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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

Striatal injection of 6-OHDA or Saline and post-operative care procedure from Mendonça et al 2024

Guidelines

All procedures were done in aseptic conditions.

Experiments were done on male mice-C57BL/6 or B6.SJL-Slc6a3tm1.1(Cre)Bkmn/J (Jax labs stock #006660).

Materials

-Stereotaxic surgery set up required (this protocol uses Kopf instruments)

-6-Hydroxydopamine hydrochloride (Sigma Aldrich AB, Sweden) was dissolved at a fixed concentration of 3.2 µg/µl free-base in 0.02% ice-cold ascorbic acid/saline and used within 2 h

Troubleshooting

Before start

Mice were kept in deep anesthesia using 1-3% isoflurane and oxygen (at 1L/min flow rate).

Stereotaxic Surgery

- 1 Place the anesthetized mouse in the stereotaxic apparatus then stabilize the head.
- 2 Make a skin incision to expose the skull.
- 3 Carefully remove any connective and muscle tissue from the exposed area.
- 4 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 Saline Injection

- 5 Find the injection coordinates and mark area for drilling: dorsolateral striatum +0.5 mm anteroposterior and ± 2.5 mm lateral from bregma and 3.0 mm deep
- 6 Drill Burr-holes in marked areas carefully.
- 7 Load glass pipette into the pipette holder (Nanojet II (Drummond Scientific)) and affix to the stereotaxic frame.
- 7.1 Set rate of injection to 4.6 nL per 5 seconds.
- 8 Inject 2 μ L of 6-OHDA or saline.
- 9 After the injection finishes, leave the pipette in place for 10-15 minutes.

Post-operative Care

- 10 After surgery each animal had access to up 4 mg of food pellets/day (some mice were on food restriction pre-surgery)



- 11 Mice that showed weight loss were hand-fed (i.e. they were presented with the food while being held by the hands of the investigator) and DietGel Boost was placed in their boxed up to 4 days after surgery. In order to avoid competition for the food, weaker mice were placed in cages other than those containing unimpaired mice. The postoperative survival rate was 100%.