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Biotechniques

Delivery of Recombinant Adeno-Associated Virus into the Rodent Brain

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Abstract
Recombinant adeno-associated virus (rAAV) has emerged as a powerful and versatile tool for manipulating gene expression in the central nervous system of experimental animals. Its utility stems from a favorable safety profile, capacity for long-term transgene expression, and ability to target specific cell types depending on serotype and promoter selection. Given the recent FDA approval of AAV for clinical use, its application in preclinical models is now even more relevant and translational, reinforcing its value in bridging experimental research and therapeutic development. In this article, we present a comprehensive overview of methodologies for rAAV-mediated gene delivery to the rodent brain. We detail protocols for intracranial, intra-cisterna magna and intravenous administration.

Keywords: Recombinant adeno-associated virus (rAAV), Central nervous system, Gene Therapy, Stereotaxy, Intracerebroventricular, Intravenous, Retroorbital, Rodent

Introduction

Recombinant adeno-associated virus (rAAV) vectors have become indispensable tools for gene delivery in therapeutic applications. Several key advantages include a strong safety profile, the potential for long-term transgene expression, and the ability to selectively target specific cell populations through strategic serotype and promoter selection (Matuszek et al., 2025; Wang and Xiao, 2025). Some vectors are injected directly into the brain parenchyma for optimal efficiency, while others can access deep brain regions via ventricular administration. In addition, some serotypes enable recombinant adeno-associated virus (rAAV) to reach the brain following systemic vascular injection (Goertsen et al., 2022; Matuszek et al., 2025; Wang and Xiao, 2025). rAAV is generally considered to be safe and non-pathogenic and exhibits a favorable immune response compared to other vectors while allowing sustained and stable protein expression (Ling et al., 2023). The durability of rAAV-mediated expression has been well documented, with transgene activity persisting over 1.5 years in rodents and up to 6 years in non-human primates (Ideno et al., 2003; Rivera et al., 2005). Notably, the therapeutic effect of Voretigene neparvovec (Luxterna), an AAV based gene therapy medication for the treatment of Leber congenital amaurosis, has lasted for at least a decade in animal models and 7.5 years in human patients (Leroy et al., 2023). In our own studies, transgene expression is typically detectable within one week of injection and stabilizes within 3 to 4 weeks, and remains consistent until euthanasia; we have performed studies ranging from 3 months to 9 months post-injection (Carty et al., 2013; Nenninger et al., 2022; Gorzek et al., 2025).

In rodent models, rAAV vectors can be administered via multiple routes, each offering distinct advantages depending on the target region and desired expression profile. Direct intracranial injection allows for precise targeting of brain structures, intra-cisterna magna delivery enables widespread CNS distribution, and intravenous injection provides a minimally invasive alternative capable of reaching the brain under specific conditions (Wang and Xiao, 2025).

This article presents a comprehensive overview of stereotaxic and non-stereotaxic techniques for rAAV-mediated gene delivery to the rodent brain, including detailed protocols for intracranial, intra-cisterna magna, and intravenous administration.

NOTE: All procedures involving live animals must be reviewed and approved by the appropriate institutional ethics committee and must adhere to established guidelines for the care and use of laboratory animals.

NOTE: Stereotaxic survival surgeries should be conducted using sterile techniques to ensure aseptic conditions.

NOTE: Rooms and procedures must adhere to specific biosafety and facility standards to ensure safety for personnel, animals, and the environment and follow the institutional biosafety committees (IBC) requirements.

Materials & Supplies

General Supplies

- Recombinant AAV vector (properly titered and stored)
- Sterile PBS or vehicle solution
- Ice bucket or cold block (for keeping virus cold)
- Timer or stopwatch
- Electric clippers/trimmers
- Heating pad (to maintain body temperature)
- Sterile gloves
- Animal identification tags or markers
- Institutional Animal Care and Use Committee (IACUC) protocol approval
- Documentation of aseptic technique for survival surgeries
- Biohazard disposal

Stereotaxic Injection

- Stereotaxic frame with digital controllers [World Precision Instruments 505322]
- Mouse adaptor [Kopf Model 923-B]
- Rat adaptor [Kopf Model 920]
- Rat anesthesia mask (Kopf Model 906)
- Anesthesia induction chamber [Patterson Scientific 78933387]
- Hamilton syringe [Hamilton 81022]
- Microdrill [RWD Life Sciences 78001]
- Microinjector [World Precision Instruments UMP3]
- Anesthesia system (e.g., isoflurane vaporizer) [Matrx 94805059]
- Oxygen tank [Airgas Healthcare UN1072]
- Oxygen pressure regulator [Western Medica 96-M2-540-P]
- Isoflurane [Piramal 66794-017-25]
- Surgical tools (scissors, forceps, scalpel, mosquito hemostats)
- Sterilized cotton swabs [Fisher Scientific 23-400-124]
- Bead sterilizer (for sterilization of surgical tools) [Braintree Scientific GER5287120V]
- Surgical drapes [Dynarex 4410]
- Iodine scrub [Covetrus 11695-2217-1]
- Scalpel [Excel 29550]
- 70% ethanol wipes (for sterilization) [Covidien 6818]
- Sutures [Ethilon 669H)
- Eye lubricant (to prevent drying during anesthesia) [Dechra 17033-211-38]
- Insulin syringes (e.g., 31G) [BD 328290]
- Analgesics (e.g.,Nocita™:bupivacaine) [Elanco YL103060A]

