Surgery Protocol

Created by Samara, August 2023. Golshani Lab UCLA

MATERIALS AND EQUIPMENT:

Items:	Notes (ie where to find etc):
● Isoflurane ● Pre and post operative drugs: carprofen, dexamethasone, lidocaine	
● Surgical instruments (scalpel, scissors, fine forceps)	
● Eye lubricant ● Cotton-tipped applicator ● Hydrogen peroxide ● Betadine ● Hair removal (Nair cream, razor)	
Virus injection: ● Nanoject syringe pump + glass pipette ● Stereotaxic accessory to level brain ● Virus + ice	
Lens implant: ● Stereotaxic accessory to level brain ● Lens ● Lens holder ● Aspiration vacuum ● ACSF (artificial cerebral spinal fluid) ie cortex buffer *Put on ice to keep cold during surgery. Not necessary but helps. ● Superglue ● Superglue accelerant ● Dental cement	
Baseplating: ● Miniscope assembly ● Miniscope holder ● Computer with miniscope software ● Superglue ● Superglue accelerant ● Dental cement	

PROTOCOL:

Before starting:

1. Retrieve animals from the animal facility and put them in the surgery room.
2. If doing a virus injection, get the virus and put on ice in the surgery room. **Optional, but recommended: Prepare Nanoject syringe pump in advance (see Nanoject manual)
3. Make sure isoflurane air supply is turned on and isoflurane tank is filled.
4. Turn on the isoflurane scavenger.
5. Turn on the heat pad for the stereotaxic rig.

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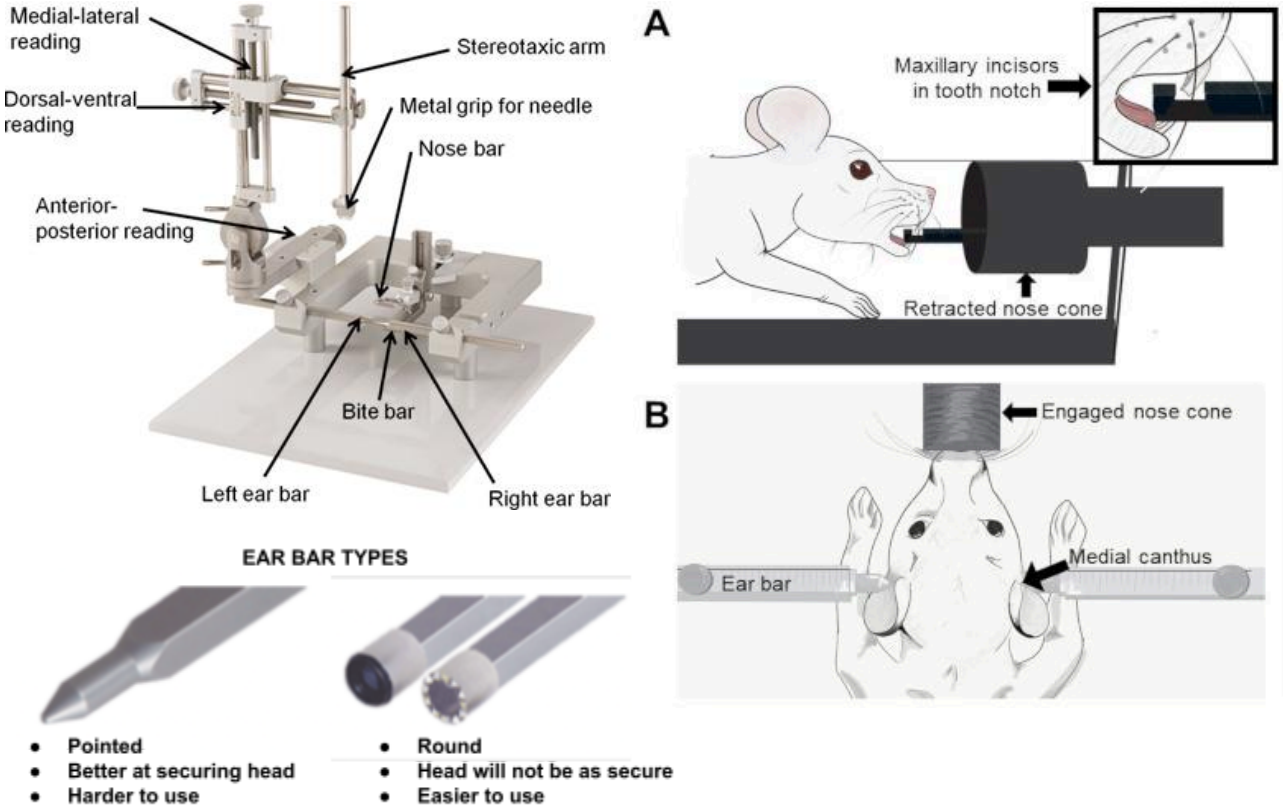
1. **Pre-operative Care:**

- Administer Carprofen (Rimadyl) subcutaneously at a dose of 5 mg/kg, 15-30 minutes before surgery
- Administer Dexamethasone subcutaneously at a dose of 0.2 mg/kg, 15-30 minutes before surgery.
- Administer Lidocaine subcutaneously at a dose of 5 mg/kg, 15-30 minutes before surgery.

