Peripheral PRV injection - Kidney & Liver Protocol

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ABSTRACT

Pseudorabies viral injections allow for retrograde tracing with fluorescent markers. This protocol demonstrates how to inject into the target organ (kidney or liver) in order to visualize and identify the neural inputs that innervate these organs.

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ABSTRACT

Pseudorabies viral injections allow for retrograde tracing with fluorescent markers. This protocol demonstrates how to inject into the target organ (kidney or liver) in order to visualize and identify the neural inputs that innervate these organs.

Pseudorabies virus (PRV) injection into the left kidney or the liver

Institutional requirements before you start:

- Approval of Institutional Biosafety Committee (IBC) to work with PRV
- The Institution may require USDA vector permit for PRV work
- Approval of Institutional Animal Care and Use Committee (IACUC) to perform PRV injections in animals

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Guidelines

- Virus stock needs to be placed on ice during surgeries.

Safety

- For virus transport one must adhere to IBC safety rules, double-containers are required for safe transport of biohazards (e.g. Eppendorf tube placed into 50mL Falcon tube).
- All containers that are in contact with virus waste need to be properly labeled as biohazard waste.

Supplies

- BSL2 surgery room and animal housing
- Container filled with ice to transport and store virus during surgery, outside clearly marked as biohazard
- Surgery cards and surgery record
- Personal protective equipment (PPE): hair bonnet, gloves, face mask, eye protection
- Ophthalmic lubricant
- Timer
- Biohazard stickers
- Bupivacaine/lidocaine/torbugesic/meloxicam
- 500nL blunt syringe with pulled glass pipette (Alternative is 1nL beveled Hamilton syringe)
- Aluminum foil (for easy cleanup of the virus work area)
- Kimtech paper
- Biohazard waste container for virus waste
- Sterile towels
- Sterile surgical tools (scissors, hemostats, tissue forceps, teethed forceps)
- Sterile wound clip remover
- Sterile wound clips (7mm)
- Sterile cotton swabs and gauze
- Isoflurane vaporizer, oxygen tanks, and anesthesia induction chamber
- Glass bead sterilizer
- Heating pads 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
- Distilled H2O
- 0.5mL syringes (bupivacaine/lidocaine, carprofen/torbugesic/meloxicam injections)
- 3mL syringes (saline injection)
- 70% ethanol spray bottle
- Mini centrifuge for spinning down virus

Room preparation

- Turn on the glass bead sterilizer
- Turn on the two heating pads (designated surgery area and designated area for recovery)
- Place a non-sterile towel on your recovery heating pad and a sterile towel on the surgery area heating pad
- Place a sterile towel to the right of surgery platform along with your rack with the three 50mL falcon tubes filled with 'Novalsan', '70% Isopropanol' and 'Empty'
- Place multiple sterile cotton swabs into each 50mL tube
- Put an empty cage half way on the recovery heating pad
- Fill one 3mL syringe completely with sterile saline, place underneath your surgery heating pad to warm fluid
- Fill your 3mL syringes with sterile saline (~1.5mL for 25g mouse), place underneath your recovery heating pad to warm fluid
- Fill your 0.5mL syringes with Bupivacaine/lidocaine (0.1mL) or others
- Open the O_2 tank valve, set the O_2 level to 1% on the anesthesia machine
- Check your isoflurane level on your anesthesia machine, set to 5% for the induction box
- Place all of your surgical tools along with your eye lubricant, wound clip applicators, spare wound clips, sterile gauze and timer on your sterile towel to the right of your stereotaxic platform

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Virus preparation

- Tape sheet of aluminum foil on clean surface
- Place rack with three 50mL falcon tubes filled with '70% Ethanol', 'dH2O', Waste
- Test flow of syringe by filling with dH2O and flushing several times