Intravenous Injection

- Anesthesia induction chamber [Patterson Scientific 78933387]

- Anesthesia system (e.g., isoflurane vaporizer) [Matrx 94805059]
- Mouse/rat nose cone and tubing for anesthesia delivery
- Oxygen tank [Airgas Healthcare UN1072]
- Oxygen pressure regulator [Western Medica 96-M2-540-P]
- Isoflurane [Piramal 66794-017-25]
- 70% ethanol wipes [Covidien 6818]
- Eye lubricant [Dechra 17033-211-38]
- Surgical drapes [Dynarex 4410]
- Sterile saline (for flushing if needed)
- Heat source (lamp or warm water) for tail vein dilation
- Diluted chlorhexidine solution for tail sterilization
- Insulin syringes (e.g., 31G) [BD 328290]
- 25G–30G needles for mice [BD 305125, 305115, 305109, 305106]
- 22G–25G for rats [305156, 305145, 305125]
- 1 mL syringes [BD 309659]
- Occlusive device or manual pressure for vein visualization

Intra-Cisterna Magna Injection
- Ketamine/Xyzaline mixture for mice [Patterson, #07-894-8462 and 07-891-9200]
- 27-gauge needle to inject IP [BD 305109]
- 1 mL syringes [BD 309659]
- Stereotaxic frame [World Precision Instruments 505322]
- Mouse adaptor [Kopf Model 923-B]
- 27GX3/4”TW Scalp Vein Set [Extel 26709]
- Eye lubricant [Dechra 17033-211-38]
- 70% ethanol wipes [Covidien 6818]
- Scissors and forceps
- DecapiCones® [Braintree Scientific DC 200]
- CSF collection tubes (optional) [USA Scientific 1615-5500]
- Rat adaptor [Kopf Model 920]
- Rat anesthesia mask (Kopf Model 906)
- Anesthesia induction chamber [Patterson Scientific 78933387]
- Anesthesia system (e.g., isoflurane vaporizer) [Matrx 94805059
- Oxygen tank [Airgas Healthcare UN1072]
- Oxygen pressure regulator [Western Medica 96-M2-540-P
- Isoflurane [Piramal 66794-017-25]
- 30-gauge needle [BD 305106]

Intracerebroventricular Injection in Neonates
- Hamilton syringe (10 µL) with 30-gauge beveled needle [Hamilton 81022]
- Metal plate or glass dish (chilled on ice)
- Folded foil sheet for pup placement during anesthesia
- 70% ethanol wipes for skull sterilization [Covidien 6818]
- Nest material for scent familiarity during pup recovery
- Surgical drape [Dynarex 4410]

Retro-orbital injections
- Anesthesia induction chamber [Patterson Scientific 78933387]
- Anesthesia system (e.g., isoflurane vaporizer) [Matrx 94805059]
- Mouse/rat nose cone and tubing for anesthesia delivery
- Oxygen tank [Airgas Healthcare UN1072]
- Oxygen pressure regulator [Western Medica 96-M2-540-P]
- Isoflurane [Piramal 66794-017-25]
- Insulin syringes (31G) [BD 328290] or 25–27G needles [BD 305125, 305115, 305109]
- Topical ophthalmic analgesic (as per IACUC protocol)

Post-Injection Monitoring
- Recovery cage with soft bedding and heating pad placed under half of the cage floor
- Analgesics (e.g., buprehorphine)
- Scale (for weight monitoring)

Observation log sheets *Biosafety Requirements for AAV Injection*
- Compliance with institutional biosafety committees (IBC) requirements.

- Biohazard signage on cages and procedure rooms
- Proper ventilation and containment to prevent aerosol exposure.
- Autoclave bedding and cages for at least 72 hours post-injection.
- Use approved disinfectants (e.g., 0.5% sodium hypochlorite or 2% glutaraldehyde).
- Ethanol is not effective against AAV

Methods

Intracranial Injections:

This protocol describes how to perform an aseptic stereotaxic survival surgery to deliver AAV vectors to the brain of a mouse.

