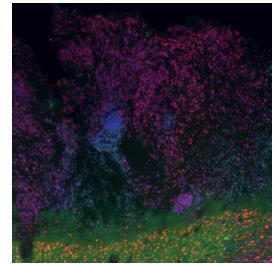


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Xenotransplantation of cortical progenitors into athymic mouse brain

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We use this protocol and it's working

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Abstract

This protocol details the surgical procedure for xenotransplantation of cortical progenitors into the striatum of athymic mice (details on generation of cortical progenitors and preparation of cells for transplantation are linked out to other protocols).

Guidelines

All animal handling should be in accordance with national and institutional animal ethics approval.

Correct management of pain relief should be overseen and documented by the responsible personnel in accordance with local and national guidelines.

Materials

Materials

- Adult (8-10 week) athymic mice (BALBc/Nu) (ARC now Ozgene)
- iPSCs differentiated into cortical progenitors (D25-30 of differentiation depending on desired experimental outcomes), typically grown in 48 well plates. Full details of the cortical differentiation protocol:
dx.doi.org/10.17504/protocols.io.bu6znzf6.

Consumables/Equipment

- Stereotaxic Frame (Stoelting with heating pad)
- Recovery cage or Thermacage with heating pad
- Sterile Surgical Instruments
- Glass micro Pipette (Hamilton 5uL)
- Microsyringe needle (recommended: 25G – 30G; 23 gauge needle may be required for a more viscous injectate).
- Non-absorbable monofilament suture material (size 5)
- physiological saline
- Size 10 scalpel blades
- Pipettes (P20, P200, P1000)
- Gowns, gloves, face masks
- Isoflurane (Gaseous anaesthetic agent)
- Buprenorphine (analgesic), meloxicam (analgesic)
- Lacrilube (eye gel)
- alcohol swabs
- iodine/povidone

Hardware

- Zeiss surgical Microscope

Safety warnings

- ! This protocol involves inhalation anaesthetic. Adequate ventilation should be ensured for both animal and operator safety.
For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

Ethics statement

All animal procedures were conducted in agreement with the Australian National Health and Medical Research Council's published Code of Practice for the Use of Animals in Research, and experiments approved by The University of Sydney (approval number: 2020/1824).

Application of this protocol will need prior approval by the users' institutional review board (IRB) or equivalent ethics committee(s).

Before start

Prior to surgery, the cortical iPSC's need to be generated and banked dx.doi.org/10.17504/protocols.io.bu6znzf6 for transplantation. Correct thawing and preparation protocol for the preparation can be found here dx.doi.org/10.17504/protocols.io.x54v9ppeqg3e/v1

All equipment should be sterilised (autoclaved or cold sterilant), and all surgical surfaces cleaned where the procedure will take place. All animals should be weighed and checked for signs of general health prior to the procedure.

Animal preparation

- 1 Heating pads on both the stereotaxic apparatus and recovery chamber are turned on to 37.8 degrees Celcius.
- 2 The mouse is placed into an induction chamber and anaesthesia induced with 5% isoflurane until there is no flexor withdrawal reflex.

The animal is moved to the stereotaxic apparatus and face placed in the nose cone where inhaled anaesthesia is redirected and reduced to 2-4% in oxygen to maintain anaesthesia. The animal is secured to the apparatus with the ear bars.

- 3 Lubricant is applied to both eyes to prevent them from drying out during anaesthesia (e.g. Lacrilube). Analgesia (Buprenorphine at a dose of 0.05mg/kg s.c.) is administered peri-operatively while the animals are maintained on heat pads.

Surgical procedure

- 4
 1. The surgical site on the dorsal surface is disinfected with an alcohol swab.
 2. A 1 – 1.5 cm long skin incision is made in the scalp using a sterile scalpel and bregma located. The stereotaxic equipment is zeroed in anterior-posterior and medio-lateral to designate Bregma.
 3. Striatal coordinates were dialled in on the stereotax as follows for a unilateral injection:
AP = +1
ML = -1.5
DV = -1.5
 4. A small burr hole is drilled into the skull at the coordinates above until a small piece of skull remains which can be lifted off to expose the dura.
Note: Extreme care is required when performing craniotomy using a surgical drill to ensure that the drill tip does not penetrate too deep and perforate brain tissue
 5. The glass syringe and capillary are flushed with saline to ensure patency.
 6. Approximately 1uL of cell suspension or 100,000 cells of cortical progenitors (prepared as per protocol <https://dx.doi.org/10.17504/protocols.io.x54v9ppcgg3e/v1>) are drawn up into the glass capillary
 7. The glass pipette or syringe needle is inserted into the brain through the hole and lowered to the depth of the target site as indicated by the coordinates above

8. Cell suspension is injected over a several minutes to ensure minimal reflux (0.5 μ l per minute). The needle or glass pipette needs to be left in place for a further 3 -5mins after injection has finished, before slowly withdrawing from the brain.
9. After completion of surgery, suture the incision site with with non-absorbable monofilament suture and paint the site with betadine/povidone solution
10. Administer an injection of Buprenorphine (0.05mg/kg s.c.) to obtain post-operative analgesia and turn off isoflurane. Remove animal from ear bars and place in recovery cage.

Post-operative care

5 ***Monitoring recovery from anaesthesia***

Check animal is maintained in a warm recovery box with constant observation until the animal is mobile.

Post-operative Monitoring

Subsequent monitoring should be done 2 hours post operatively, and once daily over the following 48 hrs.. Mash feed or recovery diet can be offered post-operatively to help aid a rodents' recovery. Rodents are returned to their home cages once they're alert and ambulatory.

The surgical procedure is considered minor and most animals will not display evidence of distress. In the days after surgery the investigators will look for: Movement disturbances, infection around the sutures, weight loss of >10%, fur disturbance, signs of dehydration or shivering in the corner. Refer to the monitoring sheets to ensure the nature and frequency of monitoring is in line with what has been approved and may vary depending on the procedure.

Protocol references

Transplantation of Fetal Midbrain Dopamine Progenitors into a Rodent Model of Parkinson's Disease (protocols.io)

Thawing and preparation for grafting: <https://dx.doi.org/10.17504/protocols.io.x54v9ppeqg3e/v1>

Generation of cortical progenitors: dx.doi.org/10.17504/protocols.io.bu6znzf6