Methods in Molecular Biology 2842

Springer Protocols

Albert Jeltsch · Marianne G. Rots *Editors*

Epigenome Editing

Methods and Protocols

Second Edition

Humana Press

Chapter 16

Stereotaxic Surgery as a Method to Deliver Epigenetic Editing Constructs in Rodent Brain

Elizabeth A. Heller and Peter J. Hamilton

Abstract

Modern neuroscience research is increasingly discovering that alterations in epigenetic states within key brain cells is correlated with brain diseases. These epigenetic alterations may include changes in histone post-translational modifications and/or DNA modifications, all of which affect transcription and other gene expression programs within the brain cells that comprise central brain regions. However, the exact causal contribution of these epigenome changes to brain disease cannot be elucidated in the absence of direct in vivo manipulations in the implicated brain areas. Combining the design and creation of epigenetic editing constructs, gene delivery strategies, and stereotaxic surgery enables neuroscience researchers to target and manipulate the epigenetic state of the brain cells of laboratory rodents in a locus-specific manner and test its causal contribution to disease-related pathology and behaviors. Here, we describe the surgical protocol utilized by our group and others, which is optimized for herpes simplex virus delivery into the mouse brain, although the protocol outlined herein could be applied for delivery of adeno-associated viruses, lentiviruses, or nonviral gene-delivery methods in both mice and rats. The method allows for the overexpression of engineered DNA-binding proteins for direct and targeted epigenome editing in rodent brain with excellent spatiotemporal control. Nearly any brain region of interest can be targeted in rodents at every stage of postnatal life. Owing to the versatility, reproducibility, and utility of this technique, it is an important method for any laboratory interested in studying the cellular, circuit, and behavioral consequences of manipulating the brain epigenome in laboratory rodents.

Key words Viral-mediated gene transfer, Synthetic biology, Neuroepigenetic editing, Stereotaxic surgery, Rodent brain

1 Introduction

Stereotaxic surgery is a powerful method used to manipulate the brain of living animals. This technique allows researchers to consistently and accurately target deep structures of the rodent brain through the use of a stereotaxic brain atlas, which provides the coordinates of a given brain area relative to Bregma, an anatomical landmark on the rodent's skull. Stereotaxic coordinates for rodent brain regions of interest can be determined from *The Mouse Brain*

Albert Jeltsch and Marianne G. Rots (eds.), *Epigenome Editing: Methods and Protocols*, Methods in Molecular Biology, vol. 2842,
https://doi.org/10.1007/978-1-0716-4051-7_16,

in Stereotaxic Coordinates [1] and *The Rat Brain in Stereotaxic Coordinates* [2]. In anesthetized animals, and facilitated through the use of a stereotaxic instrument, one can perform this surgery on large numbers of animals to reliably and accurately access structures within the rodent brain.

Combining this approach with viral-mediated gene transfer [3, 4], or even nonviral methods for delivering constructs of interest [5, 6], we and others have been successful in delivering engineered neuroepigenetic editing tools to deposit gene-locus-specific epigenome modifications in vivo to alter brain function and animal behaviors [7–14]. Epigenetic editing tools, which can exogenously catalyze epigenetic modifications at a single or multiplexed targeted genomic loci within neurons or even in a single type of neuron in an injected brain region, are necessary to establish the causal connection of such mechanisms to gene expression and neural function [15]. Given the fact that regulation of epigenetic landscapes is central to neuropsychiatric health and disease, it is crucial to combine epigenetic editing techniques with in vivo inquiry in the brains of awake and behaving animals. The technique of viral expression of epigenetic editing tools in rodent brain using stereotaxic surgery techniques facilitates the exploration of the causal impact of these targeted epigenetic modifications in these neurobiological contexts.

Here, we describe the laboratory methodology to utilize stereotaxic surgery to deliver epigenetic editing constructs in rodent brain.

