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# Optimized Stereotactic Injection Protocol for Targeting the Locus Coeruleus with Minimal Neurotoxicity

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**We use this protocol and it's working**

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## Abstract

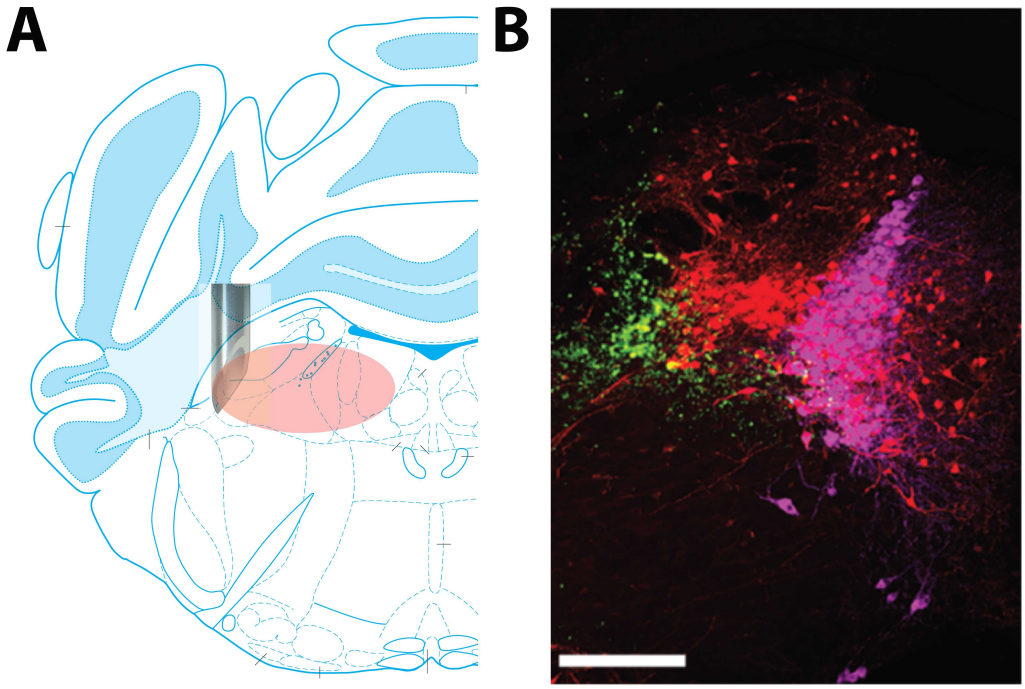
The Locus Coeruleus is a critical brain region known for its vulnerability to mechanical perturbations, neuroinflammation, and axonal disruption. This protocol presents a refined stereotactic injection procedure designed to target the LC while minimizing neurotoxicity. By positioning the injection site 0.5 mm lateral to the LC, we effectively deliver viral vectors without directly damaging the region. The protocol outlines the administration of anesthetics, including ketamine and xylazine, ensuring stable conditions during surgery. Key steps include precise head fixation, identification of anatomical landmarks, and careful drilling of the skull to facilitate accurate injections. The use of a beveled needle allows for controlled viral infusion, with a recommended volume of 500 nl per hemisphere at a rate of 50-100 nl/min. Post-operative care, including analgesic administration and monitoring for distress, is emphasized to ensure animal welfare. This optimized approach not only enhances the safety of stereotactic injections in the LC but also supports the integrity of experimental outcomes in neurobiological research.

## Materials

Stereotactic frame for mice
Injection apparatus (e.g., Hamilton syringe or similar micro-injector)
Anesthetic (ketamine/xylazine or isoflurane)
Analgesic (e.g. carprofen)
Sterile surgical tools (scissors, forceps, scalpel)
70% ethanol, sterile saline, 3% hydrogen peroxide
Betadine (for disinfection)
Suture or surgical glue
Electric or manual shaver for hair removal
Micro-drill for craniotomy
Injection material (e.g., viral vectors, drugs, or tracers)

## Optimized Stereotactic Injection Protocol for Targeting the Locus Coeruleus with Minimal Neurotoxicity

1 One of the key challenges in studying the Locus Coeruleus (LC) is its high vulnerability to perturbations, including mechanical forces, neuroinflammation, and the disruption of axonal tracts. To address this, we have developed a refined stereotactic injection strategy that targets areas further away from the LC, allowing us to infuse viruses from a lateral position. This approach minimizes the risk of damaging the LC while still effectively delivering the viral vector.




Overview of the stereotactic injection strategy positioned 1.5 mm lateral to the Locus Coeruleus using a beveled needle. In (B), green latex beads mark the opening of the beveled needle to confirm the injection site. The red fluorescence indicates the spread of a non-cre-dependent viral construct (CAG-mScarlet-dimer), demonstrating that this injection strategy surrounds the LC with viruses and induce a minimal neurotoxicity. Magenta shows tyrosine hydroxylase (TH) positive staining, highlighting the LC region.

## Animal Preparation

2 Weigh the mouse to calculate the precise dosage of anesthetic required. Administer ketamine at a dosage of 10 mg/kg and xylazine at a dosage of 20 mg/kg. Alternatively, use isoflurane as approved by your institutional animal care and use protocols. Ensure adherence to all relevant guidelines and protocols for the safe and effective administration of anesthesia.

