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Stereotaxic Injection (SNc or STR)

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol describes a precise stereotaxic injection procedure for delivery of viral vectors or α-synuclein preformed fibrils (αSyn-PFF) into the substantia nigra pars compacta (SNc) or striatum (STR) of adult mice (2–3 months old). Using a Kopf stereotaxic frame, Hamilton syringe, and nanoliter injector, animals are anesthetized with isoflurane, the skull is surgically exposed and carefully leveled, and target coordinates are identified relative to Bregma. Small craniotomies are drilled, and reagents are injected at defined depths and volumes using a slow-in/slow-out approach to minimize tissue damage and reflux. Wounds are closed under sterile conditions, followed by standardized postoperative care and recovery. Injection accuracy and spread are verified post hoc by histological analysis.

Materials

Reagents & Solutions

- AAV6-CAG-aSyn, Stock titer: 2.3 × 10^{11} vp/µL;
- αSyn-PFF, Pre-sonicate according to your validated protocol (e.g., 30–60 s, amplitude 20%)
- Sterile saline (0.9%)
- Lidocaine/prilocaine cream for pre-incision analgesia (optional).
- Ophthalmic ointment for corneal protection.
- 70% ethanol for cleaning.
- Betadine for sterilization of surgical area.
- Sutures or wound clips

Instruments

- Kopf stereotaxic frame with mouse adaptor
- Isoflurane vaporizer + O_2 supply
- Heating pad
- Electric drill with micro-burr
- Hamilton syringe (NanoNeurons #7001)
- Nanoliter injector (WPI)
- Sterile drapes
- Scissors, forceps, scalpel
- Cotton swabs, sterile gauze

Troubleshooting

Problem
SNc-specific
Solution
• DV depth is critical: o Too shallow → VTA contamination o Too deep → cerebral peduncle / blood vessel damage • Ensure skull leveling is perfect • Use slow micropump speed to avoid tissue damage

Problem
Striatum-specific
Solution
• Craniotomy must be small and centered • Inject at two depths to ensure rostro-caudal spread • Avoid lateral ventricle breach (too medial or too dorsal)

Problem
General
Solution
• Always minimize air in the syringe → incorrect volume delivery • Clean drill between surgeries to prevent cross-contamination • Keep virus/PFF on ice but avoid >2 hour room-temperature exposure

Before start

Pre-Surgical Preparation
Reagents & Solutions

- **AAV6-CAG-aSyn**

Stock titer: **2.3×10^{11} vp/µL**
Aliquot into low-binding tubes keep on ice during surgery.

- **αSyn-PFF**

Pre-sonicate according to your validated protocol (e.g., 30–60 s, amplitude 20%).
Keep on wet ice; avoid freeze–thaw.

- **Sterile saline (0.9%)**
- **Lidocaine/prilocaine cream** for pre-incision analgesia (optional).
- **Ophthalmic ointment** for corneal protection.
- **70% ethanol** for cleaning.
- **Betadine** for sterilization of surgical area.
- **Sutures or wound clips**

Preparation of the Mouse - Anesthesia

1 Place mouse in induction chamber:

1.1 3–4% isoflurane in O_2 for 1–2 min until deeply anesthetized.