2 Materials

2.1 Reagents

1. Ketamine and xylazine; or isoflurane.
2. 70% ethanol.
3. 100% acetone.
4. 10% bleach solution.
5. 70% ethanol wipes.
6. Sterile ocular lubricant.
7. Sterile PBS.
8. Sterile saline.
9. Purified virus (e.g., herpes simplex virus (HSV), adeno-associated virus (AAV), Lentivirus (LV)) or nonviral reagent (e.g., jetPEI®).
10. Betadine antiseptic.
11. Bupivacaine HCl local anesthetic.

2.2 Instruments and Materials

1. Dual small animal stereotaxic instrument (such as Kopf Model 902).
2. Small animal far infrared warming pad (such as Kent Scientific Model RT-0520).
3. Fine Science Surgical Tools, including but not necessarily limited to scalpel, scissors, forceps.
4. Laboratory scale.
5. Bead sterilizer.
6. Electric hair shaver.
7. Sterile tip cotton swabs.
8. Biohazard bags.
9. Low binding, 0.65 mL microcentrifuge tube.
10. Needles and syringes for IP injection of anesthetics and analgesics.
11. Absorbent lab bench diapers.
12. Hand-held dental drill and 0.6 mm burr.
13. Hamilton syringes (5 uL Catalog #84851) with Hamilton small-gauge RN needles (33-gauge Catalog #7762-06).
14. Tissue adhesive, surgical clips, or surgical sutures.
15. Temperature-regulated heating pads and/or heat lamp.

3 Methods

3.1 Neuroepigenetic Editing

1. Position the stereotaxic instrument in a well-lit workspace (*see* **Note 1**). Make sure the surgical area is cleaned with 70% ethanol and surgical instruments are cleaned and properly sterilized. We find that a bead sterilizer works well for this purpose. Place a small animal warming pad, set to 37 °C, in the surgical area of the stereotaxic instrument. Care should be taken to ensure the animal maintains body temperature for the duration of the surgical procedure, and many warming pads come with probes to monitor the temperature of the animal over the course of surgery. Cover the surgical area with sterile absorbent lab bench diapers. All procedures should be performed in accordance with your institution's biosafety and animal use guidelines.
2. Place Hamilton syringes in arms of the stereotaxic instrument and clear any blockages by drawing and expelling 100% acetone five times. Subsequently draw and expel sterile PBS five times to remove any residual acetone. Draw the maximum volume of sterile PBS into the Hamilton syringe, taking care to include no bubbles. Swing the stereotaxic arms to move the Hamilton

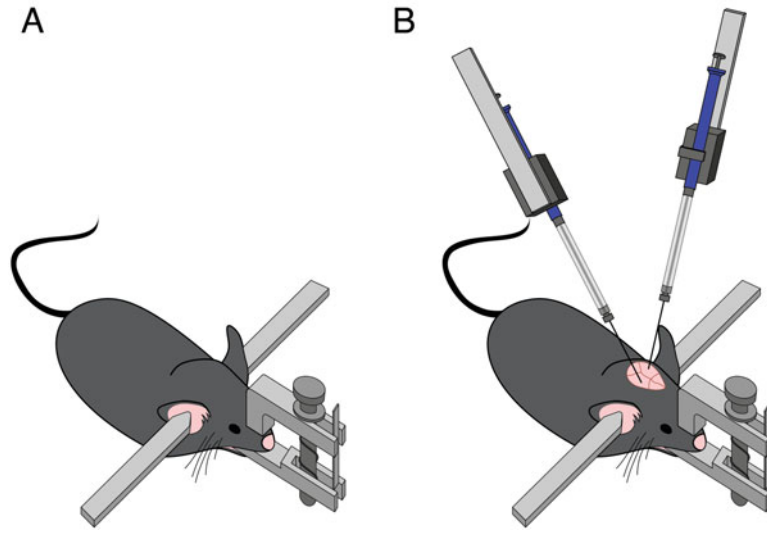

Fig. 1 Correct placement of rodent's head within stereotaxic instrument and surgical procedure for viral delivery. (**a**) Cartoon depicting the fixation of animal's head within the stereotaxic instrument. The ear bars are securely in place, preventing lateral movement of the skull. The incisor adapter restricts vertical movement, with the nose clamp is gently tightened into place. (**b**) Upon surgically exposing the stereotaxic landmarks on the skull, the stereotaxic coordinates are measured relative to bregma. Hamilton syringes are used to deliver the viral solution to desired regions within the animal's brain via small burr holes in the animal's skull