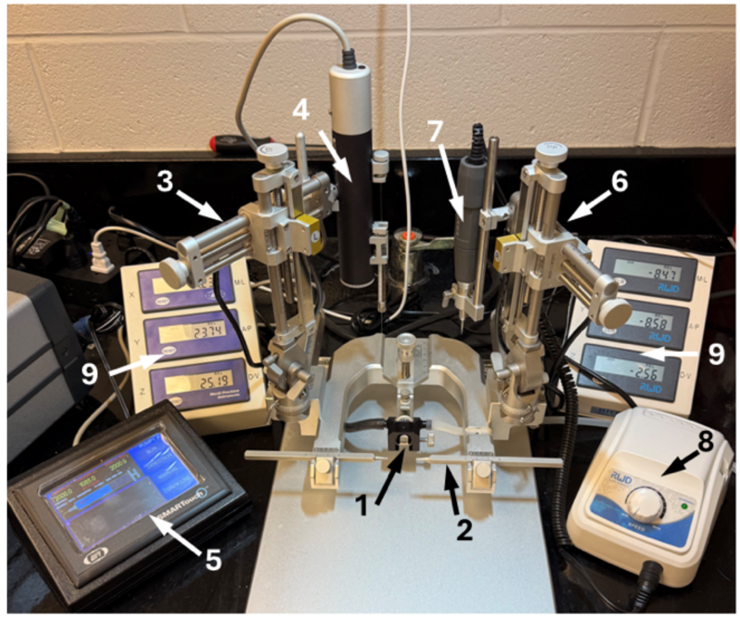

Figure 1. A typical surgical stereotaxic setup.
(1) Nose piece for positioning rodent's head with tubing delivering O2 and anesthesia. (2) Ear bars for holding rodent's head. (3) Stereotaxic left arm. (4) Syringe pump and syringe. (5) Pump controller for automated delivery. (6) Stereotaxic right arm. (7) Dremel drill. (8) Dremel controller. (9) Digital system attached to the stereotaxic arms for accurate needle and drill placement.

Adult Rodents

All procedures presented here were approved by the University of South Florida Animal Care and Use Committee. Adjustments to meet specific institutional requirements might be necessary. Animals are first acclimated for 30 min in the surgery room to minimize stress. Then, the animal is weighed (weight is recorded for post op follow up) and placed in the anesthesia induction chamber with a calibrated vaporizer delivering isoflurane anesthetic. Induction with anesthesia is typically performed with a vaporizer flow rate of 3-5% with oxygenation at 0.5%. Once anesthesia is evident via pedal withdrawal reflex test, the fur on the top of the head, from the nape of the neck up into between the eyes and ears laterally, is trimmed to prevent contaminating the operative site; paw trimmers work excellent for mice and electric clippers work well on rats. The rodent is then placed on a surgical drape atop an isothermal pad within the surgical field and mounted into the stereotaxic apparatus. Figure 1 shows a typical set-up for rodent surgery (for mice and rats, the nose piece can be easily exchanged). To mount the animal into the frame, they are first placed in the nose cone with the top front teeth placed correctly in the cone. This allows maintenance of anesthesia at a flow rate of ~2% by surgical tubing attached to the rodent-specific nose cone apparatus (Figure 2A). Subsequently, ear bars or cuffs are used to position the head in a fixed stationary position for the surgery. Then, the shaved area of the head is wiped with a 70% alcohol pad. Approved preemptive analgesic is delivered prior to the incision. We prefer to use Nocita® as an approved analgesic, which is injected under the skin on the top of the head with a 31-gauge insulin syringe. This allows for long-lasting localized effects (72 hours), thus requiring less injections and less stress for the animals. The analgesic is administered subcutaneously in the skin above the skull, and at least 2 minutes are allowed to pass prior to incision. During this 2-minute period, the top of the head can be massaged to facilitate dispersion of the Nocita® and eye lubricant is applied to prevent dryness.

The skin on top of the head is then cleaned with germicidal surgical scrub (wiping from the center of the surgical field outward). The surgical site is painted with a dilute, tamed iodine solution. A sterile surgical drape material over the animal (such as Bioclusive) is used to maintain sterility within the surgical field.