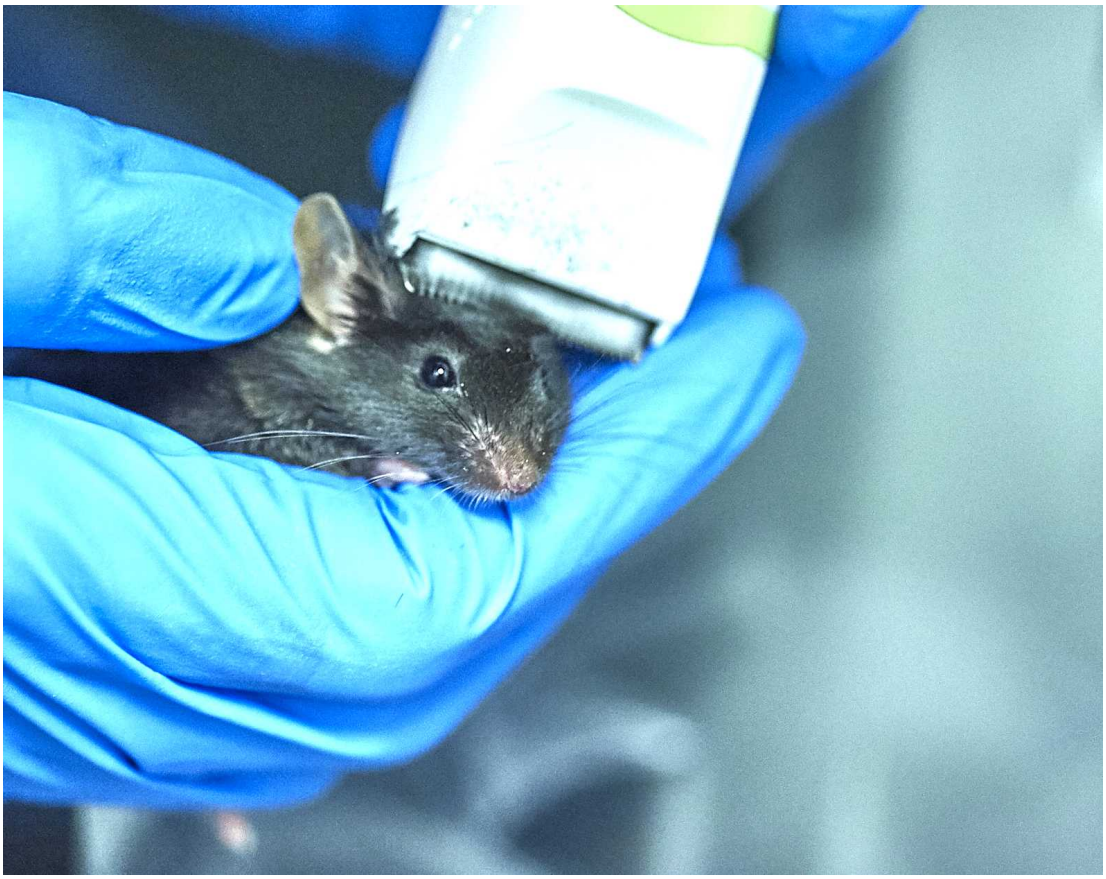
3 Anesthetize the mouse using the ketamine-xylazine mixture about 10 minutes prior to the

surgery via intraperitoneal injection for stable (reduced pain) surgery

4 Confirm the depth of anesthesia by checking the pedal withdrawal reflex

## Pre-surgical procedure

5 Shave the area over the skull



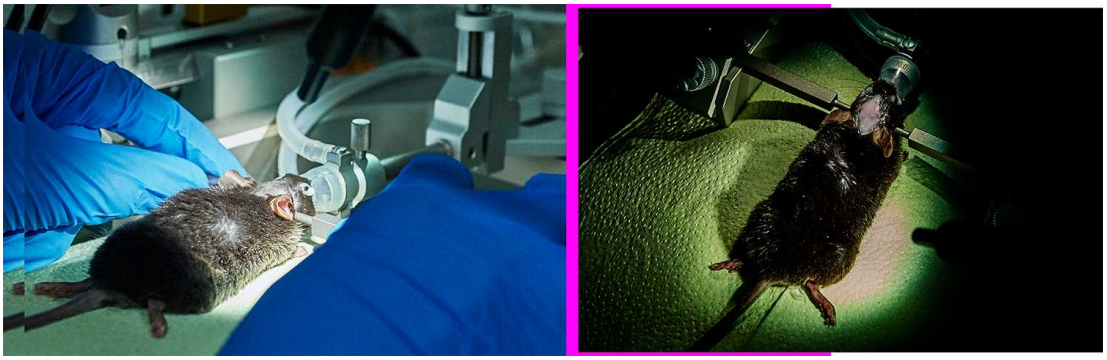
6 Clean the skin with 70% ethanol and disinfect with Betadine

7 Cover the eyes of animals with moisturising balm

8 Put 2% Lidocaine gel in the clean skin of the head

## Head fixation and Exposure of the Skull

9 Place the mouse in the stereotactic frame



Secure the animal's head firmly without causing damage to the ear canal, ensuring the head is level at this stage.