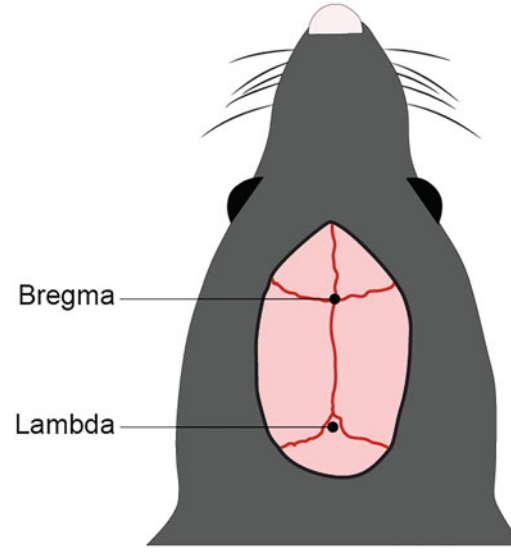

Fig. 2 Anatomical landmarks on the skull. The cartoon above depicts the stereotaxic landmarks bregma and lambda on the exposed surface of the rodent's skull

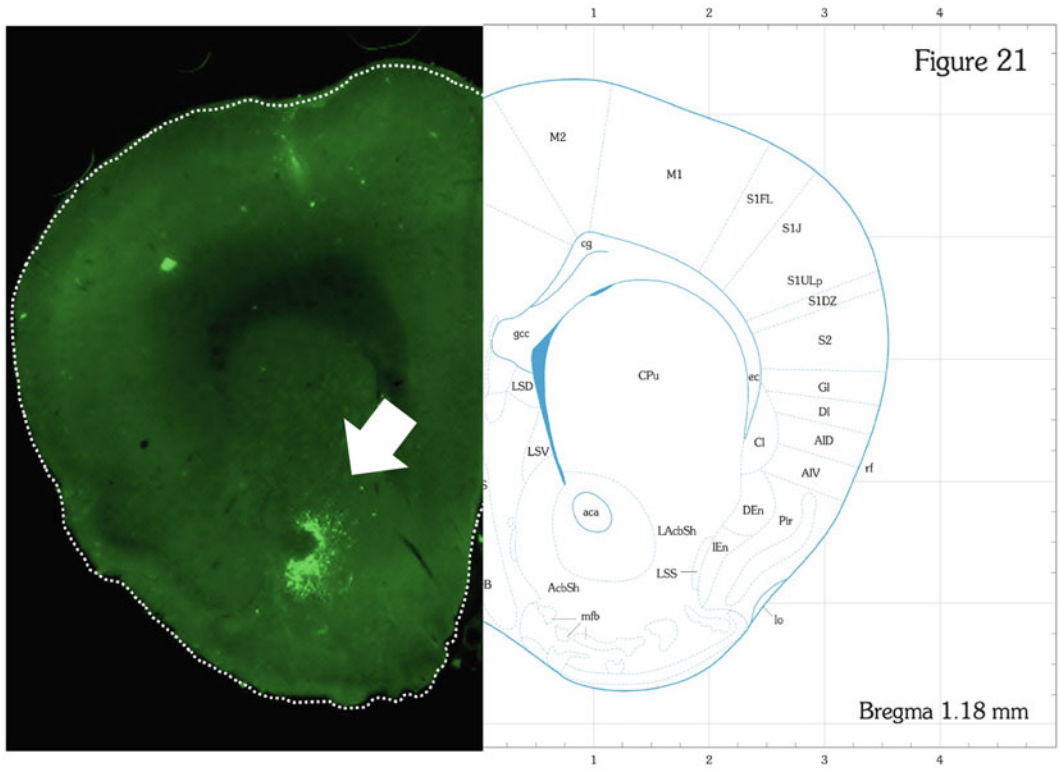

Fig. 3 Stereotaxic delivery of viral neuroepigenetic editing construct with GFP reporter to the nucleus accumbens. HSV delivered CRISPR/dCas9 fusion construct with a GFP reporter was stereotaxically injected into the nucleus accumbens (NAc) of a mouse to demonstrate the transduction efficiency and spread of the HSV viral vectors. Arrow points to targeted brain region. The injection was performed at a 10° lateral angle at +1.6 anterior/posterior, +1.5 mediolateral, and − 4.4 dorsal/ventral coordinate relative to bregma. Coronal section of manipulated mouse brain is shown on the left, the corresponding section of the mouse brain atlas is shown on the right

syringes out of the way of the workspace in the center of the instrument.

3. Anesthetize the animal with a ketamine/xylazine mixture (100 mg/kg ketamine and 5 mg/kg xylazine in sterile normal saline) delivered via intraperitoneal injection. The animal should reach surgical anesthesia within 5–10 min, and should not respond to a light pinch to the hind paw. *See* **Notes 2** and **3** for more details on assessing anesthesia. If electing to anesthetize the animal with inhaled isoflurane, *see* **Note 4**.
4. Cover the anesthetized animal's eyes with sterile ocular lubricant to keep them moist during the surgery.
5. Shave the fur off of the top of the animal's skull and clean the surface of the skin with alcohol prep wipes. Apply betadine antiseptic on sterile tip cotton swabs.

6. Place the animal upon the heat pad within the stereotaxic instrument. Secure the animal in the instrument. To do so, carefully place one ear bar in the ear canal, secure the bar and hold the animal in place as the other ear bar is placed and secured. The animal should not be able to move laterally. Next, secure the mouth in the incisor adapter of the stereotaxic instrument, taking care that the tongue is not pinched in the adapter or blocking the airway. The nose clamp can be gently tightened to firmly secure the animal's head in position (*see* **Notes 5** and **6**). Visually inspect the head and make adjustments to the pitch of the incisor adapter to make sure the head is level (Fig. 1a).