Pre-incision

1. Record the mouse's body weight and (if applicable) ear tag number.
2. Place mouse into induction chamber with isoflurane set to 5%.
3. Once the mouse is unconscious, turn your isoflurane to 1.8% (adjust this number according to the mouse's breathing), open your valve to the surgery area, close your induction box valve.
4. Secure your nose cone.
5. Gently pull one of the mouse's hind limbs to check its withdrawal reflex for anesthetic depth.
6. Lubricate the mouse's eyes
7. Spray the region along the spine for kidney injections and the abdomen for liver injections with a small amount 70% ethanol and inject carprofen/torbugesic/meloxicam (e.g., 10uL-20uL/gram body weight/depends on the drug) subcutaneously.
8. Place a kimwipe underneath the mouse and shave the left side along the spine for kidney injection or the abdominal area region of the mouse for liver injection and remove all hair.
9. Wipe off any stray hair and collect all of the hair with the kimwipe laying underneath the mouse.
10. Disinfect freshly shaved area by lightly scrubbing skin with cotton swabs soaked in isopropanol and Novalsan. Perform three alternating scrubs of isopropanol then Novalsan.

Incision

1. Start your incision by pulling straight up on the skin at the left side of the spine below the ribs to be able to expose the left kidney and make a sagittal cut to expose the muscle. In case of the liver make a transverse cut under the ribcage to be able to expose the liver.
2. Using a sterile cotton swab, remove any connective tissue underneath your incision to spread incision apart.
3. Cut the muscle layers to make the kidney or the liver lobes accessible.
4. Apply sterile saline to sterile gauze and place it over the kidney or liver to keep the tissue moist, then prepare the Hamilton syringe for injection.
5. Fill syringe with PRV-152 virus, return to the surgery platform with syringe to begin injections.
6. Remove the saline-soaked gauze from the kidney or liver lobe.
7. Administer injections (1-2x1uL) into the left kidney or one of the liver lobes (be consistent) and hold the syringe in place for ~30 seconds after each injection to minimalize backflow, slowly pull the syringe out of the tissue while holding a sterile cotton swab on the site of injection to dry of any possible virus leakage.

a.Note: Syringe should be held parallel, not perpendicular while injecting.

1. Close the injection spot with applying surgical glue on the organ to avoid backflow.
2. Place a fresh, saline-soaked gauze on top of the kidney or liver and clean the syringe with 4-5 alternating flushes of 70% ethanol and dH2O. Collect flushed liquid and dispose as biohazard waste.

Closing incision

1. Remove the saline-soaked gauze from the kidney or liver and with a sterile cotton swab and teethed forceps, reposition the organ, then suture the muscle with sterile sutures.
2. With teethed and tissue forceps, pull up on the skin surrounding the incision in order to align both sides of incision properly, and with tissue forceps, begin going along the incision ensuring that the subcutaneous layer of skin is not sticking out past the external layer of the skin to ensure proper healing of incision.
3. Using teethed forceps and wound clip applicators, start at the side of the incision closest to the midline, and administer wound clips ~25mm apart from one another to allow for proper blood circulation for healing of the incision.
4. Once you have applied a sufficient amount of wound clips (usually 4-5), use your hemostats to tighten each of the wound clips to avoid the mouse scratching them off. Make sure not to tighten them too much or they will cause irritation and poor circulation around the wound.
5. Using teethed forceps, gently lift up skin at one end of incision and inject 0.05mL of bupivacaine/lidocaine subcutaneously. Repeat at other end of incision.
6. Turn isoflurane off, loosen up the nose cone and remove the mouse's incisors with the wooden end of a cotton swab from the hole on the mount.
7. Scruff the mouse and turn it over to inject ~1.5mL (for a 25g mouse) warm saline intraperitoneally
8. Place the unconscious mouse on the warm side of the cage that is half way on the recovery heating mat. Place mouse on its side.
9. Using water from animal's cage, wet a chow pellet and place on floor of cage so mouse can easily access food.
10. Place any tools used during surgery in the glass bead sterilizer for 15-20 seconds and change gloves.

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Post-operative care

- 1 day post-op: Administer carprofen or choice of drug subcutaneously (approx. 10uL/g bw), record weight of mouse, health check.
- 2 days post-op: Administer carprofen subcutaneously (approx. 10uL/g bw), record weight of mouse, health check.
- 3 – 7 days post-op: record weight of mouse, health check.
- 10 days post-op: remove wound clips.

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