**Protocol for 6-OHDA unilateral lesioning in mice**

**Material list:**

* **Sterile saline** (Fresenius Kabi AB 9 mg/ml, 20 x 10 ml, Vnr 141856)
* **Carprofen** (Non steroidal anti-inflammatory drug, Rimadyl Bovis Vet. 50 ml Vnr 01 49 20). Stock solution 50 mg/Kg. Used at a 1:10 dilution (5 mg/ml/Kg), to renew regularly.
* **Marcain** (Local anesthetics, Bupivacaine Hydrochloride). Stock solution 5 mg/ml, aspen M130091AE). Used at a 1:3 dilution, to renew regularly.
* **Iodine** (Jodopax vet). For asepsis.
* **Isoflurane** (Attane Vnr 17 05 79, 250 ml).
* **L-Ascorbic acid** (**A92902,** Sigma)
* **NaCl** (Sigma)
* **6-OHDA hydrochloride** (**H4381,** Sigma).
* **Stereotaxic frame**
* **Anesthetic system** (isoflurane/air)
* **Tabletop microscope**
* **Parafilm** (used to put the drop of saline for withdrawing within the syringe).
* **Aluminum foil** (to protect the 6-OHDA solution from the light).
* **Cotton sticks**
* **Eye gel** (Bepanthen, Bayer, 2x5 g for nose)
* **Syringes for IP/SC injections** (Terumo Syringe Injection syringe 1ml 3d piece without needle SS + 01T1)
* **Needles for IP/SC injections** (Henke Sass Wolf, Fine-Ject 27Gx12” 0.4x12mm)
* **Syringe for 6-OHDA injection** (WPI, NanoFil 10uL syringe)
* **Needle for 6-OHDA injection** (WPI Nanofil, NF34BV, 34GA. Beveled needle)
* **Suture thread** (Ethicon Coated Vicryl rapide Plyglactin 910, 6-0 (0.7 Ph.Eur), 11 mm 3/8c, 45 cm, V32H)

**Experiment preparation:**

* Prepare a solution of 0.02% ascorbic acid / 0.9% NaCl. Make a consequent number of aliquots of 1 mL from this solution which can be stored at -80°C for many years. Once thawed, the aliquot should be used within the day and not be re-frozen.
* The 6-OHDA solution is made freshly the same day as the injections. To obtain a concentration of 1.85 mg/ml of 6-OHDA free-base, weight 2.2 mg of 6-hydroxydopamine-hydrochloride powder and add it to the ascorbic acid/NaCl 1 ml aliquot. It is important to keep the 6-OHDA solution away from the light to avoid oxidation.
* Carprofen solution: dilute the Carprofen stock solution 1:10 in sterile saline to obtain a concentration of 5 mg/mL/Kg.
* Marcain solution: dilute the Marcain stock solution 1:3 in sterile saline to obtain a concentration of 1.67 mg/mL/Kg.
* Syringe filling: The syringe is filled with first 1000 nL of saline, followed by 1000 nL of air and then the 6-OHDA solution. The amount of 6-OHDA solution withdrew in the syringe depends on the amount of animals injected within the day. Put aluminum around the syringe to protect the 6-OHDA solution from the light (oxidation) and avoid to direct lights towards the syringe.

**Experimental protocol for 6-OHDA injection:**

1. Place the mouse in the isoflurane gas chamber; turn on the isoflurane at a rate of 4% (isoflurane/air).
2. Once the mouse is deeply asleep and its respiration stable, decrease the rate to 2% isoflurane/air and switch the direction of the pump towards the mask on the stereotaxic apparatus.
3. Weigh the mouse and immediately put the animal in the mask of the stereotaxic apparatus.
4. Place the mouse in the stereotaxic apparatus and adjust the ear bars so the head of the mouse is well fixed.
5. Put eye gel on the mouse’s eyes for protection and inject Carprofen subcutaneously (5 mg/mL/kg)
6. Shave the head with a scissor or a trimmer. Decontaminate the skin with iodine and inject Marcain subcutaneously (1.7 mg/mL/Kg) at the place of the incision until you see a small bump.



1. 5 min after injecting the Marcain, incise the skin with the scalpel along the sagittal plan. Clean the skull’s surface until the bone sutures are clean and clearly visible.
2. Look for the sagittal vein and define the midline. The sagittal vein is used as the zero in the medio-lateral (MD) plan. Zero the MD coordinate (X). Then, define your bregma (meeting point between the coronal sutures and the sagittal vein) and zero the antero-posterior (AP) coordinate (Y).
3. Verify that the head is well positioned horizontally: Zero the dorso-ventral (DV) coordinates on the bregma (skull). Move the needle to the lambda and look at the Z coordinate. The acceptable error of the Z value is ± 0.07 mm between the bregma and the lambda.
4. Go back to bregma and zero all the coordinates. Take your AP coordinate (MFB: -1.20 mm for AP) and ML coordinate (MFB: -1.10 mm). Mark the AP and ML coordinates on the skull and drill the hole.
5. Clean the hole making sure there is no dura matter, pieces of bone left or clogged blood. Place the needle on the brain surface and zero the Z coordinate. Slowly lower the needle to -4.75 mm to reach the MFB.
6. Inject 1 µL of the 6-OHDA solution at a rate of 100 nl.min-1.
7. Let the needle in place for 10 min before lifting it up slowly.
8. Stich the skin of the head back and apply iodine.
9. Inject subcutaneously 1 mL of sterile saline.
10. Put the mouse in a “waking” cage on top of a heating pad until the mouse is fully awake (normal locomotion and exploratory behavior, food consumption). The mouse is then put back in its home cage.
11. 20 to 24 hours after the first Carprofen injection, a second subcutaneously injection of Carprofen (5 mg/ml/Kg) is performed.

**Post-operative care:**

* Mice are weighted daily to keep track on potential weight loss.
* The 6-OHDA injected mice are injected daily with 1 ml of saline, subcutaneously, until sacrifice. The saline is warmed up before injecting (around 35-37°C) to avoid hypothermia.
* A petri dish is available in the cage, filled with 15% sucrose solution. A couple of food pellets is placed in the petri dish so they get softer and easier to eat.
* Hydrating caloric gel can be placed within the cage if needed.
* Sun flower seeds can also be placed in the cage.
* Cages are changed to clean cages at least once a week.