7. Make a midline incision to the top of the animal's skull with small surgical scissors or a scalpel. Use small surgical clips to gently keep the incision open, providing access to the skull. Optionally, sterile saline can be used with sterile swabs to clean the skull to aid in visualization of stereotaxic landmarks on the skull (Fig. 2).
8. Measure the *z* coordinates of Bregma and Lambda on the animal's skull and adjust the position of the head with the incisor adapter until they become equal. This serves to level the skull. Adjust the pitch of the ear bar to ensure that the skull is completely flat.
9. Position the tip of the Hamilton syringes to Bregma and record the *x*, *y*, and *z* coordinates on the Vernier scale located on the arms of the stereotaxic instrument. Subtract the coordinates of the targeted brain region to calculate the site of targeted viral injection. These coordinates can be determined from a stereotaxic brain atlas (*see* Introduction and **Note 7**). Note that the angle of the stereotaxic arm is an important consideration when determining the coordinates for targeting a desired brain region.
10. Position the tip of the Hamilton syringes according to the calculated *x* and *y* coordinates. Using a dental drill with a 0.6 mm burr, thin the area of the skull directly under the Hamilton syringe tip. Do not apply much downward force, as it may result in drilling through the skull and damaging the surface of the brain. Lower the Hamilton syringe on the *z* coordinate until it slides through the thinned skull, and raise the Hamilton syringe above the surface of the skull.
11. Proper safety attire and handling techniques should be applied based on the biosafety level of the virus being used (*see* **Note 8**). Defer to your institutional biosafety requirements for proper safety attire and handling techniques. The use of HSV vectors for our epigenome engineering experiments necessitates the use of a lab coat, gloves, and goggles when handling

the virus. Place a viral aliquot in a low binding, 0.65 mL microcentrifuge tube on wet ice, allowing it to thaw. If electing to use nonviral gene delivery methods, *see* **Note 9**. An abridged consideration of the relative strengths and weaknesses of each gene delivery method is included in **Note 10**.

