. Genome-wide specificity of DNA binding, gene regulation, and chromatin remodeling by TALE- and CRISPR/Cas9-based transcriptional activators. *Genome research* 25, 1158–1169, doi:10.1101/gr.179044.114 (2015). [PubMed: 26025803]
- 61**. Liao HK et al. In Vivo Target Gene Activation via CRISPR/Cas9-Mediated Trans-epigenetic Modulation. *Cell* 171, 1495–1507 e1415, doi:10.1016/j.cell.2017.10.025 (2017). [PubMed: 29224783]
62. Ingles CJ, Shales M, Cress WD, Triezenberg SJ & Greenblatt J Reduced binding of TFIID to transcriptionally compromised mutants of VP16. *Nature* 351, 588–590, doi:10.1038/351588a0 (1991). [PubMed: 1646402]
63. Stege JT, Guan X, Ho T, Beachy RN & Barbas CF 3rd. Controlling gene expression in plants using synthetic zinc finger transcription factors. *The Plant journal : for cell and molecular biology* 32, 1077–1086 (2002). [PubMed: 12492848]
64. Crocker J & Stern DL TALE-mediated modulation of transcriptional enhancers in vivo. *Nature methods* 10, 762–767, doi:10.1038/nmeth.2543 (2013). [PubMed: 23817068]
65. Black JB et al. Targeted Epigenetic Remodeling of Endogenous Loci by CRISPR/Cas9-Based Transcriptional Activators Directly Converts Fibroblasts to Neuronal Cells. *Cell stem cell* 19, 406–414, doi:10.1016/j.stem.2016.07.001 (2016). [PubMed: 27524438]
- 66**. Zhou H et al. In vivo simultaneous transcriptional activation of multiple genes in the brain using CRISPR-dCas9-activator transgenic mice. *Nature neuroscience* 21, 440–446, doi:10.1038/s41593-017-0060-6 (2018). [PubMed: 29335603]
67. Tanenbaum ME, Gilbert LA, Qi LS, Weissman JS & Vale RD A protein-tagging system for signal amplification in gene expression and fluorescence imaging. *Cell* 159, 635–646, doi:10.1016/j.cell.2014.09.039 (2014). [PubMed: 25307933]
68. Kim SS et al. A novel member of the RING finger family, KRIP-1, associates with the KRAB-A transcriptional repressor domain of zinc finger proteins. *Proceedings of the National Academy of Sciences of the United States of America* 93, 15299–15304 (1996). [PubMed: 8986806]
69. Groner AC et al. KRAB-zinc finger proteins and KAP1 can mediate long-range transcriptional repression through heterochromatin spreading. *PLoS genetics* 6, e1000869, doi:10.1371/journal.pgen.1000869 (2010). [PubMed: 20221260]
70. Zheng Y et al. CRISPR interference-based specific and efficient gene inactivation in the brain. *Nature neuroscience* 21, 447–454, doi:10.1038/s41593-018-0077-5 (2018). [PubMed: 29403034]
71. Vojta A et al. Repurposing the CRISPR-Cas9 system for targeted DNA methylation. *Nucleic acids research* 44, 5615–5628, doi:10.1093/nar/gkw159 (2016). [PubMed: 26969735]
72. Stepper P et al. Efficient targeted DNA methylation with chimeric dCas9-Dnmt3a-Dnmt3L methyltransferase. *Nucleic acids research* 45, 1703–1713, doi:10.1093/nar/gkw1112 (2017). [PubMed: 27899645]
- 73*. Liu XS et al. Editing DNA Methylation in the Mammalian Genome. *Cell* 167, 233–247 e217, doi:10.1016/j.cell.2016.08.056 (2016). [PubMed: 27662091]
74. Liu XS et al. Rescue of Fragile X Syndrome Neurons by DNA Methylation Editing of the FMR1 Gene. *Cell* 172, 979–992 e976, doi:10.1016/j.cell.2018.01.012 (2018). [PubMed: 29456084]
75. Cong L, Zhou R, Kuo YC, Cunniff M & Zhang F Comprehensive interrogation of natural TALE DNA-binding modules and transcriptional repressor domains. *Nature communications* 3, 968, doi:10.1038/ncomms1962 (2012).
76. Konermann S et al. Optical control of mammalian endogenous transcription and epigenetic states. *Nature* 500, 472–476, doi:10.1038/nature12466 (2013). [PubMed: 23877069]