2. **Preparation:**

- Anesthetize the mouse using an induction chamber with 4-5% isoflurane for induction *Make sure isoflurane valves are pointed in the correct direction.
- Confirm anesthesia with a toe pinch test.
- Remove as much hair as possible with an electric razor. Note: the hair removal cream used later on does a better job of removing hair, so it's ok if razor cut is not perfect.

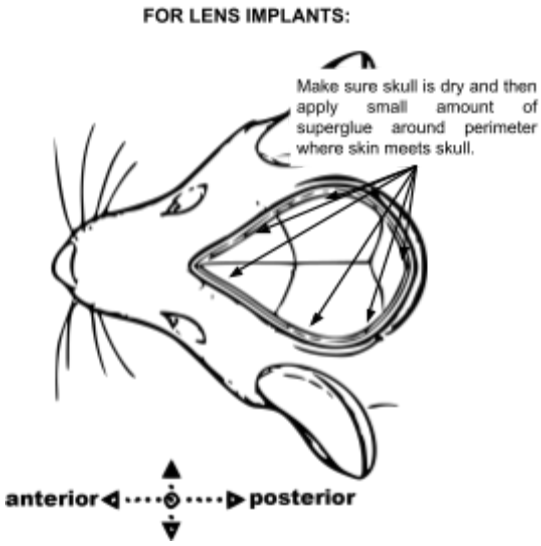
3. **Positioning and Securing:**



- Place the anesthetized mouse in the stereotaxic head holder and maintain at 1-2% isoflurane.*Make sure isoflurane valves are pointed in the correct direction
- Gently secure the head holder to the skull using ear bars and a nose clamp to ensure stability.
    - There are two varieties of ear bars in the Golshani lab. The pointed ones and the round ones. Feel free to use whichever works better for you. My experience is that the round ones are easier to use, but don't secure the brain as well.
- Generously apply eye lubricant to eyes of mouse using cotton applicator (ie Q-tip)
- Generously apply hair removal cream (Nair) to the head of the mouse with a cotton applicator, wait 1-2 minutes before removing with a clean cotton applicator. (The longer you wait, the better job it does at removing hair)
- Monitor anesthesia with a toe pinch test.

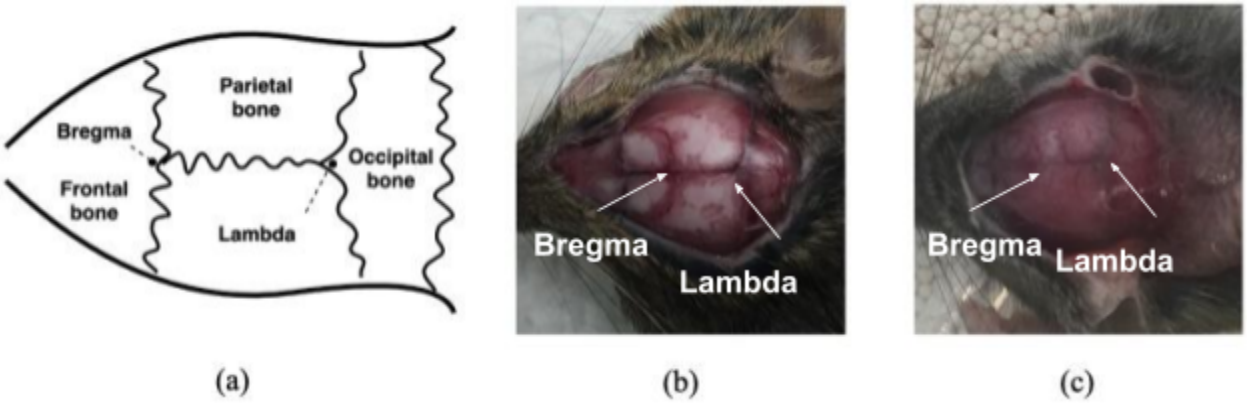
4. **Exposure of Skull:**

- Disinfect the surgical area by swabbing the hairless region with 1) betadine and 2) 70% EtOH.
- Make a midline incision with a scalpel to expose the skull. If doing a lens implant, use scissors to cut off a significant portion of skin flap to expose the entire skull.
- Use a cotton applicator to swab hydrogen peroxide over the exposed skull to carefully remove connective tissue and expose the bregma and lambda points. *If the mouse is showing signs of wakefulness, turn up the isoflurane to 2-2.5%.