12. Taking care not to alter the *x* or *y* coordinates, expel 2.5 μL of sterile PBS from the Hamilton syringe. This volume will accumulate on the tip of the syringe, indicating unobstructed flow through the syringe tip (*see* **Note 11**). This volume can be removed with a sterile tip cotton swab. Draw the plunger up by an increment of 0.5 μL to introduce a small air bubble into the barrel of the syringe. This serves to separate the viral solution from the sterile PBS. Finally, pull up the desired volume of virus to inject (typically 0.2–1 μL of a viral solution diluted to an appropriate concentration based on the type of virus being used) and place the microcentrifuge tube back on wet ice.
13. Slowly lower the Hamilton syringe through the burr hole in the animal's skull to the calculated z coordinate to the desired injection site within the brain (Fig. 1b).
14. Deliver the viral solution by lowering the plunger of the Hamilton syringe at a rate of 0.1 μL per min or less. Once the full volume of the viral solution has been dispensed, wait 5 min for the virus to diffuse through the tissue (*see* **Note 12**).
15. To avoid backflow of the virus to the surface of the brain, *slowly* raise the Hamilton syringe out of the skull.
16. Expel the remaining contents of the Hamilton syringe into a flask containing a 10% bleach solution and use an alcohol prep wipe to remove any material that may have accumulated on the syringe tip.
17. Remove the animal from the stereotaxic instrument and close the incision via surgical suture or tissue adhesive. Small burr holes (less than 1 mm in diameter) do not need to be covered with bone wax. Apply antibiotic ointment to the wound and inject the local anesthetic bupivacaine subcutaneously near the wound, to reduce discomfort during the recovery period.
18. Place the animal in a clean cage that is warmed by a temperature-regulated heating pad until the animal fully recovers. This should take approximately 20 min, depending on the duration of the surgery.
19. Return the animal to a clean age with moistened food pellets for easy access to food. Monitor the animal's recovery, looking for any signs of distress, which can include a lack of grooming, wound scratching, inflammation, altered locomotion, or

reduced weight gain. Monitor the animals daily for these signs (*see* **Note 13**).

20. Clean the Hamilton syringes with 100% acetone and sterile PBS and according to manufacturer's instructions (*see* **Note 14**). Discard the lab bench diapers into a biohazard receptacle and clean the workspace with 70% ethanol.
21. The time to maximal in vivo expression of our HSV-delivered, engineered transcription factors is approximately 2–3 days and persists through days 8–10 (Fig. 3). However, the trans-gene expression profile, viral spread, and cell tropism depends largely on the type of virus being used. Following stereotaxic surgery, any number of molecular or behavioral experiments can be performed.

3.2 Validation of Neuroepigenetic Editing Tools

It is essential to validate the use of epigenetic editing tools in several ways to ensure their effectiveness and selectivity in vivo. The outline below provides a general list of suggested validations. Depending on your application, the essential validations may vary.

1. Validate expression of the synthetic construct (e.g., ZFP, dCas9, etc.) in the brain in vivo. This includes validating selective expression in neurons when using a neurotrophic vector like HSVs, as well as selective expression within a single type of neuron if using a Cre-dependent vector in a mouse line that expresses Cre recombinase in a given cell type.
2. Validate that the epigenetic editing tool produces the designated epigenetic modification at the targeted locus. For example, that the p65 effector domain induces histone acetylation, G9a induces H3 Lys9 dimethylation, Tet1 induces DNA hydroxymethylcytosine, and Dnmt induces DNA methylcytosine, etc.
3. Determine whether the designated epigenetic modification is associated with altered expression of the targeted gene.
4. Study whether the designated epigenetic modification is associated with other forms of epigenetic regulation, transcription factor binding, or changes in chromatin architecture.
5. Validate that the epigenetic editing tool selectively acts at the targeted locus. This inquiry can be informed by identifying regions of the genome that are most highly homologous to the targeted region and demonstrating lack of epigenetic modification of these other regions and lack of altered expression of any nearby genes. Predicted off-target genomic regions can be identified on the basis of their DNA sequence similarity to the targeted genomic region. The ideal validation is experimentation to demonstrate direct and selective binding of the epigenetic editing tool to the targeted locus at levels much greater