2 Transfer to nose cone on stereotaxic frame:

2.1 Maintain at ~2% isoflurane

2.2 Confirm anesthesia depth (toe pinch)

Preparation of the Mouse - Positioning

3 Secure ear bars gently into external auditory canals

3.1 Verify the skull is stable and mouse is centered

4 Keep mouse on heating pad to maintain **37°C**

Preparation of the Mouse - Eye & Analgesia

5 Apply ophthalmic ointment to both eyes

5.1 Reapply as needed throughout procedure to ensure sufficient lubrication.

6 Optionally apply local anesthetic cream to scalp (10 min prior)

Surgical Exposure of the Skull

7 **Shaving**

7.1 Shave entire top of head, approx 150x inscision sight

7.2 Clean area with **3× alternating betadine and ethanol**

8 **Incision**

8.1 Make a **~1.5–2 cm midline incision** using a scalpel

8.2 Clear connective tissue from bone using cotton swabs

9 **Leveling the Skull**

9.1 Using digital readout:

9.2 Position the manipulator tip on **Bregma**, set Z = 0

9.3 Move to **Lambda**, check DV difference

9.4 Adjust nose clamp until **Bregma–Lambda are within ≤0.05 mm DV**

10 This is essential for hitting SNc reliably.

Identification of Coordinates

11 Coordinates are relative to **Bregma** and **dura surface**:

12 **SNc Injection Coordinates**

12.1 **AP**: −3.6 mm

12.2 **ML**: ±1.5 mm

12.3 **DV**: −4.2 mm

12.4 **Volume**: 250 nL

13 Laterality:

13.1 Bilateral for behavioral/ephys

13.2 Unilateral for mapping

14 **Striatum Injection Coordinates** (*two-depth injection for volume spread*)

14.1 **AP**: +0.5 mm

14.2 **ML**: ±1 mm or ±2 mm

14.3 **DV**: −2.5 mm (first) and −3.5 mm (second)

14.4 **Volume**: 500 nL total per site

Drilling the Skull

15 Mark Injection Site

15.1 Using digital coordinates, lower the needle to hover above the skull

15.2 Mark the site using the needle tip or fine marker

16 Drill

16.1 Use micro-burr to create a **small craniotomy (~0.5 mm)**

16.2 Avoid damaging dura

16.3 Clean bone dust with sterile saline + cotton swab

Preparing the Hamilton Syringe

17 Load virus or αSyn-PFF **slowly** to avoid bubbles

18 Flick gently to remove air

19 Insert syringe into nanoinjector

20 Set injection rate corresponding to **10 min delivery**:

20.1 Example: for 250 nL → **25 nL/min**

20.2 For 500 nL → **50 nL/min**

Injection Procedure

21 **Lowering the Needle**

21.1 Move needle to AP and ML

21.2 Lower slowly until touching dura

21.3 Zero the Z coordinate

21.4 Lower to target **DV depth** (SNc: −4.2 mm)

22 **Injection ("slow-in slow-out" technique)**

22.1 Begin nanoinjection: **total duration 10 minutes**

22.2 Maintain stable isoflurane (2%)

22.3 **Do not move the animal or frame** during injection

23 **Post-Injection Diffusion Period**

24 After injection completes:

24.1 **Leave needle in place for 5 minutes** to allow diffusion

24.2 Prevent backflow by maintaining stable positive pressure

25 **Needle Withdrawal**

25.1 Withdraw **very slowly**, over ~1–2 minutes

25.2 This dramatically reduces reflux, especially in striatum

26 **Repeat for Bilateral Injections**

26.1 Move to next coordinate and repeat drilling + injection

26.2 Maintain sterile field at all times

Closing the Wound

27 Rinse skull with sterile saline

28 Gently lower scalp over skull

29 Close with:

29.1 **Sutures** (preferred) or **wound clips**

30 Apply topical antibiotic ointment

Postoperative Care

31 **Recovery**

31.1 Move mouse to warmed recovery cage (37°C)

31.2 Maintain monitoring until animal is fully ambulatory

31.3 Do not leave unattended until righting reflex returns

32 **Analgesia**

32.1 According to IACUC approved protocol, adminisiter **meloxicam and/or buprenorphine**

32.2 Continue to give for 48-72 hours

33 **Surgical Recovery Time**

33.1 Allow **at least 2 weeks** before behavioral testing or physiology

Verification of Injection Accuracy - SNc / Str Target Validation

34 Perfuse at experimental endpoint

35 Perform **TH immunostaining**

35.1 SNc: dense TH+ dopaminergic zone

35.2 STR: characteristic TH+ terminal fields

36 Check for:

36.1 Injection tract position

36.2 Spread of virus/PFF

36.3 Hemispheric symmetry (if bilateral)