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Stereotaxic surgery

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We use this protocol and it's working

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Abstract

Stereotaxic surgery

Materials

O2 tank, sterile instruments, drugs: isoflurane, buprenex, meloxicam, 70% ethanol, Betadine, warming pad for stereotax and for recovery, eye moistener, lidocaine for ears, solution to inject, syringes, wound clips, scalpel

Prior to surgery

- 1 Transfer cages to conventional or containment
- 2 Autoclave surgical instruments
- 3 Prepare everything needed for surgery (sterile instruments, drugs: isoflurane, buprenex, meloxicam, 70% ethanol, Betadine, warming pad for stereotax and for recovery, eye moistener, lidocaine for ears, solution to inject, syringes, wound clips, scalpel)

Pre-op

- 4 Ensure isoflurane reservoir is filled between the two black lines.
- 5 Open O2 tank
- 6 Adjust O2 flow rate to 0.8-1.5
- 7 Put mice in anesthesia chamber
- 8 Ensure line to stereotaxic apparatus is pinched
- 9 Set isoflurane to 3%
- 10 Once the mouse is anesthetized switch flow line to mask and set isofluorane to 1.5-2 and O2 rate to 0.8-1.5.
- 11 Inject mice with Buprenex subcutaneously at 0.1mg/kg; Buprenex, buprenorphine hydrochloride, comes in 0.3mg/mL solution. (a 30g mouse would be injected with 0.1mL)

Surgery

- 12 Unpinch isoflurane line to the stereotaxic apparatus, pinch line toward chamber
- 13 Install animal in the stereotaxic apparatus, use the mouthpiece and adjust the mask
- 14 Set isoflurane concentration to 1.8-2% and O2 rate to 0.5-0.8
- 15 Put eye moistener and ensure there's plenty on the eye throughout surgery
- 16 Once the animal is anesthetized, secure the head in place with the ear pieces
- 17 Shave head
- 18 Prepare head with Betadine, followed by 70% ethanol
- 19 Ensure deep anesthesia by pinching one foot
- 20 Make a 5 mm incision in a rostro-caudal direction in between the ears
- 21 Expose the skull via blunt dissection of the overlying musculature and scrape off connective tissue
- 22 Identify landmark point bregma and calculate stereotaxic coordinates
- 23 Use the microdrill to puncture a small hole in the skull at the rostro-caudal/medio-lateral stereotaxic coordinates
- 24 Lower needle to the dorso-ventral coordinate over 30s to 1min (can add 0.02mm to create a pocket, then retract to the calculated z coordinate)

- 25 For viral vector solution, inject 0.2-0.4uL over a period of 5min (0.08uL/min), for cell solutions inject 1 to 2 ul over 5 min.
- 26 Wait 5 min after the injection, then withdraw the needle slowly
- 27 Close the skin with wound clips (make sure they're not close to the eye and that they are tight)
- 28 Wash Hamilton syringe 10 times with RO water and 10 times with Acetone.
- 29 Spray table and all instruments with 70% EtOH and then with fresh 10% bleach.
- 30 Wash and autoclave surgical tools.

Post-op

- 31 Put mouse in clean cage with chow on the floor cage and a water bottle. The cage should be on a heating pad in low.
- 32 During recovery from anesthesia, observe mice carefully (at least every five minutes).
- 33 4-6 hours after Buprenex injection, inject mouse with subcutaneous Rimadyl (5 mg/kg); Stock injection is 0.5mg/mL (for a 30g mice volume injected is 0.3mL)
- 34 Once the mice are returned to their holding room, they will be monitored on a daily basis for signs of pain or distress: including decreased activity, hunched posture, decreased grooming, and teeth chattering. Signs of infection, like heat, swelling, redness, and exudation will also be monitored for.