



Apr 27, 2021

Pseudorabies virus (PRV) injection into inguinal white adipose tissue

Clara Huesing¹, Heike Muenzberg¹, Rui Zhang¹, Nathan Lee¹, Emily Qualls-Creekmore¹, Marie Francois¹

¹Pennington Biomedical Research Center

1

Works for me

dx.doi.org/10.17504/protocols.io.baamiac6

SPARC

Tech. support email: info@neuinfo.org

Clara Huesing

Pennington Biomedical Research Center

ABSTRACT

We utilize pseudorabies virus (PRV) retrograde tracing in combination with reporter mice, iDISCO, and confocal/light sheet microscopy to identify the location of pre- and post-ganglionic neurons as well as nerves that selectively innervate the iWAT in the mouse.

DOI

dx.doi.org/10.17504/protocols.io.baamiac6

PROTOCOL CITATION

Clara Huesing, Heike Muenzberg, Rui Zhang, Nathan Lee, Emily Qualls-Creekmore, Marie Francois 2021. Pseudorabies virus (PRV) injection into inguinal white adipose tissue. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.baamiac6>

LICENSE

————— This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Dec 09, 2019

LAST MODIFIED

Apr 27, 2021

PROTOCOL INTEGER ID

30765

GUIDELINES

Virus stock needs to be placed on ice during surgeries.

MATERIALS TEXT

- 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
- 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, toothed 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 and carprofen injections)
- 3mL syringes (saline injection)
- 70% ethanol spray bottle
- Mini centrifuge for spinning down virus

SAFETY WARNINGS

- 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.
- Surgery must be conducted in a BSL2-designated area

BEFORE STARTING


- 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

Room preparation

- 1 Turn on the glass bead sterilizer
- 2 Turn on the two heating pads (stereotaxic platform and designated area for recovery)

- 3 Place a non-sterile towel on your recovery heating pad and a sterile towel on the stereotaxic platform heating pad
- 4 Place a sterile towel to the right of the stereotaxic platform along with your rack with the three tubes filled with 'Novalsan', '70% Isopropanol' and 'Empty'
- 5 Place multiple sterile cotton swabs into each 50mL tube
- 6 Put an empty cage half way on the recovery heating pad
- 7 Fill one 3mL syringe completely with sterile saline, place underneath your stereotaxic heating pad to warm fluid
- 8 Fill your 3mL syringes with sterile saline (~1.5ml for 25g mouse), place underneath your recovery heating pad to warm fluid
- 9 Fill your 0.5ml syringes with Bupivacaine/lidocaine (0.1ml)
- 10 Open the O2 tank valve, set the O2 level to 1% on the anesthesia machine
- 11 Check your isoflurane level on your anesthesia machine, set to 5% for the induction box
- 12 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

Virus preparation

- 13 Place aluminum foil on clean surface, this will be the designated area to test and fill syringe with PRV
- 14 Place rack with three tubes filled with '70% Ethanol', 'dH2O', Waste
- 15 

Test flow of syringe by filling with dH₂O and flushing several times. *If you do not observe a steady flow of water out of the syringe, one must replace the syringe as this indicates the virus will not be properly administered.*

Pre-incision

- 16 Record the mouse's body weight and (if applicable) ear tag number.
- 17 Place mouse into induction chamber with isoflurane set to 5%.
- 18 Once the mouse is unconscious, turn your isoflurane to 1.8% (adjust this number according to the mouse's breathing), open your stereotaxic valve, close your induction box valve and place into the stereotaxic nose cone, using a cotton swab to align its incisors into the hole.
- 19 Secure your nose cone by tightening the top screw, **gently** tug on the base of the mouse's tail to check if its incisors are inside the hole and the mouse is secured in the cone.
- 20 Gently pull one of the mouse's hind limbs to check its withdrawal reflex for anesthetic depth.
- 21 Lubricate the mouse's eyes
- 22 Spray interscapular region with a small amount (enough to cover carprofen injection spot) 70% ethanol and inject carprofen (10ul-20ul/gram body weight) subcutaneously.
- 23 Place a kimwipe underneath the mouse and shave the right dorsolumbar region of the mouse and remove all hair
- 24 Wipe loose fur off mouse using kimwipe placed underneath mouse
- 25 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

- 26 Start your incision by pulling straight up on the skin at the midline of the back, parallel to the hip with forceps and make a transverse cut that extends the length of the dorsolumbar portion of the iWAT fat pad.
- 27 Using a sterile cotton swab, remove any connective tissue underneath your incision to spread incision apart.

- 28 Remove connective tissue underneath the skin incision using a sterile cotton swab until the iWAT depot is fully accessible.
- 29 Apply sterile saline to sterile gauze and place it over the iWAT to keep the tissue moist, then prepare the Hamilton syringe for injection.
- 30 Fill syringe with PRV-152 virus, return to the surgery platform with syringe to begin injections.
- 31 Remove the saline-soaked gauze from the iWAT
- 32 Administer injections (10x200nl) into the right iWAT pad and hold the syringe in place for ~30 seconds after each injection to minimize backflow, slowly pull the syringe out of the iWAT while holding a sterile cotton swab on the site of injection to dry of any possible virus leakage.

32.1

Note: Syringe should be held parallel, not perpendicular, to the fat pad while injecting. This will help prevent piercing into the peritoneal wall, causing off-target labeling

- 33 Place a fresh, saline-soaked gauze on top of the iWAT and clean the syringe with 4-5 alternating flushes of 70% ethanol and dH2O. Collect flushed liquid and dispose as biohazard waste

Closing incision

- 34 Remove the saline-soaked gauze from the iWAT and with a sterile cotton swab and toothed forceps, reposition the overlying skin back to its original location prior to incision.
- 35 With toothed 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.
- 36 Using toothed 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.
- 37 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.
- 38 Using toothed forceps, gently lift up skin at one end of incision and inject 0.05mL of bupivacaine/lidocaine subcutaneously. Repeat at other end of incision.
- 39 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.

- 40 Scruff the mouse and turn it over to inject ~1.5mL (for a 25g mouse) of warm saline intraperitoneally
- 41 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.
- 42 Using water from animal's cage, wet a chow pellet and place on floor of cage so mouse can easily access food.
- 43 Place any tools used during surgery in the glass bead sterilizer for 15-20 seconds and change gloves.

Post-operative Care

- 44 1 day post-op: Administer carprofen subcutaneously (approx. 10ul/g bw), record weight of mouse, health check
- 45 2 days post-op: Administer carprofen subcutaneously (approx. 10ul/g bw), record weight of mouse, health check
- 46 3 – 7 days post-op: record weight of mouse, health check
- 47 10 days post-op: remove wound clips, replace PVC pipe in cage