77. Kearns NA et al. Functional annotation of native enhancers with a Cas9-histone demethylase fusion. *Nature methods* 12, 401–403, doi:10.1038/nmeth.3325 (2015). [PubMed: 25775043]
78. Cano-Rodriguez D et al. Writing of H3K4Me3 overcomes epigenetic silencing in a sustained but context-dependent manner. *Nature communications* 7, 12284, doi:10.1038/ncomms12284 (2016).
79. Lei Y et al. Targeted DNA methylation in vivo using an engineered dCas9-MQ1 fusion protein. *Nature communications* 8, 16026, doi:10.1038/ncomms16026 (2017).
80. Kwon DY, Zhao YT, Lamonica JM & Zhou Z Locus-specific histone deacetylation using a synthetic CRISPR-Cas9-based HDAC. *Nature communications* 8, 15315, doi:10.1038/ncomms15315 (2017).
- 81**. Lorsch ZS et al. Zfp189 Mediates Stress Resilience Through a CREB-Regulated Transcriptional Network in Prefrontal Cortex. *bioRxiv*, 403733, doi:10.1101/403733 (2018).
82. Lino CA, Harper JC, Carney JP & Timlin JA Delivering CRISPR: a review of the challenges and approaches. *Drug delivery* 25, 1234–1257, doi:10.1080/10717544.2018.1474964 (2018). [PubMed: 29801422]
83. Mali P et al. RNA-guided human genome engineering via Cas9. *Science* 339, 823–826, doi:10.1126/science.1232033 (2013). [PubMed: 23287722]
84. Ran FA et al. In vivo genome editing using *Staphylococcus aureus* Cas9. *Nature* 520, 186–191, doi:10.1038/nature14299 (2015). [PubMed: 25830891]
85. Chen LF et al. Enhancer Histone Acetylation Modulates Transcriptional Bursting Dynamics of Neuronal Activity-Inducible Genes. *Cell reports* 26, 1174–1188 e1175, doi:10.1016/j.celrep.2019.01.032 (2019). [PubMed: 30699347]

Highlights:

- Chronic drug use alters the epigenetic state of neurons and other cell types within brain reward regions.
- Epigenetic adaptations are correlated with addiction, but their causal contributions are incompletely characterized.
- Neuroepigenetic editing enables locus-targeted epigenetic manipulations in specific brain cells.
- Such locus-directed epigenome editing reveals specific causal contributions to addiction pathogenesis.