Using a scalpel (#10 or #11), a single incision is made on the top of the skull. Two sterile surgical swabs are used to clean and dry the skull while moving the skin apart to sufficiently expose the skull (Figure 2B). Alternatively, the skin can be held open using two mosquito hemostats clamped to the subcutaneous material. For targeted intracranial injections, stereotaxic coordinates are determined using a mouse brain atlas, which presents coronal or sagittal sections overlaid with a millimeter-scale grid. Coordinates are referenced from bregma, the intersection of the coronal and sagittal sutures, across three dimensions: the x-axis (anterior-posterior), y-axis (medial-lateral), and z-axis (depth) (Paxinos and Watson, 2013; Paxinos and Franklin, 2019). It is essential to verify that the skull is properly leveled within the stereotaxic frame. The microdrill attached to one stereotaxic arm can be used to confirm that the skull is level in both the X and Y planes. To confirm that the skull is level on the Y plane, position the drill bit slightly ahead of bregma, bring the drill bit down until it is just barely touching the skull, and zero out the Z coordinate. Bring the drill bit back up, position it slightly behind bregma, and then bring the drill bit down the same distance as before. The Z coordinate should be zero. The position of the nose can be adjusted up or down in the frame to adjust the skull to a level position, and the positioning checked again. Repeat this procedure to test that the skull is level on the X plane by moving the drill bit slightly to the left and then to the right of bregma. Bregma and Lamba could also be used as points of zero reference. The microdrill is then placed at bregma, and the coordinates are zeroed out. Afterwards, the microdrill is then moved under stereotaxic guidance to the region of interest, and the hole is drilled through the skull (Figure 2C). Multiple drill holes can be performed, e.g. one may target the hippocampus and anterior cortex within one animal. A hand-held microdrill may be used if the stereotaxic equipment does not have a separate arm for the drill attachment. In this case, the needle would be used to identify the stereotaxic coordinates from bregma for correct hole placement.

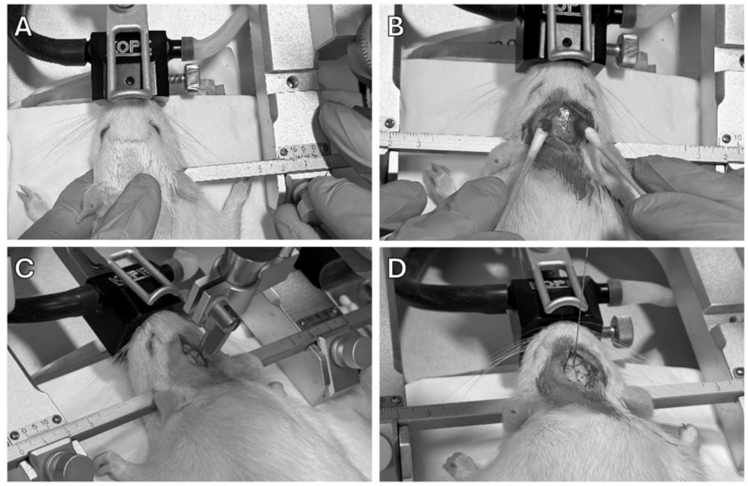

Figure 2. Stereotaxic intracranial injection in an adult rat.
(A) Positioning of rat in nose cone and placement of ear bars. (B) Cleaning and drying of the skull after incision. (C) Drilling of hole with Dremel in target area. (D). Positioning of syringe at bregma before guiding it to the injection site.

Performing pilot injections may be of value to ensure correct targeting. This could be performed with a dye or ink, immediately followed by gross brain sectioning to identify correct injection placement. Examples of routinely used coordinates within our laboratory for adult mice and rats are indicated in Table 1. Coordinates will need to be adjusted for smaller or younger animals.

Mouse	Lateral (X)	Anterior-Posterior (Y)	Dorsal-Ventral (Z)
Hippocampus	±2.7	-2.7	-3.0
Anterior cortex	±2.0	+2.0	-2.0
Substantia nigra	±1.4	-2.8	-4.6
Lateral Ventricle	±1.0	-0.4	-2.4
Cerebellum	±1.7	-6.4	-3.7
Rat	Lateral (X)	Anterior-Posterior (Y)	Dorsal-Ventral (Z)
Hippocampus	±4.6	-6.0	-5.0
Substantia nigra	±2.0	-5.2	-8.2
Lateral Ventricle	±1.5	-0.5	-4.3
Thalamus	±2.7	-3.3	-6.3

Table 1: Stereotaxic coordinates of adult rodents from bregma for viral injections to commonly targeted brain regions used in our laboratory.

A Hamilton syringe is placed in the injector on the opposite stereotaxic arm and the needle point is moved to bregma to zero out the coordinates (Figure 2D). The needle is then moved to the desired anatomical coordinates for injection. For parenchymal injections 2 μL of viral vector are dispensed per site at a flow rate of 2.5 μL/min using convection enhanced delivery (CED). We have published using bilateral injections in the hippocampus and anterior cortex and published sustained expression in both rats and mice models (Carty et al., 2013; Joly-Amado et al., 2020;

Nenninger et al., 2022). CED injections can be reviewed here (Nash and Gordon, 2016). A timer set to 2 min is started concomitantly with the injection, allowing the needle to be kept in position for one minute. This reduces the chance of back flow and allow complete dispersion of the virus. Longer times can be used to leave the needle in place and other typical viral injections have been reported with flow rates of 0.5 µL/min (Cearley et al., 2008; Jaworski et al., 2009).