than at off-target genomic loci. This is increasingly achieved by in vivo precipitation of the epigenetic editing tool followed by sequencing (i.e., ChIP-sequencing). This has been successfully performed in cultured cell lines [16, 17], but is not easily achieved in brain since every cell only has two specific binding locations (alleles) and the quantity of infected tissue can be limiting. Alternatively, the deposited epigenetic mark and its associated DNA can be precipitated followed by sequencing, and enrichment for the epigenetic modification can be demonstrated at the targeted genomic locus. This has been performed in brain following delivery of epigenetic editing constructs [18].

4 Notes

1. Ensure that the stereotaxic frame and accessories including the ear bars and incisor adaptor are appropriate for the type of animal to receive surgery.
2. If the animal does not reach a sufficient level of surgical anesthesia after 10–15 min, inject an additional 20% dose of Ketamine/Xylazine. Closely monitor the animal to confirm that the anesthesia deepens.
3. If the animal begins to awaken during surgery, remove the animal from the stereotaxic instrument, and reapply the anesthesia. The early signs of an animal awakening from anesthesia include twitches of the large facial whiskers and twitching of the tail. With careful monitoring, this occurrence can be avoided.
4. Animals may also be anesthetized by inhaled isoflurane. To do so, specialized equipment (such as the Kent Scientific Somno-Flo) is required to vaporize concentrated isoflurane and deliver a constant flow of 1–3% isoflurane mixed with air to be inhaled by the rodent over the course of surgery. Specialized stereotaxic mask adaptors are required to accept the in- and out-flow of the isoflurane gas over the nose of the rodent. Care should be taken to ensure that these anesthetic gasses are scavenged properly either by direct exhaust or an activated charcoal canister.
5. It is essential that the animal is firmly secured in the ear bars. Visually validate that the car bars are in the ear canal and not pinching the jaw, neck, or skull. Animals appropriately positioned in the ear bars will be able to move their snout up and down in the incisor adapter but will not be able to move side to side.
6. If the animal is not securely placed in the stereotaxic instrument, then it is possible that the skull's position will shift when drilling burr holes. This invalidates all recorded coordinates. To

be sure this does not occur, when the animal is first positioned in the instrument, apply light pressure to the skull with a sterile tip cotton swab. If the animal's skull shifts in response to the pressure, resecure the animal within the stereotaxic instrument.

7. The stereotaxic coordinates provided in atlases are optimized for adult, male animals. If experiments involve varying from these average metrics, it becomes important to validate and/or alter targeting coordinates through pilot experiments. In short, use the stereotaxic atlas coordinates as initial values, perform surgeries, and validate viral targeting with fluorescent microscopy. Adjust the stereotaxic coordinates as needed.
8. Selecting the appropriate viral vector for delivery of neuroepigenomic editing tools is paramount to the success of these experiments. Each viral vector varies in its spread, packaging capacity, tropism, transgene expression timing, and duration of expression. These variables should be carefully considered, and pilot studies should be performed to empirically validate viral function.
9. Increasingly, nonviral plasmid delivery methods are becoming available. An example includes charged polymer-based transfection reagents, like jetPEI®, which deliver nucleic acids to the central nervous system by forming charged interactions with the negatively charged plasmid and negatively charged cell surface proteoglycans, and facilitates the intracellular delivery of these plasmid constructs via endocytosis [5]. Additional considerations when using nonviral delivery strategies include validating cell tropism and time course of trans-gene expression, as these nonviral methods are not limited by viral tropism or the time course of viral processing and trans-gene delivery. This may present advantages or disadvantages depending on the research question of interest, so careful consideration on the part of the researcher and thorough in vivo validation is required.
10. There are multiple viral vector and nonviral delivery methods for delivering epigenetic editing constructs in rodent brain. Briefly, the most commonly applied methods are: (1) Lentiviruses (LVs). LVs are RNA retroviruses capable of transducing mitotic and post-mitotic cells, can carry gene payloads up to 8 kb, and randomly integrate their trans-gene into the host genome [19]. Strengths of LVs include large packaging capacity, which is a particularly important consideration as epigenetic editing constructs tend to be quite large, the ability to transduce a variety of CNS cell types, which can be modified by pseudotyping [20], and long-term trans-gene expression of greater than 12 months. Weaknesses include potential unintended consequences of insertional mutagenesis, slow onset of

