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6-OHDA lesion in medial forebrain bundle

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We use this protocol and it's working

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Abstract

Lesion of the medial forebrain bundle using 6-OHDA to induce parkinsonian-like deficits in mice.

Guidelines

Note that sterile technique must be followed during this procedure to avoid health complications.

Troubleshooting


Anesthesia

- 1 Weigh mice.
- 2 Administer intraperitoneally a mixture of ketamine (Butler Animal Health Supply) and xylazine (Lloyd Laboratories) at concentrations of 110 and 10 mg/kg of body weight, respectively.
- 3 Administer desipramine 25mg/kg intraperitoneally 30 minutes prior to the intracerebral injection of 6-OHDA.

6-OHDA Preparation

- 4 Prepare 6-OHDA hydrobromide solution:
6-OHDA hydrobromide (Sigma-Aldrich) in PBS with 0.1% ascorbate at a concentration of 3.0 mg/mL

Each mouse will be administered injected with a total volume of 0.6 μ L.

*Note that this solution is light-sensitive and loses efficacy after 2 hours. Stock solution can be made and stored in  -80 °C and used for 2 hours before discarding.

Surgical Procedure

- 5 Place mouse into stereotactic frame (David Kopf Instruments) and place eye lubricant.
- 6 Remove fur from skull area using either electric shaver or depilatory cream.
- 7 Sterilize skull area and then cut open with scalpel.
- 8 Adjust brain so that it is level. Locate bregma, and zero X and Y axes on stereotaxic frame.
- 9 Move to injection coordinates and drill small hole into the skull.
Coordinates: AP -1.1 mm, ML -1.1 mm, DV -5.0 mm relative to bregma and the dural surface



- 10 Zero the Z axis on the stereotaxic frame.
- 11 Using a 10 μ L stereotactic syringe with a 30 G needle attached to a micro-infusion pump (World Precision Instruments), withdraw the 6-OHDA solution.
- 12 With the loaded syringe, navigate the needle to the desired Z coordinate.
- 13 Inject 0.6 μ L of 6-OHDA at a flow rate of 0.1 μ L/min.
- 14 To prevent reflux, leave the injection needle in place for 5 min, withdraw a short distance (0.3 - 0.5 mm), and then leave in the new position for an additional 2 min before complete removal of the needle.

Post-surgery Procedure

- 15 Close skin with non-absorbable sutures.
- 16 Give meloxicam 2mg/kg subcutaneously immediately after surgery and 24 hours post-surgery.
- 17 Give 1-2mL of dextrose 0.833% S.C. This is made by diluting 5% stock dextrose 1:6.
- 18 *Note that mice will weaken and lose body weight. Extra care must be given until body weight stabilizes to ensure survival.
 - Perform health checks twice a day for 15 days post-surgery or until the mice fully recover.
 - Check for any sign of distress, infection, and inflammation.
 - Give 1-2mL of dextrose 0.83% S.C. every day for the first 15 days post-injection.
 - Give diet supplements (e.g. condensed milk solution, moistened pellets).
 - Remove any bacterial plaques that form on genitals.
- 19 Remove sutures 15 days post-injections.

Apomorphine-induced Behavioral Test



- 20 Mice were allowed 4 to 6 weeks to recover before being subjected to the apomorphine-induced a behavioral test to estimate the extent of dopamine depletion in the substantia nigra. See protocol on protocols.io for more details.