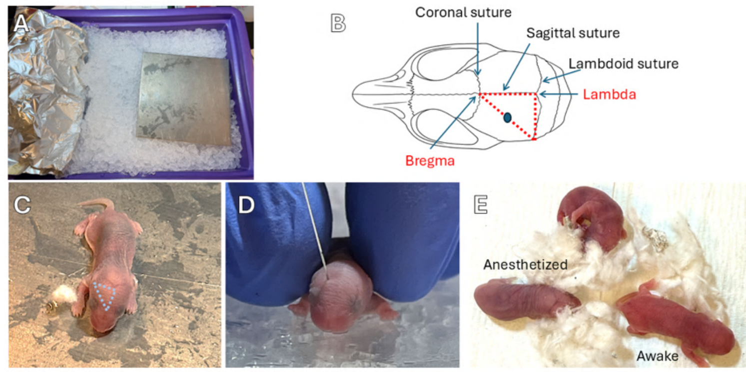

Figure 3. Intracerebroventricular injection into mouse neonates.
(A) Foil and metal plate setup on ice. (B) Diagram of targeted injection site. (C) Overlay of targeted injection site in a pup. Note the color of the pup is indicative of anesthesia. (D) Injection into skull with syringe. (E) Phenotypical comparison of blueish anesthetized versus pink awake pups.

After injection (single or multiple), the incision is closed with nylon (Ethilon® or an equivalent product) sutures. The rodent is placed into a clean home cage with at least half of the cage on an isothermal/warming pad. The animals are monitored until recovery (typically within 10-20 min), with the time of induction and wakefulness recorded.

Intracerebroventricular Injection into Postnatal Day 0 (P0) Pups

On ice, place a metal plate or glass dish along with a folded piece of foil (Figure 3A). Prepare a warming pad for recovery after the injections. Load a Hamilton syringe (10 µL) with a 30-gauge beveled needle with the desired amount of AAV for injection (2 µL of viral vector per lateral ventricle).

The mother can be removed to another cage to limit her exposure to the removal of the pups. Alternatively, half of the pups can be removed from the mother first, so that she remains associated with some pups at all times. P0 pups are placed in the foil on ice to induce anesthesia. After a few minutes (no more than five minutes), the pups will begin to show a purple-bluish hue and become inactive, which indicates they are anesthetized. A pup is then moved from the foil onto the metal/glass plate. This step is also performed on ice to maintain anesthesia during injection. The top of the head is gently swabbed with a 70% alcohol wipe. The suture lines of the skull should be clear. Forming a right-angled triangle from bregma to lambda to the intersection of the parietal-interparietal and interparietal-occipital sutures, find the midpoint along the hypotenuse to perform the injection (Figures 3B and 3C). The needle is depressed through the skull 2 millimeters (Figure 3D). A 2 millimeter mark can be made on the needle so as not to depress too deeply into the brain. The AAV solution is injected slowly and consistently. Once everything has been dispensed, the needle is left in place for approximately 10 seconds prior to removal. This process is repeated on the contralateral side.

The pup is then immediately placed on the warming pad to restore its body temperature. A small amount of nest material can be placed with the pup so that it has a familiar scent around it. Once the pink color returns and the mouse is warm and active, it can be placed back into the home cage (Figure 3E). Observe mother to ensure care of the pups is continued and she is not rejecting them. In the past we have occasionally attempted to use foster moms when animals do not seem to take care of their offspring, but unless there is a relatively large breeding colony this may not be feasible for most labs.

Intravenous Injections in Adult Rodents:

Tail Vein

Tail vein injections are the primary method of performing intravenous injections in rodents. To start, the animal is placed in an anesthesia induction chamber which delivers a 3-5% flow rate of isoflurane with 0.5% oxygenation. Following successful pedal withdrawal reflex test to verify the animal has been anesthetized, it is placed atop a surgical drape on a warming/isothermal pad and attached to an appropriate nose cone for sustained delivery of anesthesia during injection (Figure 4A). Lubricant may be placed on the eyes, so they do not dry out during injection. To make the vein more visible, the animal's tail is warmed. This can be performed with a heat lamp or using warm water (a damp paper towel soaked in warm water can be used). The appropriate needle size for tail vein injections is 25G-30G and 22G-25G for mice and rats, respectively.

