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Unilateral 6-hydroxydopamine lesion mouse model of Parkinson's disease

Forked from 6-Hydroxydopamine lesion and virus injection

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Abstract

To generate a mouse model of Parkinson's disease, unilateral injection of 6-OHDA(6-hydroxydopamine) into the medial forebrain bundle (MFB) was performed. Three to four weeks later, a drug-free cylinder test was used to assess the extent of lesion.

Guidelines

All procedures were done in aseptic conditions with proper PPEs.

Materials

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

-Surgical tools
-Ethanol wipes (sterile)
-Iodine wipes (sterile)
-Food color
-6-Hydroxydopamine hydrochloride (Sigma Aldrich) was dissolved in 0.02% ice-cold L-ascorbic acid/saline (3.2 µg free-base 6-OHDA/µL)
-Glass calibrated micropipette (Drummond Scientific Company, 200005)
-Surgical suture (sterile)
-Sterile saline
-Lidocaine cream
-500 ml glass beaker (e.g. VWR low form Griffin beaker)

Before start

Anesthesia of mice was induced at 5% isofluorane and maintained at ~2% isoflurane supplemented with oxygen and mouse body temperature was maintained at 37 degrees Celsius using a temperature controller (ATC1000, World Precision Instruments). Mice were administered with
analgesics meloxicam (METACAM®, 0.1mg/kg, s.c., Covetrus) before surgery. Surgeries were performed on a stereotactic frame (David Kopf Instruments, Model 940).

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 0.05 mm by comparing the height sof Bregma and Lambda, and also in medial-lateral directions.

6-OHDA or Vehicle Injection

7 Find the drill position on the skull (the coordinates of the medial forebrain bundle are: AP= - 0.7 mm from Bregma, ML= - 1.2 mm from Bregma, DV= - 4.75 from the dura surface) and mark the position with food color.

8 Drill Burr-hole in marked areas carefully.

9 Attach glass calibrated micropipette (Drummond Scientific) to the pipette holder.

9.1 Front-fill the pipette with freshly dissolved 6-OHDA. Avoid any bubbles.

10 Bring the pipette tip to Bregma and zero the coordinates

10.1 Move the pipette to the position right above the MFB and slowly insert the pipette tip into the brain until it reaches the MFB coordinates.

10.2 Leave the pipette in place for at least 2 minutes before injection.

11 Inject 1 ul of 6-OHDA at a speed < 0.5 ul/min. Wait 10 minutes after injection before slowly removing the pipette.

Post-operative Care

12 Close the incision with surgical suture (). Apply sterile lidocaine cream on the wound.

13 Inject 0.5 ml sterile saline i.p. to prevent dehydration.

14 Keep the mouse on a clean cage bottom (on a heating pad) until it recovers from the anesthesia. Transfer the mouse back to its clean home cage.

14.1 The cage should be kept on the heating pad during the first 2 weeks post-surgery to avoid hypothermia.

15 During the first 2 to 3 weeks of surgery, mice receive daily subcutaneous injections of sterile saline and dietary supplementations.

Cylinder test

16 Three to four weeks after 6-OHDA lesion, cylinder test is performed.

16.1 Place the mouse in 500 ml beaker and start the timer.

17 Count the numbers of weight-bearing touches by left and right forepaws in 3 min.

18 Calculate the percentage of (right paw touches/total touches). If this percentage is less than 25%, the mouse is considered near-complete lesioned and used for experiments.

Protocol references

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