trans-gene expression, and safety concerns which typically necessitate LVs be handled at biosafety level two. (2) Herpes simplex viruses (HSVs). HSVs are double-stranded DNA viruses that preferentially transduce neurons, can carry gene payloads >40 kb, and rapidly, but transiently, express their trans-genes in vivo. For these reasons, our group has heavily utilized HSVs for neuroepigenetic editing approaches. Strengths of HSVs include episomal integration, large packaging capacity, high transduction efficiency in neurons, and rapid trans-gene expression occurring within 1–3 days of viral delivery [3]. Weaknesses include potential for cytotoxicity and immune response, inability to transduce nonneuronal cells, and transient transduction profile lasting only about 10 days. (3) Adeno-associated viruses (AAVs). AAVs are small single-stranded DNA viruses that can infect many tissue and cell types due to the multitude of AAV serotypes, which confer distinct cell tropism [21]. AAVs are the most commonly used viral vector in neuroscience research. Strengths include episomal integration (although instances of genomic integration are noted [22]), broad cell tropism that can be tailored via serotype selection, long duration of trans-gene expression (up to 6 months), minimal immune response in vivo, and minimal safety concerns that often allow AAVs to be handled at biosafety level one. Weaknesses include a small packaging capacity of <4.8 kb and slow expression profile of around 3 weeks before trans-genes can be detected. The limited packaging capacity of AAVs is the primary limitation for using AAVs in epigenetic editing, and researchers have been seeking to overcome this limitation by designing split DNA-binding proteins like dCas9 or identifying/designing smaller dCas9 proteins that can be packaged in AAVs. (4) Nonviral delivery methods, which can include physical methods like electroporation or microinjection or chemical methods like lipid, polymer, or inorganic delivery [22]. The properties of nonviral delivery vectors depend on the method selected. Broadly, and when compared to viral delivery strategies, the strengths of nonviral delivery strategies include simpler production methods, less expense, more flexibility in delivery, less immune response, and fewer safety concerns. Weaknesses include shorter trans-gene expression duration, less gene-transfer efficiency, and no cell-targeted tropism.

11. If the Hamilton syringe clogs, it will prevent dispersion of the virus into the brain. Always visually confirm that the flow from the syringe is not impeded by expelling a very small volume of the viral solution back into the microcentrifuge tube before lowering into the rodent brain. If the syringe does not appear to work, expel the contents of the syringe into a 10% bleach

solution, clean with 100% acetone and/or replace the Hamilton needle, and restart the process of loading viral solution into the Hamilton syringe.

12. The Hamilton syringe tip is left in place during the 5-min rest after delivering virus in order to prevent backflow of viral solution up the needle track. However, we have found it beneficial during this time to slightly retract the Hamilton syringe along the z axis (<1 mm) to provide a small space for the more even dispersion of the viral solution in the tissue.
13. If signs of distress emerge over the course of daily monitoring post-surgery, then removal from the experiment and humane euthanasia may be warranted. Consult with your institution's veterinary staff on how to best care for an animal showing these symptoms. If the protocol is performed correctly, nearly all animals should recover within 1–3 days post-surgery.
14. If using surfactant-based cleaning solutions for cleaning and maintaining Hamilton syringes (as is often suggested according to manufacturer instructions), be extremely vigilant to thoroughly remove all traces of soap, as it can be damaging to viral function and titers. There should be no formation of soap bubbles when pipetting sterile PBS.

Acknowledgments

This work was supported by NIH grants R01MH126027 and R01DA052465 to EAH, as well as R00DA045795, R01DA058089, and R01DA058958 to PJH.