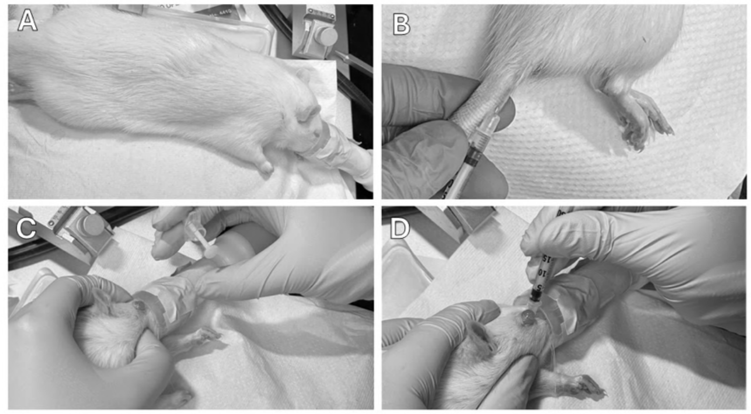

Figure 4. Intravenous tail and retro-orbital injections in a rat.
(A) Maintenance of anesthesia with a nose cone. (B) Injection into the tail vein. (C) Restraint of rat's head to shift eyeball out of the socket. (D) Injection into the medial canthus to access the retro-orbital sinus.

Preceding injection, the tail is sterilized with 70% ethanol wipes. For rats, scrubbing with chlorohexidine prior to sterilization may help remove some of the sebum accumulated as they age. When the tail has been sufficiently cleaned and sterilized, apply occlusive pressure above the intended injection site to aid in visualization of the tail vein. Once ready for injection, puncture the vein and draw back the syringe. The appearance of a blood flash in the syringe indicates successful entry into the vein. Upon confirmed access to the vein, slowly inject the viral solution, applying consistent pressure (Figure 4B). Successful injection is indicated by the vein clearing upon virus administration. Standard injection volumes do not exceed 200 µL in mice and 500 µL in rats.

Retro-orbital injection in adult rodent

Retro-orbital injections are an alternative to intravenous tail injections. It is recommended that no more than 6 injections (3 per eye) be administered within a rodent's lifespan. Injection volumes should be limited to 0.1 to 0.3 mL. Start by placing the rodent in an anesthesia induction chamber delivering isoflurane at a flow rate of 3-5% with oxygenation at 0.5%. Isoflurane is preferred for fast recovery. Once the rodent is anesthetized, as indicated by pedal withdrawal reflex test, it is removed from the chamber and attached to a nose cone delivering isoflurane at a flow rate of 2-3% with oxygenation at 0.5%. The anesthetized rodent should be placed laterally on a surgical drape on top of an isothermal pad (Figure 4A).

Using the forefinger and thumb, obtain a secure hold of the forehead and jaw, applying pressure on the head to make the eyeball pop out from the eye socket (Figure 4C). Care should be taken to not overexert pressure on the trachea, as breathing can be interrupted and/or collapse of the trachea can occur. With the other hand, insert the needle straight down into the medial canthus, with the bevel facing away from the eye (Figure 4D). A 31-gauge insulin syringes is preferred, but 25-27 gauge needles are also appropriate. Ensure that even and consistent pressure is applied onto the head and jaw to prevent the eyeball from sinking back down into the socket. Continue moving the needle downwards until the bone at the back of the eye is felt. When the needle is fully in the retro-orbital sinus, the full volume of the syringe is dispensed. Avoid pushing down or applying pressure with the needle, as this may lead the retro-orbital sinus to be punctured or ruptured. This may cause the injected material to leak from the nasal cavity, in which case veterinary assistance should be sought immediately. Proper injection will result in a visible bulging or blanching of the eye, with no leakage or bleeding. Once the full volume has been dispensed, move the needle backwards out of the medial canthus and swiftly use the fingers that were holding the jaw and forehead to roll the eyelids over the eyeball, situating it back into the eye socket. After the injection is complete, the rodent should be returned to a clean cage for recovery, with movement returning in less than a minute.

Lubricant can be placed onto the eye to prevent the cornea from drying out. A topical ophthalmic analgesic can be administered as per the institution's IACUC protocol. If the eye appears ruptured, damaged, or excessively dry during or after the injection, the institution's veterinarian should be promptly consulted to ensure appropriate care for the rodent.

Intra-Cisterna Magna Injection in Adult Rodents:

Intra-cisterna magna (ICM) injections allow for injection of virus directly into the cerebrospinal fluid (CSF), allowing for delivery of the viral vector throughout the central nervous system (Figure 5A). This is considered a less invasive administration route compared to direct injection into the lateral ventricles.

Adult Mice

To begin, a 27GX3/4"TW Scalp Vein Set attached to a 1mL syringe will be needed. The scalp vein (butterfly) needle is attached to the arm of the stereotaxic instrument. Slight bending of the needle will occur. Position the ear bars on the equipment so that they are equal in distance (symmetrical) and the ends of the ear bars are touching. Position the needle until it is directly above where the ear bars meet and then zero out the X/Y coordinates (Figure 5B). This establishes the X coordinate on the midline of the mouse skull.