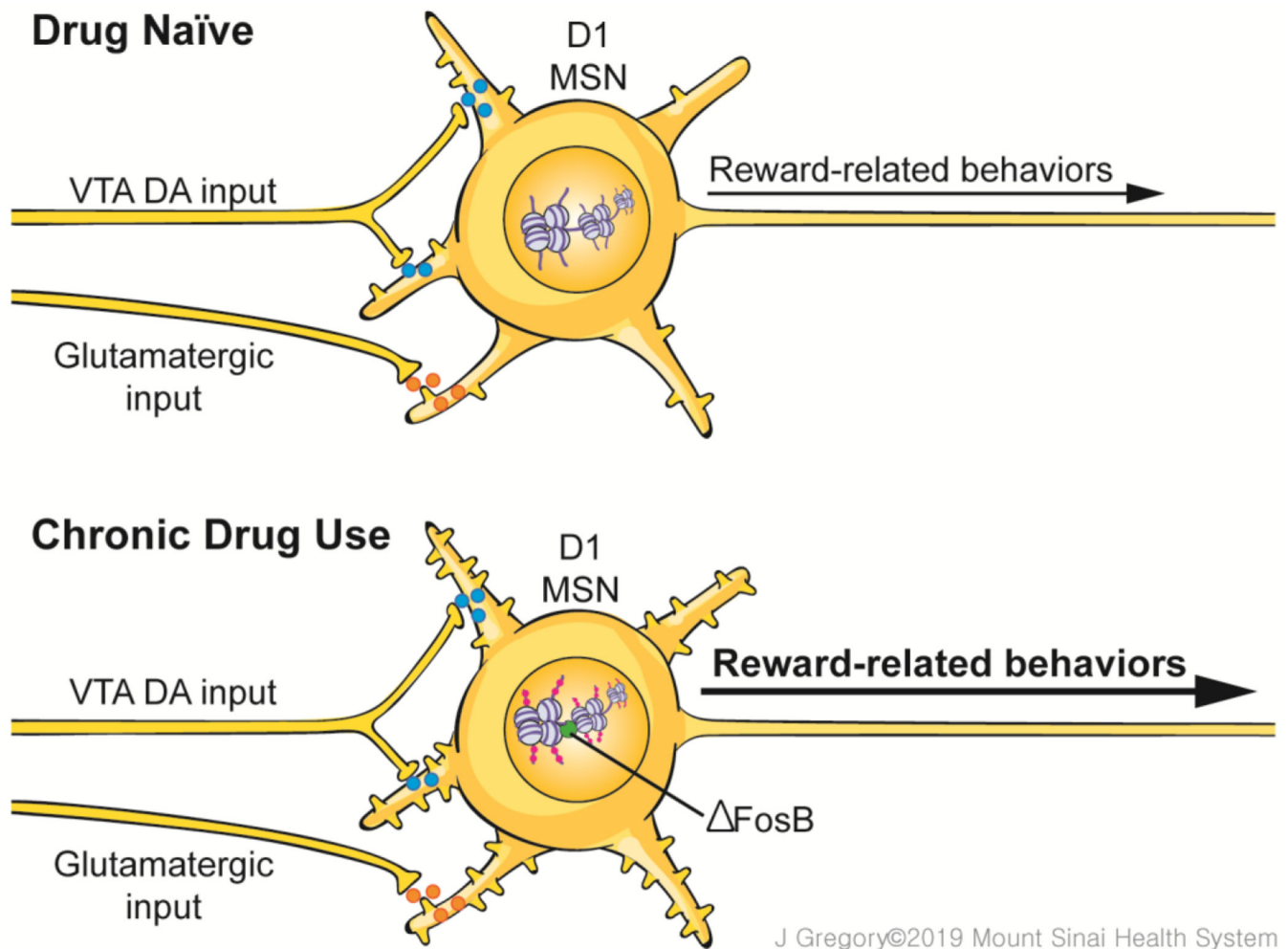


Figure 1. Chronic drug use alters the epigenome in reward processing neurons.

Top: In drug naïve conditions, medium spiny neurons (MSNs) within the nucleus accumbens (NAc) receive dopaminergic inputs from the ventral tegmental area (VTA) and glutamatergic inputs from several cortical and thalamic brain regions. These MSNs receive and integrate reward-related signals, and the homeostasis in nuclear epigenome writer and eraser enzymes within these MSNs enable normal reward-processing required for organismal survival. The NAc contains two types of MSNs, D1- and D2-type, named for the dopamine receptor they predominantly express. Only a D1-type MSN is shown. **Bottom:** Chronic drug use perturbs the balance in writer and eraser enzymes, resulting in a multitude of epigenetic adaptations at specific loci within the MSN nucleus. These adaptations, in conjunction with drug induction of certain transcription factors (e.g., FosB), mediate transcriptional changes at many genes, including those that encode neurotransmitter receptors, cytoskeletal proteins, and ion channels, among many others. The cumulative consequence of these transcriptional adaptations is altered MSN morphology (e.g., increased dendritic spine density is shown) and physiological function in relation to reward processing which underlies behavioral maladaptations that define addiction.

