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Rodent intracerebroventricular AAV injections

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Protocol status: Working

We use this protocol and it's working

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Abstract

This abstract outlines the procedure for AAV injections into the lateral ventricle of C57BL/6J mice (intracerebroventricular injections)

Protocol materials

⊗ DBA/2J mice The Jackson Laboratory Catalog #000671

⊗ C57BL/6J The Jackson Laboratory Catalog #000664

⊗ BALB/cJ The Jackson Laboratory Catalog #000651

Troubleshooting

Rodent surgery

- 1 Raise and house mice in compliance with your local Institutional Animal Care and Use Committee (IACUC) and ensure that all protocols and procedures have been approved by the appropriate ethical and regulatory committees.

Obtain mice from The Jackson Laboratory (male, 6-8 weeks).

☒ C57BL/6J The Jackson Laboratory Catalog #000664

☒ BALB/cJ The Jackson Laboratory Catalog #000651

☒ DBA/2J mice The Jackson Laboratory Catalog #000671

- 2 Anesthetize mice using isoflurane (5% for induction, 1-3% for maintenance) with 95% O₂/5% CO₂ (1 L/min).
- 3 Head fix mice in a stereotaxic frame, shave head using clippers, and sterilize the area with chlorohexidine.
- 4 Administer 0.05 mL of 2.5 mg/mL bupivacaine subcutaneously above the skull.
- 5 Using a surgical blade, make an midline incision to reveal the skull. Clean the skull of blood and connective tissue and level the head in the stereotaxic frame.
- 6 Intraoperatively administer 1 mg/kg of buprenorphine SR and 5 mg/kg of ketoprofen via subcutaneous injection.
- 7 Drill 2 bilateral burr holes above the lateral ventricles using the following coordinates:
 - Anterior-posterior: 0.6 mm posterior to bregma
 - Medial-lateral: 1.15 mm from the midline
- 8 Using a 10 µL NanoFil syringe with a 33 gauge beveled needle, aspirate viral vectors (5×10^{10} or 1.5×10^{11} vector genomes in 3-5 µL) using a microsyringe pump.

Equipment

SUB-MICROLITER INJECTION SYSTEM

NAME

NanoFil Syringe

TYPE

World Precision Instruments

BRAND

NANOFIL

SKU

<https://www.wpiinc.com/var-3167-sub-microliter-injection-system>

LINK

Equipment

33 guage beveled NanoFil needle

NAME

NANOFIL NEEDLES

TYPE

World Precision Instruments

BRAND

NF33BV-2

SKU

<https://www.wpiinc.com/var-3184-nanofil-needles>

LINK

Equipment

UMP3 ULTRAMICROPUMP

NAME

Microsyringe pump and controller

TYPE

World Precision Instruments

BRAND

UMP3-3

SKU

<https://www.wpiinc.com/var-3735-ump3-ultramicropump>^{LINK}

- 9 Slowly lower the needle through the burr hole into the lateral ventricle (1.6 mm from the pial surface) and allow the needle to sit in place for approximately 5 minute. Inject viral vector at a rate of 300 nL/min.
- 10 Allow needle to stay in place for 5 minutes after injection has been complete. Slowly remove needle, and suture.
- 11 For 5-days post-surgery, mice were treated with 30 mg/kg of ibuprofen and 60 mg/kg of trimethoprim/sulfamethoxazole via water.