Inject the mouse with a Ketamine/Xylazine mixture (1.75 mL Ketamine (100mg/mL), 0.25 mL Xylazine (100mg/mL), 8 mL saline) at a dose of 0.1mL/20g intraperitoneally. Once the mouse is anesthetized, shave the top of the head down to the neck. Place the mouse on a heating pad and insert the ear bars symmetrically (Figure 5C). Clean the shaved area of the head with an ethanol wipe and apply lubricant to the eyes.

Tilt the head forward (70°) over the edge of the heating pad. Brace the mouthpiece against the forehead (Figure 5D). Use a finger to feel for the soft tissue directly below the occipital protuberance of the skull. Adjust the position of the needle in the Y axis until it is touching the very top of the skin below the occipital protuberance (Figure 5E). The Z coordinate can be zeroed out for reference. Begin to slowly lower the stereotaxic arm with the needle attached. As the needle enters the skin, there should be no resistance or shifting of the skull. Continue to lower the needle through the tissue, while simultaneously gently pulling back on the 1 mL syringe. This will allow for identification of the presence of CSF and allow for CSF sampling (Figure 5F). We observed that the depth of Z coordinate where we obtained CSF ranged from 2-5 mm. Once CSF is observed in the tubing of the needle, halt the descent of the needle. Clamp the tubing using forceps, leaving a small gap for injections (Figure 5G). Cut the tubing directly above the clamped forceps and immediately place the collected CSF in a tube on ice. To deliver the virus, insert the needle into the tubing, below where the forceps are clamped, and slowly dispense the desired volume of treatment, which should be no greater than 10 μL (Figure 5H). Wait 1 minute after all the virus has been dispensed, then unclamp the forceps

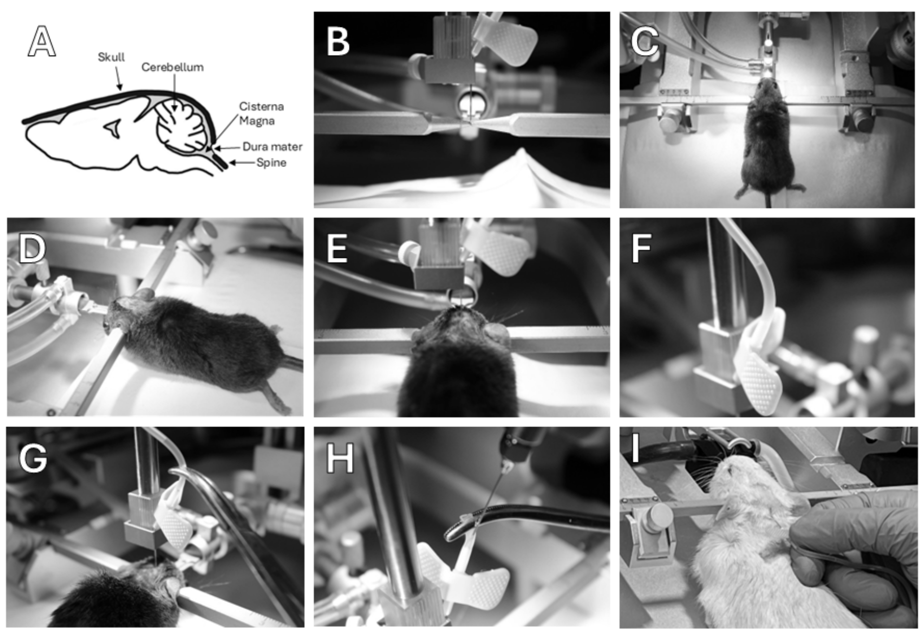

Figure 5. Procedural steps of intra-cisterna magna injections in rodents.
(A) Diagram of where the cisterna magna is located. (B) Positioning of needle where ear bars meet so that the X/Y coordinates can be zeroed out. (C) Adult mouse situated into stereotaxic apparatus with ear bars inserted. (D) Mouthpiece of the stereotaxic equipment pushed up against the forehead of the mouse. (E) Needle tip touching the top of the tissue below the occipital protuberance. (F) CSF flowing through the tubing. (G) Clamping of the tube directly below where the CSF collected. (H) The tubing is cut above where it is clamped and the syringe with the virus is inserted into the tubing below the clamping. (I) Adult rat with a butterfly needle inserted into the cisterna magna for CSF collection.

and allow the solution to flow down into the cisterna magna. Wait an additional 2 minutes after unclamping the forceps. To further enhance delivery, mice can be placed in the Trendelenburg position. We have placed mice in DecapiCones® at 30° decline on top of a heating pad (the cone has an open end for easy breathing but gives support to the animal, holding it steady in a decline).

