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Rodent intracerebroventricular AAV injections

Miguel Chuapoco¹

¹California Institute of Technology



Miguel Chuapoco
California Institute of Technology

ABSTRACT

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

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

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Rodent surgery

Raise and house mice in compliance with your local Institutional Animal Care and Use

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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% $O_2/5\%$ CO_2 (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.
- 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 μ L NanoFil syringe with a 33 guage beveled needle, aspirate viral vectors (5 x 10¹⁰ or 1.5 x 10¹¹ vector genomes in 3-5 μ L) 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.
- 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.