**Calcium imaging pipeline protocol**

1. **Inject virus to region of interest**
2. **Implant lens + baseplate**
3. **Apply metabond and attach cover plate**
4. **Let animal recover**
5. **3 weeks later check for cells**
6. **If cells are present, run behavior!**

**Virus order information from Addgene:**

*162376-AAV1* pGP-AAV-syn-jGCaMP8f-WPRE (AAV1) 100ul – For imaging

Make 5ul aliquots and store in -80

Virus (titrate if necessary; we titrate by ½ (3ul virus, 3ul PBS))

500nl to region of interest

Lens order information: 6.1mm Inscopix

Metbond order information: Parkell

Camera information: Dino-Lite

Viral injection: Nanoject III

Set up isoflurane delivery

* + Weigh isoflurane canister
  + Reload isoflurane syringe and set delivery rates for nose cone and induction chamber
    1. 5% for induction
    2. ~2.7% for maintenance
  + Layout surgery tools
  + Prepare betadine and EtOH in conical tubes
  + Load carprofen into 1ml syringes (0.3ml s.c. per animal)

Prepare and load the virus into the pipette (*to make pipettes- see protocol in folder*)

* + Fill the glass pipette with mineral oil
  + Carefully fasten the pipette tip to the Nanojector and look for the tip of the plunger sticking out from the base of the pipette
  + Eject some mineral oil so there is room in the pipette to uptake virus
  + Set the Nanoject to the appropriate injection rate for your experiment (50nl per ejection pump for us)
  + Attach the Nanoinjector to the left stereotaxic arm
  + Stretch a piece of parafilm flat over the flat side of one of the earbars or use weigh boat
  + Load the total amount of virus needed for the day onto a piece of parafilm with a micropipette
  + Using the microscope camera, center the pipette tip at the centermost, top part of the droplet.
  + Then slowly lower the tip so it is just above the bottom of the droplet; It SHOULD NOT touch the parafilm, in case it shatters the tip
  + Press “FILL” to uptake the virus; watch for the uptake using the microscope
  + Recenter the pipette tip if the centermost point of the droplet begins to change
  + Once the virus is loaded, raise the pipette out of the way and lock it.

Anesthetic induction:

* Load mouse into induction chamber. Press “deliver” to turn on isoflurane after loading mouse into the chamber (5% delivery for induction)
* Always lock the isoflurane delivery tubes opposite to the one you are using
* Check breathing rate as a measure of appropriate anesthesia level

Transfer mouse to stereotax:

* Shut off isoflurane delivery from induction chamber
* Change the isoflurane delivery tubes
* Quickly grab mouse from chamber; minimize time the door is open
* Using the end of a cotton tip applicator, open the mouse’s mouse and roll its tongue out of the way
* Touch incisors to the stereotax mouthpiece and slide the incisors (and rest of mouse’s head) up till it latches into the divot in the mouthpiece.
* Secure the nose cone & tug on mouse’s tail to check that it is secure
* Turn on maintenance isoflurane for the nose cone (~2.7% was good for B6, may need tweaking for other mouse strains)
* Secure the mouse’s head in the stereotaxic ear bars.
* Use the ruler ticks in ear bar arms as reference
* You may need to raise height of nose cone
* Use the evenness in level of the mouse’s eyes to judge whether there is any left/right asymmetry
* Apply ophthalmic lubrication
* Use clippers to shave the hair above the surgical sight and the skin between the ears.
* Apply the surgical scrub with betadine and EtOH in a circular motion (3X)
* Make a surgical incision with either scalpel or with surgical scissors, then use cotton tipped applicators to further widen the surgical site
* Use the pipette tip to level the head using bregma and lambda
* DV difference between bregma and lambra should be no more than 0.05
* Zero at Bregma & move pipette tip to the AP and ML coordinates of the injection site (vCA1: AP -3.16, ML 3.25) \*\*potentially 3.35 ML\*\*
* Using the drill, perform a craniotomy. Create a hole large enough for both the pipette and the GRIN lens

Inject the virus:

* First, create a track for the pipette
* Place a blunted needle tip (SIZE HERE) on the arm for the lens implant
* Touch the tip to the dura and zero DV
* Lower the tip slowly to the ventral-most injection coordinate (vCA1 DV -3.85, -3.50. -3.0)
* Move our pipette to the injection site
* Lower slowly and inject 3 pumps (50nl/pump) per DV site, wait 10 sec between pumps at the same DV coordinate
* Raise the tip slowly to the next DV coordinate
* Wait ~ 1 min between injections between DV site
* Wait 7 minutes to raise the pipette from the final injection coordinate
* Lock pipette arm safely out of the way.

Perform duratomy with needle tip:

* Clean the injection site very carefully to prep for lens implant
* Position the GRIN lens into the lens holder and fasten to the right stereotaxic arm in the following position
* Lower the lens slowly to the DV coordinate (vCA1 DV -3.5) using a “seesaw” motion (down 0.2, up 0.1). This allows us to create a lens tract when lowering the lens and avoids depressing the brain tissue.
* Before surgery, make sure the ceramic MetBond plate has been sitting in the -20 degree freezer for at least a few hours. The MetBond solution will not work if used in a non-chilled plate.
* To make a MetBond solution, add 2 scoops of clear MetBond powder to the ceramic plate using the scoop included in the box.
* Next, add 4 drops of primer and 1 drop of catalyst to the powder (catalyst is the syringe looking thing. Twist it to dispense)
* Mix the powder and catalyst together using a green applicator tip included in the box.
* Apply the solution around the lens where it is entering the skull. Apply a good amount all around the lens so that it is evenly distributed.
* Wait 5 minutes or so for the MetBond to dry before starting to apply dental cement.
* Break a cotton tipped applicator to get a sharp point. Mix the dental cement with the catalyst till you get the appropriate consistency (not too runny, but not too clumpy)
* Carefully dental cement over the posterior-most screw and cement the lens that remains outside the skull **WITHOUT getting cement on the GRIN lens holder or the stereotax arm**. Build a cement ‘mound’ as best you can around the lens. It’s fine at this stage if it’s incomplete.
* Wait ~10 minutes till the cement has dried. CHECK AROUND YOUR CEMENT MOUND TO MAKE SURE EVERYTHING HAS DRIED**.** Carefully raise the sterotax arm up so that the GRIN lens holder separates from the GRIN lens.
* Turn off the isoflurane and return the mouse to the cage.
* Add HydroGel and DietGel to the cage. Change the cage’s dry pellet container to one that reaches the floor.