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Stereotactic injection of viral vectors

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We use this protocol and it's working

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Abstract

Here we describe stereotactic injection of AAV into ventral tegmental area (VTA) of mice.

Attachments



surgery.docx

24KB

Materials

Material	Supplier/Catalog Number
Glass bead sterilizer	Germinator 500, CellPoint Scientific
Heating pumps (x2)	T/Pump, Stryker
Autoclaved paper towels, cottonswabs, and absorbent pads	-
Autoclaved surgical tools	-
Glass pipette	3-000-203-G/X Drummond Scientific
Pipette puller	P-1000, Sutter Instrument
Nanoject III	Drummond Scientific
Stereotactic frame	Kopf 1900

Materials for Section A

Isoflurane	502017, Primal Critical Care
Eye ointment	Optixcare, Aventix
Carprofen	NDC 13985-748-20, Norbrook Laboratories Limited
Betadine	NDC 67618-151-17, Atlantis Consumer Healthcare Inc.
Drill	1911, Kopf
Micro drill bit	SGS 10227907
0.9% Sodium Chloride	00409-4888-10, Pfizer Hospital
Eye Spears	DeRoyal, 30-049

Section B

A	B
AAV1-EF1a-FLEX-HTB	Salk Institute
Parafilm	PM-996, Bemis
Mineral Oil	BP 2629-1, Fisher Bioreagents

Section C

Uncoated polyester non-absorbable sutures	1176B, Look
Triple antibiotic ointment	22335, Medique Products

Section D

A. Pre-surgery Preparation

- 1 Pull pipettes and cut at 9.0 mm from start of glass bend with microdissection scissors.
- 2 Wash hands and put on clean lab coat and gloves
- 3 Turn on glass bead sterilizer, heating pads (for operation and recovery), stereotactic coordinate display, nanoject, and drill.
- 4 Wipe stereotactic frame and surrounding area with 70% EtOH.
- 5 Place autoclaved tools in sterile field.
- 6 Place autoclaved absorbent pad on top of operation heating pad and place in frame.

B. Animal placement and craniometry

- 7 Weigh mouse then place in induction chamber at 4% isoflurane for five minutes.
- 8 Once mouse is anesthetized and displaying slowed, deep breathing, place mouse into nose cone of stereotactic frame with 2% isoflurane.
- 9 Fix mouse into place with ear bars.
- 10 Add ophthalmic ointment to eyes.
- 11 Inject carprofen (5mg/kg s.c.)



- 12 Wipe top of head with betadine.
- 13 Check for anesthesia.
- 14 Use scalpel to cut scalp and periosteum in one motion, in rostral to caudal direction.
- 15 Gently push two sides of periosteum apart with autoclaved cotton swabs to expose skull.
- 16 Clean skull with cotton swabs and sterile saline if needed.
- 17 Once the skull is dry, attach drill and locate bregma using the stereotax binocular. Use the drill to lightly mark bregma. Zero the medial-lateral (ML), anterior-posterior (AP), and dorsal-ventral (DV) coordinates.
- 18 Locate lambda. If change in AP axis is greater than 0.05 mm, adjust skull's pitch. Return to bregma, re-zero coordinates, and locate lambda again.
- 19 Ensure that there is less than 0.05mm of tilt in ML axis by measuring DV at AP: -2 between ML:+2 vs -2. Otherwise adjust skull's tilt.
- 20 Bring skull to ML and AP coordinates of region of interest. For VTA we used ML: ± 0.35 and AP: -3.4 mm. Turn drill on and slowly lower into skull. Stop before reaching dura.
- 21 Wipe skull clean with sterile saline and cotton swabs.
- 22 Pierce dura with the tip of a 32 gauge needle. Use eye-spears to stop bleeding. Clean area with saline and cotton swabs as needed.

C. Viral Injection

- 23 Partially fill a 1 ml syringe with mineral oil, then plunge into pulled glass pipette until full. Ensure no bubbles are in pipette. Wipe clean with cotton swab. Be careful to not break pipette tip for the duration of the surgery.



- 24 Insert glass pipet into nanoject and empty the nanoject
- 25 Remove drill from stereotactic holder and replace with nanoject.
- 26 Pipet ~2 uL of virus onto parafilm.
- 27 Use nanoject controller to draw virus into pipette. Ensure no air is pulled into pipette. Mark oil/virus meniscus on glass pipette
- 28 Bring pipette to bregma and re-zero coordinates.
- 29 Ensure pipette is working by injecting a small amount of virus with pipette tip visible. Wipe virus away with cotton swab.
- 30 Use coordinates to bring pipette to region of interest. Slowly lower pipette into brain. For VTA, we use coordinates ML: ± 0.35 , AP: -3.4 , DV: -4.4 mm relative to Bregma.
- 31 Begin injection program. We inject 150 nl virus per side at 10 nl/sec, 5 sec delay between 15 injection cycles.
- 32 Leave pipette in place for 10 minutes after injection. Slowly withdraw pipette by 0.05 mm, wait one minute, then slowly and complete withdraw pipette.
- 33 Try injecting a small amount of virus with the pipette tip visible to ensure no blood is clogging it. If no virus comes out, gently wipe tip with saline and cotton swab until the clog is removed.
- 34 Ensure meniscus is lower than marked and mark it again if you continue with additional surgeries

D. Suturing and recovery

- 35 Rehydrate skin near skull with saline.



- 36 Suture skin together.
- 37 Apply antibiotic ointment.
- 38 Remove animal from frame and place in recovery box with heating pad. Turn off isoflurane flow to stereotactic frame.
- 39 Once animal is ambulating and responsive, place it back into its home cage with clean bedding.
- 40 Monitor the animal's weight and behavior for at least 5 days after surgery. Inject carprofen (5mg/kg s.c.) first day post-operation.
- 41 Wait at least 3 weeks for viral expression and surgery recovery.