Adult Rats

In rats, we can take a different approach because of their larger size. A rat can be anesthetized with a vaporizer flow rate of 3-5% with oxygenation at 0.5%. Once anesthesia is evident, the fur from the top of the head and down the neck is shaved. The anesthetized rat is placed in the stereotaxic frame on top of a surgical drape and heating pad. Insert the ear bars symmetrically and disinfect the shaved area with an ethanol wipe. Remove the heating pad from below the animal so that the body is now lower than the head, allowing the neck to be exposed in a more vertical position (Figure 5I). Using the index finger of the hand not doing the injection (typically nondominant), feel for the soft tissue directly below the occipital protuberance of the skull. Using a fingernail as a guide, position the 30 gauges needle on top of the fingernail and under the occipital protuberance, then penetrate through the skin (Figure 5I). There should be a resistance as the needle passes through the dura mater into the cisterna magna space. Then, withdraw the syringe to observe CSF and inject the virus. If a hard surface is encountered while attempting to pass through the dura, indicating contact with the skull, reposition the needle at a lower angle and proceed through the dura mater. We administer 20 µL of virus sample, but a typical range of 10-50 µL has been reported for CSF injections in rats. This technique can be used for mice but due to their significantly smaller size, it is difficult to complete freehand, so we have used the method described above. We do however use this technique in mice when we sample CSF. Using 27GX3/4"TW Scalp Vein Set attached to a 1mL syringe as above, the needle can be positioned into the cisterna magna and the syringe gently withdrawn. If it is a terminal procedure, we can obtain ~200 µL of clean CSF with this method.

Summary

Direct intracranial injections, while inherently invasive, offer a significant advantage: precise targeting of specific brain regions. In our studies, targeted delivery to areas such as the hippocampus (Carty et al., 2010), anterior cortex (Joly-Amado et al., 2020) and substantia nigra (Nash et al., 2015) has consistently resulted in robust and widespread distribution of viral vectors within these regions. Conversely, intracerebroventricular injections using AAV serotypes such as 9 or 10 have proven highly effective for achieving broad, global transduction across the central nervous system, making them a powerful tool for systemic gene delivery (Finneran et al., 2019).

P0 intracranial injections of rAAV offer several compelling advantages for gene delivery to the brain. At this early developmental stage, the blood-brain barrier is still immature, allowing rAAV vectors to cross more readily and achieve widespread distribution throughout the central nervous system. This results in efficient transduction of both neurons and glial cells across various brain regions. The procedure is minimally invasive compared to intracranial injections, reducing surgical risks and making it particularly suitable for early-life interventions. Additionally, the neonatal immune system is less reactive, which may lower the likelihood of immune responses against the viral vector, enhancing safety and long-term transgene expression. Administering gene therapy at P0 also enables early correction of genetic disorders, potentially preventing the onset or progression of neurological diseases. Some examples from our work and that of others can be found here (Chakrabarty et al., 2013; Cook et al., 2015; Carlomagno et al., 2019).

Recent developments in capsid engineering by directed evolution have led to the creation of rAAV variants that can cross the blood-brain barrier more efficiently than standard AAV serotypes administered intravenously. This has opened this route of administration for rAAV gene delivery to the brain. Such vectors include PhP.eB and CAP-B22, which were derived from the original AAV9 serotype (Chan et al., 2017; Goertsen et al., 2022). These vectors can achieve wide spread brain transduction (Finneran et al., 2024), but currently do not target all neurons. Another limitation to these vectors is the requirement for a larger amount of virus to be injected to achieve significant brain transduction, however, improvements in vector design continue in the field.

Intracisternal injections offer a potentially minimally invasive and effective route for delivering rAAV vectors directly into the CSF of rodents. It has been proposed that this method enables widespread distribution of the viral vector throughout the central nervous system via CSF flow, facilitating transduction of brain and spinal cord tissues (Bailey et al., 2020; Chatterjee et al., 2022). However, some studies report that cisterna magna injections can lead to limited transduction efficiency in certain brain regions due to restricted CSF flow or anatomical barriers (Ye et al., 2024). Perhaps transduction efficiency differences between studies may depend on the AAV serotype, dose, and infusion parameters. This method has been shown to be effective in large animal models which does suggest a potential translatability to human applications (Taghian et al., 2020; Nakamura et al., 2021; Hunter et al., 2025). The Trendelenburg position, where the animal is placed with its head lower than its body, has been proposed to offer several benefits during cisterna magna injections. Trendelenburg positioning after injection has been shown to enhance virus transduction to the brain and spinal cord (Castle et al., 2018; Chatterjee et al., 2022). However, the amount of time inclined and the species of animal may contribute to the effectiveness of this method.

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