1. Materials
   1. Anesthesia : Inhalant Isoflurane
      1. Induction: 3.0 - 3.5% and
      2. Maintenance: 2.0 – 2.5%
   2. Analgesia:
      1. **Marcaine (Local)**
      2. Ethiqa XR / buprenorphine extended-release (Systemic)
   3. Sterile 0.9% saline
   4. Sterile ophthalmic ointment
   5. Electric hair clippers
   6. 70% ethanol
   7. 3% Hydrogen peroxide
   8. Motorized stereotaxic frame
   9. Heating pad
   10. Sterile surgical instruments
   11. Sterile gauze and swabs
   12. Surgical drill
   13. Hamilton syringe
   14. Steel needle
   15. Surgical tubing
   16. Pump
   17. Surgical glue

Procedure:

1. Turn on the heating pad to and set to 36.9°C
2. Weigh mouse and record weight before starting surgery
3. Place mouse in isoflurane chamber (3% isoflurane)
   1. Record time of anesthesia
4. After mouse is fully anesthetized, use hair clippers to shave head
5. Place mouse back into isoflurane chamber (3% isoflurane) until fully anesthetized
6. Transfer mouse onto stereotaxic frame and place the mouth / nose into nose cone with isoflurane
   1. Turn down isoflurane to 2%
7. Apply sterile ophthalmic ointment to eyes using a sterile swab (this will prevent desiccation)
8. Use ear bars to secure mouse in stereotaxic frame ensuring head is level in all directions
9. Using a sterile swab apply 70% EtOH to the scalp
10. Using a sterile swab, apply povidone-iodine solution to the scalp
    1. *Wait for povidone-iodine solution to dry before making surgical incision*
11. Perform a subcutaneous injection of Ethiqa XR (buprenorphine extended-release) into the leg
    1. **Use 0.05ml per 20g mouse.**
12. **Inject 30 uL of Marcaine in 2-3 locations underneath the scalp near the incision site. This is a local analgesic.**
    1. **Wait 30-60 seconds for the Marcaine to diffuse before performing incision.**
13. Using a sterile scalpel, make a surgical incision to expose the skull
    1. Minimize the size of the incision as much as possible. You will need to see bregma and have access to the injection site. For substantia nigra injections this will be located at the caudal region of the skull.
14. Position your injection needle at bregma and save location in the AP and XY axis
15. Enter and save injection coordinates into the motorized stereotaxic frame
    1. Coordinates for right substantia nigra
       1. Anterior-posterior (AP): -2.9 mm
       2. Medial-lateral (ML): -1.3 mm
       3. Dorso-ventral (DV): -4.2
16. Raise the needle away from the skull slightly and move to AP and ML injection coordinates
17. Slowly lower the needle to touch the skull. Raise the needle and drill a small hole where the needle touched the skull
    1. Slowly drill through the skull keeping the drill shallow enough to not damage brain tissue
18. Once the hole is drilled lower the needle to the surface of the brain ensuring that the needle is not deflected by the skull. The needle should be completely straight.
19. Raise the needle 30-40mm providing space to flush the needle and load with virus.
    1. Flush the needle with sterile room temp H2O
       1. Cover the mouse’s head with sterile gauze to absorb the H2O
    2. Draw up a 1µL air bubble
    3. Load needle with 2.5µL of virus
       1. Slowly draw up virus and watch the air / liquid interface to determine volume
       2. Needle / tubing should be marked with 1µL intervals using a sharpie to help with this step
20. Move the prepared needle down to the surface of the brain. Move the needle to the desired DV coordinate at a slow speed (250µm / sec).
21. Wait 30 seconds and then start the virus injections at 0.2µL / min.
22. After the injection is finished wait for 5 minutes, leaving the needle in place and allowing the virus to diffuse away from injection site.
23. Remove the needle at slow speed (250µm / sec).
24. Move the needle up away from the skull and then release it and rotate it out of the way.
25. Close the incision site using sterile forceps and surgical glue.
26. Administer 0.5mL sterile saline solution subcutaneously to avoid dehydration.
27. Place the mouse back into the home cage and monitor recovery.
    1. Record time at recovery
28. Monitor post operation recover for 3 days and record any observations of pain or distress onto surgery cards.