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6-Hydroxydopamine lesion and virus injection



Forked from [Striatal injection of 6-OHDA or Saline and post-operative care \(Mendonça et al 2024\)](#)

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We use this protocol and it's working

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Abstract

Injection of 6-OHDA(6-Hydroxydopamine) or vehicle and virus injection protocol used in Alcacer et al.

Guidelines

All procedures were done in aseptic conditions.

Materials

- Stereotaxic surgery set up required (this protocol uses Kopf instruments)
- Nanojet II Injector (Drummond Scientific, USA)
- Surgical Tools
- 6-Hydroxydopamine hydrochloride (Sigma Aldrich, Portugal) was dissolved in 0.02% ice-cold L-ascorbic acid/saline (3.2 µg free-base 6-OHDA/µL)
- AAV5.CAG.Flex.GCaMP6f.WPRE.SV40 virus (made by University of Pennsylvania Vector Core, Addgene Cat# 100835-AAV5)

Before start

Mice were kept in deep anesthesia using 1-3% isoflurane and oxygen (at 1L/min flow rate) and mouse body temperature was maintained at 37 degrees Celsius using a temperature controller (ATC1000, World Precision Instruments). Surgeries were performed on a stereotactic frame (David Kopf Instruments, Model 962LS) with a mouse adaptor (David Kopf Instruments, Model 923-B Mouse gas anesthesia heads holder).

Stereotaxic Surgery

- 1 Anesthetize mouse with isoflurane.
- 2 Shave head and transfer to stereotaxic apparatus and secure the head and keep mouse anesthetized for length of procedure.
- 3 Disinfect surgical area of the mouse head with 70% ethanol and iodine.
- 4 Make a small incision in the skin to expose the skull.
- 5 Carefully remove any connective and muscle tissue from the exposed area.
- 6 Level the skull surface to be at less than 0.05mm by comparing the height of bregma and lambda, and also in medial-lateral directions.

6-OHDA or Vehicle Injection

- 7 Find the injection coordinates (coordinates used for medial forebrain bundle : AP= - 0.7, ML= - 1.2, DV= - 4.7) and mark area for drilling.
- 8 Drill Burr-hole in marked areas carefully.
- 9 Load glass pipette into the pipette holder (Nanojet II (Drummond Scientific)) and affix to the stereotaxic frame.
- 9.1 Set rate of injection to 4.6 nL per pulse every 5 seconds.
- 10 Leave pipette in place for 2 minutes before injection.
- 11 Inject 1 ul of 6-OHDA or vehicle solution then wait 10 minutes after injection before removing.

Virus Injection

- 12 The capillary was replaced with a new one to inject the virus.
- 13 Inject 600 nL AAV5.CAG.Flex.GCaMP6f.WPRE.SV40 into right dorsal striatum, ipsilateral to the lesion (coordinates AP= + 0.5, ML= -2.3, DV= 2.3).

Post-operative Care

- 14 Close incision with tissue glue (Vetbond tissue adhesive, 3M, USA).
- 15 Inject carprofen (5mg/kg; 10uL/10g body weight) as an analgesia. Then inject 0.6 mL sterile glucose-ringer acetate (subcutaneously) to prevent dehydration.
- 16 Add that the mice in a warming cabinet at 27 °C to keep a constant body temperature avoiding hypothermia immediately after the surgery and during the first 2 weeks post-surgery.
- 17 During the first 2 to 3 weeks of surgery, mice receive daily subcutaneous injections of sterile glucose-ringer acetate solution (0.1 mL/10g body weight) and dietary supplementations.