10 position the nose in the nose clamp ensuring that the head is straight and stable

11 secure the head by adjusting the ear bars into the external auditory canals

12 check the pedal withdrawal reflex

13 using sterile scalpel, make a midline incision (ca 1 cm) to exposed the skull

14 retract the skin to exposed the bregma and lambda on the skull

15 clean the skull with sterile saline and 3% hydrogen peroxide

## Measuring coordinates

16 Identify the lambda point and measure its depth (dorsoventral level) using a needle or injection syringe. To ensure accurate measurement, gently touch the skull with the needle or syringe, and confirm contact through the stereoscope.

17 Move from lambda to bregma while maintaining the same mediolateral position of the arm. Measure the depth at bregma, ensuring that both lambda and bregma are aligned on the same midline. Adjust the head position in the left-right direction if needed to align them. Ensure that the dorsoventral (DV) levels of bregma and lambda are similar, allowing for a difference of up to 50 micrometers.
At the lambda position, verify the plane along the mediolateral axis. To do this, move 1.5 mm to the left and right, and check if the DV depths at these points are similar. A difference of up to 50 micrometers is acceptable between the left and right points.

18 zero the stereotactic manipulator on bregma and move to desired coordinates. For locus coeruleus injections use

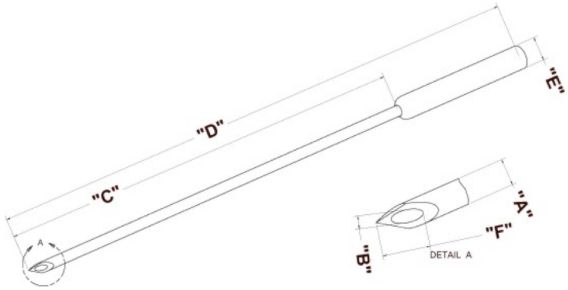
**AP -5.4, mm**
**ML +/- 1.5 mm**
**DV: - 3.75 mm** coordinates relative to bregma

**Important:** We are using a bevel needle, such as the WPI-NF34BV from World Precision Instruments (WPI) or equivalent needles from other companies.

**Important:** To prevent overexpression in the LC and subsequent degeneration, it is crucial to test the titer and efficiency of viral constructs by using different AAV serotypes and titers before conducting an experiment. The injection strategy described here can accommodate higher viral loads, as the injection site is 0.5 mm away from the LC. This distance reduces the amount of virus that reaches the LC, allowing for more controlled dosing.

A

B




C

| Tip Order code | Guage | Needle O.D. "A" (µm) | Avg. Neelde I.D. "B" (µm) | Range of ID for B (µm)* | Range of OD for B (µm) | Tip Length "C" (mm) | Total Length "D" (mm) | Shank O.D. "E" (µm) | Bevel Length "F" (µm) | Total Dead Volume (µL) | Dead Vol Range (µL) | Tip Material |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **NF34BL** | 34 | 185 | 85 | 64-100 | 178-190 | 5 | 35 | 460 | ≈0 | 0.199 | 0.1126-0.2759 | Stainless Steel |

Picture taken from WPI website (https://www.wpiinc.com/var-3184-nanofil-needles.html?srsltid=AfmBOooP8yjCs3-6cLYDJprVGTamPO5Ml3nUrzVFZ2qrAELROV6AXlF2)) to illustrate nature of. bevel neele

## Craniotomy and Injections

19 Using a microdrill, carefully drill a small hole in the skull at the identified coordinates. Ensure that the holes are clean and stop any bleedings, by treating the holes with sterile saline and clean tissues.

20 Fill the Hamilton syringe or microinjector with the prepared injection material. Ensure that the opening of the bevel needle (e.g., WPI-NF34BV) is facing the midline, as the viral injection will diffuse in that direction. Confirm the needle's orientation using a stereoscope. Before starting the injection, expel a small amount of the viral solution outside the brain to check that the needle is not clogged and to confirm the bevel's orientation.

21 slowly lower the syringe needle into the brain to the desired depth and allow the needle to rest for 2 minutes

22 slowly inject the substance (For LC: 500 nl per hemisphere) at a rate of 50-100 nl / min

23 after injection, allow the needle to rest for 5 minutes to allow for viral diffusion and prevent reflux of the injected material

24 slowly withdraw the needle

## Post-surgical procedure

25 Use sterile saline to moisture the skull and clean the area around the craniotomy. Make sure no lose hair are left on the skull or muscule tissue

26 close the incision using sutures or surgical glue

27 Inject analgesic under the skin around the neck area (e.g. Carprofen 0.2 ml of 1mg/ml) and inject saline (0.5 ml s.c.) to protect from dehydration.

28 place the mouse back to the home cage placed on the heating pad. Monitor the animal until it regains consciosness.

29 provide analgesic (e.g. Carprofen chewing tablets) for 3 consecutive days post surgery. Monitor the animal for signs of distress and infection for a few days.