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Stereotaxic Viral Injections Into VTA and PnO (No Implant)

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Protocol status: Working

We use this protocol and it's working



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Abstract

This protocol describes stereotaxic delivery of viral vectors into the ventral tegmental area (VTA) and the oral pontine reticular nucleus (PnO) under isoflurane anesthesia. This version covers injections only (no fiber or optrode implantation).

Materials

- Isoflurane anesthesia system.
- Stereotaxic frame and mouse adaptor.
- Heating pad set to 37 C.
- Micropipettes or nanoliter injector suitable for small-volume injections.
- Surgical instruments and sterile supplies.
- Viral vectors kept on ice during setup.
- Analgesic and supportive care supplies.

Step-by-Step Procedure

- 1 Induce anesthesia with isoflurane (3-4% induction) and maintain at 1.0-1.5% in room air.
- 2 Place the mouse in a stereotaxic frame on a 37 C heating pad. Protect eyes.
- 3 Shave and disinfect the scalp. Make a midline incision and expose the skull.
- 4 Level the skull at bregma and lambda.
- 5 Drill a small craniotomy above the target coordinate(s).
- 6 Load viral solution into the injector. Lower to the target DV coordinate slowly.
- 7 Inject virus using a slow, controlled infusion.
- 8 Wait briefly after injection before retracting the injector to reduce reflux.
- 9 Repeat for additional targets as required (VTA and/or PnO).
- 10 Close the scalp with sutures or wound clips.
- 11 Monitor the animal until fully recovered from anesthesia and provide post-operative care.

Critical Steps

- 12 Confirm skull leveling before targeting coordinates.



- 13 Use slow injections and a dwell time to reduce backflow.
- 14 Keep injection approach consistent across animals and conditions.

Acknowledgements

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