



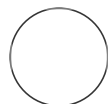
OCT 30, 2023

## 6-OHDA mouse model of Parkinson's disease

In 1 collection

Beatriz E Nielsen<sup>1</sup>

<sup>1</sup>University of Colorado Anschutz Medical Campus



Beatriz E Nielsen

University of Colorado Anschutz Medical Campus

### ABSTRACT

This protocol describes the steps for generating a 6-hydroxy-dopamine (6-OHDA) mouse model of Parkinson's disease.

Low or high doses of 6-OHDA are injected into the medial forebrain bundle to induce a partial or a more complete dopaminergic lesion respectively.

### MATERIALS

Desipramine hydrochloride

Pargyline hydrochloride

6-hydroxydopamine hydrobromide, Sigma-Aldrich, 162957.

### PROTOCOL MATERIALS

Dietgel ClearH2O Catalog #72-06-5022 In 2 steps

OPEN ACCESS



DOI:

[dx.doi.org/10.17504/protocols.io.81wgbxyjlpk/v1](https://dx.doi.org/10.17504/protocols.io.81wgbxyjlpk/v1)

**Protocol Citation:** Beatriz E Nielsen 2023. 6-OHDA mouse model of Parkinson's disease.

**protocols.io**

<https://dx.doi.org/10.17504/protocols.io.81wgbxyjlpk/v1>

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**Protocol status:** Working

**Created:** Aug 11, 2023

**Last Modified:** Oct 30, 2023

## Pre-operative care

- 1 1 to 3 days prior to surgery, provide mice (7-8 week-old, preferably in groups of at least 3 mice/cage) with:
  - Cage enrichment with hiding places (e.g. igloos) and nesting material (avoid cage changes the day before surgery).
  - Supplementary food to prevent food neophobia post-surgery (e.g. food pellets soaked in water, sunflower seeds, nutritionally fortified water gel [Dietgel ClearH2O Catalog #72-06-5022](#)).

## Drugs preparation

- 2 Preparation of desipramine ( $[M]$  2.5 mg/mL) and pargyline ( $[M]$  0.5 mg/mL) solution
  - 2.1 For a final volume of 10 mL, weigh the appropriate amount of each drug, accounting for the weight of the salt component such that the following concentrations are achieved:
    - Desipramine: 25 mg
    - Pargyline: 5 mg
  - 2.2 Add 7-8 mL of sterile saline and sonicate with heat  $37-45^{\circ}\text{C}$  until the mixture is completely dissolved.
  - 2.3 As the pH of this solution will be acid ( $\sim 3$ ), add  $[M]$  1 Molarity (m) NaOH until pH=7.4.
  - 2.4 Bring volume up to 10 mL with sterile saline.

2.5 Aliquot in 1.5 mL tubes and store them at  $-80^{\circ}\text{C}$ .

3 Preparation of low ( $1\ \mu\text{g}/\mu\text{L}$ ) and high dose ( $4\ \mu\text{g}/\mu\text{L}$ ) 6-OHDA solutions (freshly immediately before surgery):

3.1 For a final volume of 1 mL, weigh the appropriate amount of drug accounting for the weight of the salt component such that the following concentrations are achieved:

- 6-OHDA high dose: 5 mg 6-OHDA
- 6-OHDA low dose: 1 mg 6-OHDA

#### Note

As 6-OHDA is light and heat sensitive, avoid exposure to light, and place on ice prior to weighing.

When injecting high and low doses of 6-OHDA the same day, the low dose solution can be prepared by dilution from high dose solution. For 1 mL of low dose 6-OHDA, take 250  $\mu\text{L}$  of the high dose solution and add 750  $\mu\text{L}$  of vehicle.

3.2 Add 1 mL of sterile saline or sterile saline containing ascorbic acid ( $0.2\%$  volume).

#### Note

6-OHDA solutions must be used within approximately 4-5 hours after preparation. If the solution turns dark brown, this indicates that 6-OHDA has become oxidized and it must be discarded.

3.3 Wrap in aluminum foil, vortex until drug is dissolved and place in an ice bucket.

## Stereotaxic surgery

4 Gather supplies needed for surgery plus desipramine/pargyline and 6-OHDA solutions.

### Note

Unthaw the desipramine-pargyline solution aliquot and warm it at **37 °C**. Vortex and check there is no precipitation of pargyline.  
6-OHDA solutions are freshly prepared.

- 5 30 minutes prior to surgery, weigh each mouse and administer desipramine/pargyline solution at 10 mL/kg by intraperitoneal injection (i.p). (Final doses per mouse: desipramine 25 mg/kg and pargyline 5 mg/kg).
- 6 Start stereotaxic surgery. The full detailed protocol for performing stereotaxic surgery is available below, and specific considerations for 6-OHDA lesion model of Parkinson's disease are described here:
  - After loading injector with 6-OHDA solutions, carefully wrap the micropipette with aluminum foil, but leaving the tip exposed to enter the brain.
  - Inject 1 µL of high or low dose 6-OHDA for experimental mice and 1 µL of vehicle (saline or saline + ascorbic acid) for control mice, into the medial forebrain bundle (MFB) (coordinates: AP: -1.2 ML: +/- 1.3 DV: -4.75).

### Protocol



NAME

**Stereotaxic Surgery**

CREATED BY



Beatriz E Nielsen

**PREVIEW**

- 7 To prevent dehydration, inject 1 mL of sterile saline (or **5 % volume** sterile glucose) subcutaneously (s.c.).

## Post-operative care

- 8 As 6-OHDA lesion is associated to significant mortality rates, post-operative care is conducted for at least 1 week post-surgery to increase survival rates:

- 8.1** Check mice daily and monitor general health, weight (every 3-4 days) and signs of pain/distress, dehydration, hypothermia, aphagia and adipsia.
- 8.2** Keep house cages halfway onto the heating pad.
- 8.3** Keep providing supplementary (e.g. food pellets soaked in water, sunflower seeds, nutritionally fortified water gel  Dietgel ClearH2O Catalog #72-06-5022 ).
- 8.4** Dehydration is a common complication (slow retraction of the skin following skin pinch). Inject daily 1 mL of sterile saline i.p. or s.c. (or  5 % volume sterile glucose).
- 8.5** In male mice, also monitor genitals, since penis prolapse (paraphimosis) and urethral plugs (obstructive uropathy) are usual complications. If there is an urethral plug, apply a lubricant (e.g. ophthalmic ointment, mineral oil) and local analgesic cream if needed and try to remove it. Then use a lubricated stainless-steel probe to reinsert the penis into the prepuce if possible, or leave the penis lubricated and check for spontaneous resolution.