References

1. Paxinos G, Franklin KBJ (2019) Paxinos and Franklin's the mouse brain in stereotaxic coordinates, 5th Ed
2. Paxinos G, Watson C (2013) The rat brain in stereotaxic coordinates, 7th Ed
3. Neve RL, Neve KA, Nestler EJ, Carlezon WA (2005) Use of herpes virus amplicon vectors to study brain disorders. BioTechniques 39:381–391
4. Davidson BL, Breakefield XO (2003) Viral vectors for gene delivery to the nervous system. Nat Rev Neurosci 4:353–364. https://doi.org/10.1038/nrn1104
5. Puhl DL, D'Amato AR, Gilbert RJ (2019) Challenges of gene delivery to the central nervous system and the growing use of biomaterial vectors. Brain Res Bull 150:216–230
6. Tasset A, Bellamkonda A, Wang W et al (2022) Overcoming barriers in non-viral gene delivery for neurological applications. Nanoscale 14:3698–3719
7. Heller EA, Cates HM, Peña CJ et al (2014) Locus-specific epigenetic remodeling controls addiction- and depression-related behaviors (NN-A50213-T). Nat Neurosci 17:1720–1727
8. Heller EA, Hamilton PJ, Burek DD et al (2016) Targeted epigenetic remodeling of the Cdk5 gene in nucleus accumbens regulates cocaine- and stress-evoked behavior. J Neurosci 36:4690–4697
9. Hamilton PJ, Burek DJ, Lombroso SI et al (2018) Cell-type-specific epigenetic editing at the Fosb gene controls susceptibility to social defeat stress. Neuropsychopharmacology 43:272–284

10. Sase AS, Lombroso SI, Santhumayor BA et al (2019) Sex-specific regulation of fear memory by targeted epigenetic editing of Cdk5. Biol Psychiatry 85:623–634
11. Carpenter MD, Hu Q, Bond AM et al (2020) Nr4a1 suppresses cocaine-induced behavior via epigenetic regulation of homeostatic target genes. Nat Commun 11:504
12. Lorsch ZS, Hamilton PJ, Ramakrishnan A et al (2019) Stress resilience is promoted by a Zfp189 -driven transcriptional network in prefrontal cortex. Nat Neurosci 22:1413–1423
13. Lardner CK, van der Zee Y, Estill MS et al (2021) Gene-targeted, CREB-mediated induction of ΔFosB controls distinct downstream transcriptional patterns within D1 and D2 medium spiny neurons. Biol Psychiatry 90: 540–549
14. Teague CD, Picone JA, Wright WJ, et al (2022) CREB binding at the Zfp189 promoter within medium spiny neuron subtypes differentially regulates behavioral and physiological adaptations over the course of cocaine use. Biol Psychiatry S0006322322014780
15. Hamilton PJ, Nestler EJ (2019) Epigenetics and addiction. Curr Opin Neurobiol 59:128–136
16. O'Geen H, Henry IM, Bhakta MS et al (2015) A genome-wide analysis of Cas9 binding specificity using ChIP-seq and targeted sequence capture. Nucleic Acids Res 43: 3389–3404
17. Tsui C, Inouye C, Levy M et al (2018) dCas9-targeted locus-specific protein isolation method identifies histone gene regulators. Proc Natl Acad Sci USA 115:E2734–E2741
18. Xu S-J, Lombroso SI, Fischer DK et al (2021) Chromatin-mediated alternative splicing regulates cocaine-reward behavior. Neuron 109: 2943–2966.e8
19. Dong W, Kantor B (2021) Lentiviral vectors for delivery of gene-editing systems based on CRISPR/Cas: current state and perspectives. Viruses 13:1288
20. Parr-Brownlie LC, Bosch-Bouju C, Schoderboeck L et al (2015) Lentiviral vectors as tools to understand central nervous system biology in mammalian model organisms. Front Mol Neurosci 8:14
21. Haggerty DL, Grecco GG, Reeves KC, Atwood B (2020) Adeno-associated viral vectors in neuroscience research. Mol Ther Methods Clin Dev 17:69–82
22. Butt MH, Zaman M, Ahmad A et al (2022) Appraisal for the potential of viral and nonviral vectors in gene therapy: a review. Genes 13: 1370