**Set Up:**

1. The Hamilton syringes should be angled 12º on the stereotax.
2. Clean the Hamilton syringe needles 3X with acetone, then 3X with ddH2O. Fill the needles with ddH2O and maintain the water in the needle.
3. Place the heat pad and surgical drape and/or gauze.
4. Induce anesthesia with an IP injection of ketamine (100mg/kg) with xylazine (10mg/kg), diluted with sterile saline. Record the “start time” for the surgery at induction.
5. Start the isoflurane in O2 at 1% isoflurane and place the nose of the mouse in the nose cone; secure.
6. Block the head with the ear bars, blocking at the jaw under the ears. Secure.
7. Add ointment (e.g., Optixcare) to the animal’s eyes.

**Pre-Incision Animal Prep:**

1. Apply Nair with a cotton tip and let set for 3-5 minutes. Wipe the Nair away with gauze and clean with an alcohol wipe.
2. Apply betadine or chlorhexidine surgical scrub or solution with clean gauze in a circular fashion starting at the surgical incision site and rotating outward.
3. Alternate surgical scrub or solution with 70% alcohol or sterile saline (best practice but not required unless using surgical scrub). Repeat a minimum of three times discarding cotton pad or swab after each use. End with betadine.
4. Cover rodent with sterile drape to avoid contamination of the incision, instruments, and supplies.

**Craniotomy:**

1. Open skin with surgical blade, and clean the exposed skull with an alcohol wipe.
2. Check that the head is flat:

14a. put the needle on Bregma, set all coordinates to 0.

14b. Move the needle to Lambda, check that the DL measurement is 0.0 when the needle touches the skull.

14c. Adjust head as needed.

1. For the mPOA, move the needles back to Bregma (coordinate: 0,0,0). Move the **AP position to bregma +1.35 mm**. Adjust the **ML position to ±1.42 mm.**
2. Place tiny drops of water on the skull with both needles to mark drill locations. Set the drill speed to setting 15. Raise both needles. Drill injection site(s).
3. Eject water level to 3.0µL. Pull air into the needles up to 3.5 µL. **Pull up 0.150µL (150nL) of virus**. Clean the exterior of the needles with an alcohol wipe.
4. Slowly lower the needles to a **DV coordinate of -5.8mm**. Pause briefly. Lower the needles down to **-5.9mm** and pause for 1 minute. Raise the needles **back up to -5.8mm** and pause for 1 minute.
5. Proceed as follows:
   * Inject **0.050µL (50nL)** wait **1** minutes.
   * Inject another **0.050µL (50nL)** wait **1** minutes.
   * Inject final **0.050µL (50nL)** wait **5** minutes.
   * Raise the needles to **-5.7 mm** wait **3** minutes.
   * Raise the needles to **-5.2 mm** wait **3** minutes.
   * Raise the needles to **-5.00 mm** wait **3** minutes.
   * Raise the needles to **-4.80 mm** wait **5** minutes.
   * Raise the needles incrementally, from -4.80 to -4.00 in 0.10 mm increments with 20 second pauses.
   * Remove the needles slowly.
6. Pinch the skin together with curved forceps and add 2 small drops of vet glue. Pinch firmly yet gently to close the incision site.
7. Inject 10mg/kg Ketoprofen into the animal’s scruff. Once the glue has settled, add a drop of bupivacaine.
8. Allow the mouse to recover on a heating pad. Clean the surgical area with MB10 (chlorine dioxide).