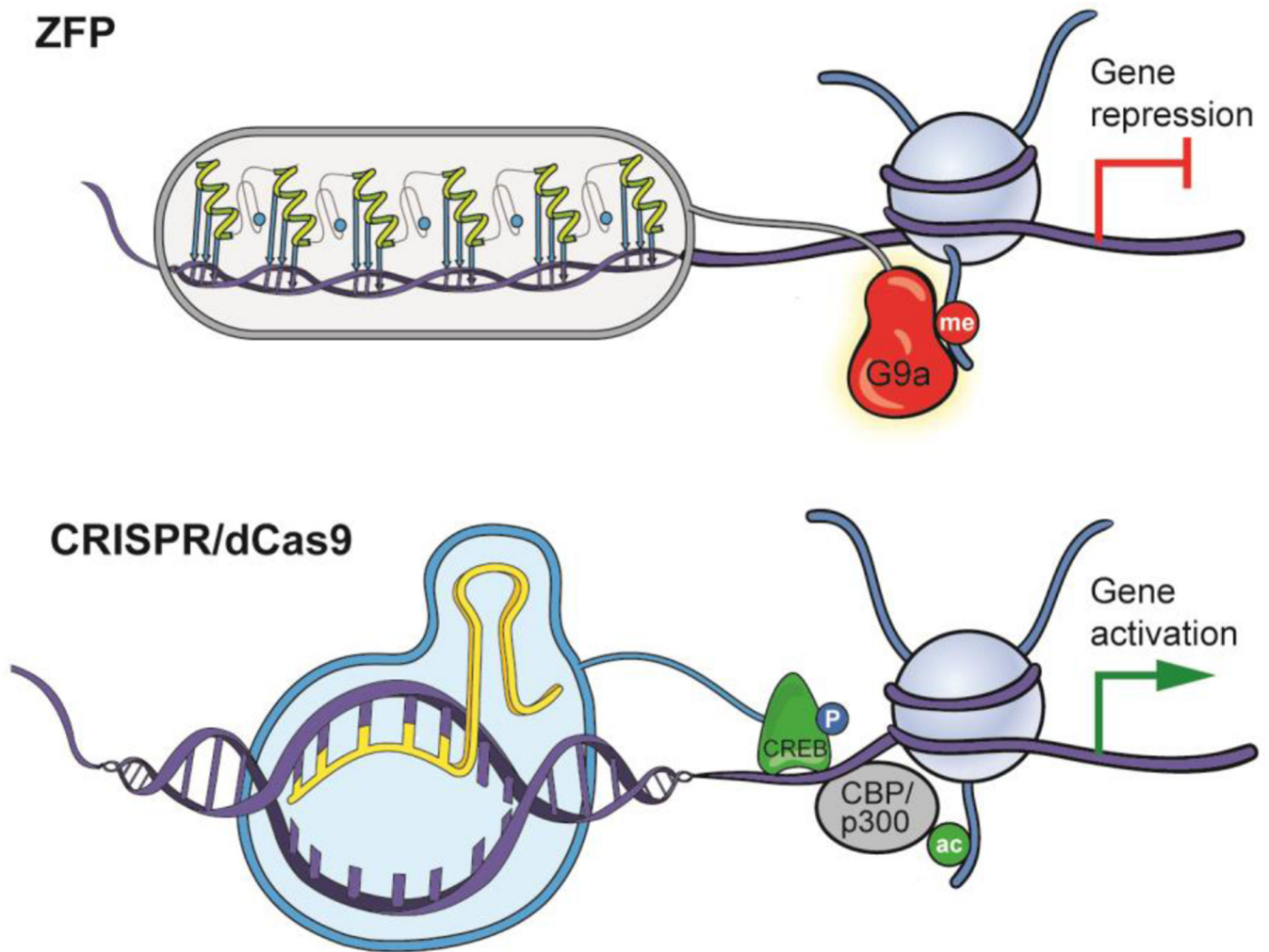
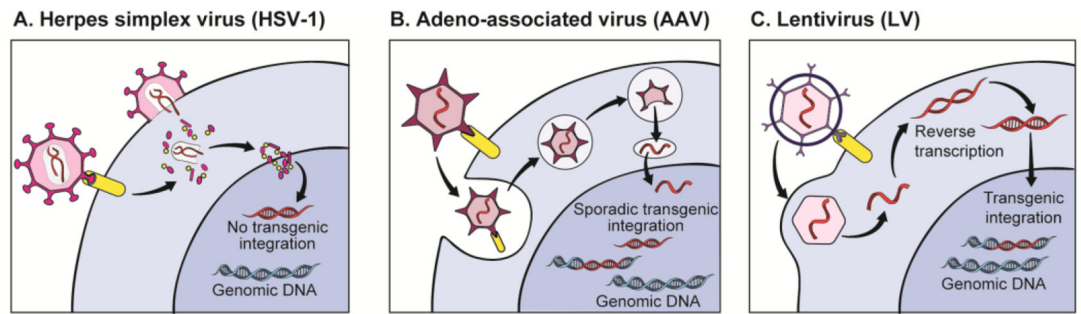


Figure 2. Neuroepigenetic editing tools.

Such tools are bi-functional constructs that consist of a DNA-binding domain that is designed to target a desired sequence of genomic DNA with high affinity and specificity and an effector moiety that mediates an epigenetic modification proximal to the region of DNA-binding. This results in epigenetic editing restricted to a single genomic locus, the specificity of which must be validated extensively to ensure minimal off-target binding. The most widely used DNA-binding domains are zinc finger proteins (ZFPs) and, more recently, RNA-guided CRISPR/dCas9. Here, we highlight a ZFP-G9a fusion that our group has generated for targeted gene repression in brain⁵² (top), as well as a dCas9-CREB fusion for *in vivo* gene-targeted activation⁸¹ (bottom). Figure modified with permission from reference 44: Hamilton, P. J., Lim, C. J., Nestler, E. J. & Heller, E. A. Neuroepigenetic Editing. *Methods in molecular biology* **1767**, 113-136, doi:10.1007/978-1-4939-7774-1_5 (2018).

**Feature**

Genetic material	dsDNA	ssDNA	ssRNA (positive strand)
Cloning capacity (Kb)	40	~4.9	9
Chromosomal integration	No	Yes (in about 10% of infected cells)	Yes
Vector production yield	High	High	High
Expression onset (in vivo)	More than 24 hours	1-2 weeks	1-2 weeks
Expression duration (in vivo)	7-10 days	More than six months	More than six months
Emergence of replicating vectors (in vivo)?	Low risk	Possible but not a concern	Risk is a concern
Insertional mutagenesis?	No	No	Yes

Figure 3: *In vivo* delivery methods for neuroepigenetic editing tools.

A summary of the properties of routinely used viral vectors available for delivery of neuroepigenetic editing tools to the brain of awake, behaving animals. While other approaches exist, the vast majority of published work on neuroepigenetic editing in brain cells utilize these viral delivery strategies coupled with stereotaxic surgery. Figure reproduced with permission from reference 44: Hamilton, P. J., Lim, C. J., Nestler, E. J. & Heller, E. A. Neuroepigenetic Editing. *Methods in molecular biology* **1767**, 113-136, doi: 10.1007/978-1-4939-7774-1_5 (2018).