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Pseudorabies Virus (PRV) injection into interscapular brown adipose tissue

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ABSTRACT

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MATERIALS TEXT

Supplies

- BSL2 surgery room and animal housing
- Surgery cards and surgery record
- Personal protective equipment (PPE): coverall, bonnet, gloves mask, eye protection
- Eye lubricant
- Timer
- Biohazard stickers
- Bupivacaine/lidocaine
- 500nl blunt syringe with pulled glass pipette (Alternative is 1ul beveled Hamilton syringe)
- Aluminum foil (for easy cleanup of the virus work area)
- Kimtech paper
- Biohazard waste container for virus waste
- Sterile towels
- Surgery tools (scissors, hemostats, tissue forceps, teethed forceps)
- Sterile wound clip remover
- Sterile wound clips
- Sterile cotton swabs and gauze
- Isoflurane vaporizer, oxygen tanks, and anesthesia induction chamber
- Glass bead sterilizer
- Heating mats for surgery and recovery
- Pseudorabies virus stock (PRV-152)(http://www.cnnv.pitt.edu/PRVtable.pdf)
- Bupivacaine/lidocaine
- Carprofen
- Sterile Saline
- Novalsan
- 70% isopropyl rubbing alcohol
- 70% Ethanol
- Sterile H2O
- 0.5ml syringes (for bupivacaine/lidocaine and carprofen)
- 3ml syringes (for saline)
- 70% ethanol spray

BEFORE STARTING

Institutional requirements before you start:

- Approval of Institutional Biosafety Committee (IBC) to work with PRV.
- Approval of Institutional Animal Care and Use Committee (IACUC) to perform PRV injections in animals.

1

- All PRV work is performed in BSL2 laboratories, and all PRV injected mice are kept in BSL2 housing till euthanasia.
- All containers that are in contact with virus waste need to be properly labeled as biohazard waste.
- Virus stock needs to be placed on ice during surgeries.
- For virus transport make sure to follow IBC safety rules, double-containers are required for safe transport of biohazards (e.g. Eppendorf tube placed into 50ml Falcon tube).

Place a sterile towel on the surgery platform over the heating mat, another sterile towel in the anesthesia induction chamber.

2 Record the mouse's body weight to prepare syringes for saline and analgesia treatment.Warm saline (1-1.5 ml for 25 g body weight); bupivacaine/lidocaine (2.5-12.5 mg/kg body weight); carprofen (5-10 mg/kg body weight).

3 Induce anesthesia with 5% isoflurane in the induction chamber.

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4 Place the mouse on the surgery platform and maintain anesthesia via a nose cone at 1.5-2% Isoflurane/oxygen (adjust for each mouse accordingly to breathing and anesthetic depth (e.g. loss of withdrawal reflex)).

5 Apply eye lubricant to the mouse's eyes.

6 Disinfect the lower back of the mouse with 70% ethanol prior to subcutaneous carprofen injection.

7 Check for full withdrawal reflex to ensure anesthetic depth.

8 Use a clipper to shave the interscapular region of the mouse and remove all hair.

9 Disinfect the skin by three alternating scrubs of Novalsan followed by 70% Isopropyl rubbing alcohol.

10 Make a midline incision with sharp scissors from the scapula to the neck.

11 Remove connective tissue underneath the skin incision using a sterile cotton swab and teethed forceps until the interscapular brown adipose tissue (iBAT) depot is fully accessible.

The iBAT pad (light brown color) is surrounded by a layer of white adipose tissue. We have successfully injected virus dorsally into the iBAT, however, we could increase our PRV infection success rate by placing the injections into the iBAT core and accessing the ventral iBAT site. The rostral portion of the iBAT depot is detached from the interscapular muscle and the iBAT lobe is flipped back from the rostral side. This will allow visibility of the main iBAT vein (Sulzer vein) and injections are placed from the ventral iBAT side (see Figure 1).

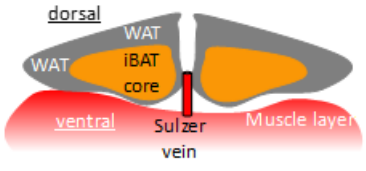

Figure 1: Schematic of iBAT depot

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12 Cover the iBAT with a saline soaked sterile gauze to keep the tissue from drying, then prepare the Hamilton syringe for injection.

13

Virus injections are prepared under a biological safety cabinet, while we handle surgeries and injections outside the biological safety cabinet.

Fill the Hamilton syringe with 500nl PRV-152 virus, return to the surgery platform with the Hamilton syringe to begin the injections.

14 Remove the saline-soaked gauze from the brown adipose tissue and locate the brown adipose tissue pad.

15 Place injections (5x100nl) into the right BAT and hold the syringe in place for ~30 seconds after each injection to minimalize backflow, slowly pull the syringe out of the BAT while holding a sterile cotton swab on the site of injection to dry of any possible virus leakage.

16 Place sterile saline-soaked gauze on top of the brown adipose tissue and clean the syringe under the biological safety cabinet with 70% ethanol. Flush the syringe about 10x with 70% ethanol. After that flush the syringe about 10x with sterile H2O.

Note: Collect all liquid as biohazard waste.

17 Remove the saline-soaked gauze from the brown adipose tissue and with a sterile cotton swab and teethed forceps, reposition the overlaying white adipose tissue back to its original location prior to your incision.

18 With the teethed and tissue forceps align both sides of the incision properly, and apply wound clips (~25 mm apart, to ensure proper blood circulation).

19 Inject bupivacaine/lidocaine subcutaneously around the incision site.

20 Inject warm saline intraperitoneal for rehydration.

21 Place the unconscious mouse in a fresh cage with approximately one half of the cage on a heating mat and monitor until startling reflexes are restored.

Use 70% ethanol to clean surgery tools and place in glass bead sterilizer for 15-20 seconds before additional surgeries.

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