5. **Leveling the head, coordinates and targeting: *Using Neurostar Robot Stereotax**

- Open Neurostar software on computer, and ensure that the stereotax markings align with the software readings (if not, manually enter the coordinates from stereotax)
- Mount desired stereotaxic arm
    - For virus injections, it is most efficient to use the Nanoject syringe pump pipette tip but this can be tricky because of how fragile it is. Another option is to use one of the designated “levelers” which is just a needle attached to a stereotaxic bar
- Visualize through the microscope as you carefully lower the tip of the leveling device to bregma and lambda on the skull of the mouse and mark these locations in the Neurostar software.



(a) (b) (c)

- Use the Neurostar software to enter desired target coordinates, move the leveling device to this location and mark with a pen.

Commonly used coordinates (mm) *From The Mouse Brain Atlas						
Brain region	**AP (anterior-posterior)**		**ML (medial-lateral)**		**DV (dorsal-ventral)**	
	Virus	Lens	Virus	Lens	Virus	Lens
CA2	-1.8	-1.8	-1.9	Craniotomy -2.0 - 3.0 (angle requires large hole)	-1.7	-1.7 (30° away from midline)
dCA1	-2.1	-2.1	2	1.8	-1.65	-1.25
DG	-1.8		-0.75		-2.25	

6. **VIRUS INJECTIONS - Drilling and Injection:**

- Drill a small hole in the skull at the target coordinates using a micro drill with a fine drill bit.
- Lower the Nanoject needle into the hole for drug delivery
- Administer the desired solution slowly and carefully, adhering to the recommended infusion rate (typically 1nl/sec)

7. **Withdrawal and Closing:**

- After the injection has finished, wait 10 minutes.
- Gradually withdraw the needle from the brain.
- To prepare for lens implant, wait an additional 40 minutes or more to make sure the virus is not aspirated during implantation.
- Otherwise close the incision using sterile sutures (vetbond - veterinary glue will also work).

8. **LENS IMPLANT - Drilling, aspiration, injection:**

- Reposition the microscope to be exactly over coordinates of craniotomy and zoom in as much as possible.
- Carefully drill a craniotomy in the skull around the target coordinates using a micro drill with a fine drill bit. If the lens will be angled, drill a hole ~2 times the diameter of the lens around the injection coordinates. Otherwise, the drill hole is just slightly larger than the lens around the injection coordinates.

- To start, attach a larger (gray) gauge blunt needle tip to the flask-vacuum. Carefully aspirate off brain tissue / skull bone bits. As much as possible, keep constant flow of cortex buffer to the craniotomy to keep the exposed brain wet.
- When the top layers of brain tissue have been aspirated, attach a smaller gauge (purple) blunt tip needle to flask-vacuum and very very carefully continue to aspirate off brain tissue until you reach the white striations of the corpus callosum. Very slowly and carefully remove the horizontal white striations of the corpus callosum until you reach the striations that go vertical.
- When you reach these striations, stop aspirating and clean up the sides so that the lens can be placed into the brain. Continue washing with cortex buffer until all bleeding stops and keep the brain constantly flushed with cortex buffer.
- Connect vacuum to top of lens holder on stereotax and insert lens lens . Carefully touch the lens down at bregma and record DV coordinate. Move lens back over to craniotomy and lower down to desired DV coordinate of brain region. Remove any excess liquid with the aspirator. Connect the outside of the GRIN lens with the skull using vetbond and a little superglue. Be careful not to suck glue into the lens holder.
- After just a few seconds, remove the lens holder by simply removing the vacuum suction and withdrawing the holder. The lens should remain in the brain.
- Next, cover the entire skull with superglue, and then cover with dental cement. Let dry completely and cover with Kwik-Sil to protect the lens.

9. Recovery:

- Gently remove the mouse from the stereotaxic apparatus.
- Place the mouse in a clean, warmed cage on a heating pad.
- Monitor the mouse until it regains consciousness.

10. Post-operative care:

- Carprofen (Rimadyl)
    - Dose: 5 mg/kg
    - Injected subcutaneously
    - Duration: every 12-24 hours for 48 hours
    - Administered “once at the time of the procedure, and then every 12 - 24 hours for 48 hours. Additional doses will be administered after the 48 hour time point as needed for pain.”
- Dexamethasone
    - Dose: 0.2 mg/kg
    - Injected subcutaneously
    - Duration: every 12-24 hours for 48 hours
    - “Once at the time of the procedure, and then every 12 - 24 hours for 48 hours. Additional doses will be administered after the 48 hour time point as needed for pain.”

- Amoxicillin
    - Dose: 0.25mg/ml
    - Diluted in drinking water
    - Duration: 7 days
    - Fill out purple “Investigator Will” card
    - Replace with normal water after 7 days
- TMS (amoxicillin alternative)
    - 1 mL TMS in 100 mL drinking water
    - 7 days
    - Fill out purple “Investigator Will” card
    - Replace with normal water after 7 days

COORDINATES:

From Mouse Brain Atlas

VIRUS			
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LENS			
Commonly used coordinates (mm)			
Brain region	**AP (anterior-posterior)**	**ML (medial-lateral)**	**DV (dorsal-ventral)**
	Lens	Lens	Lens
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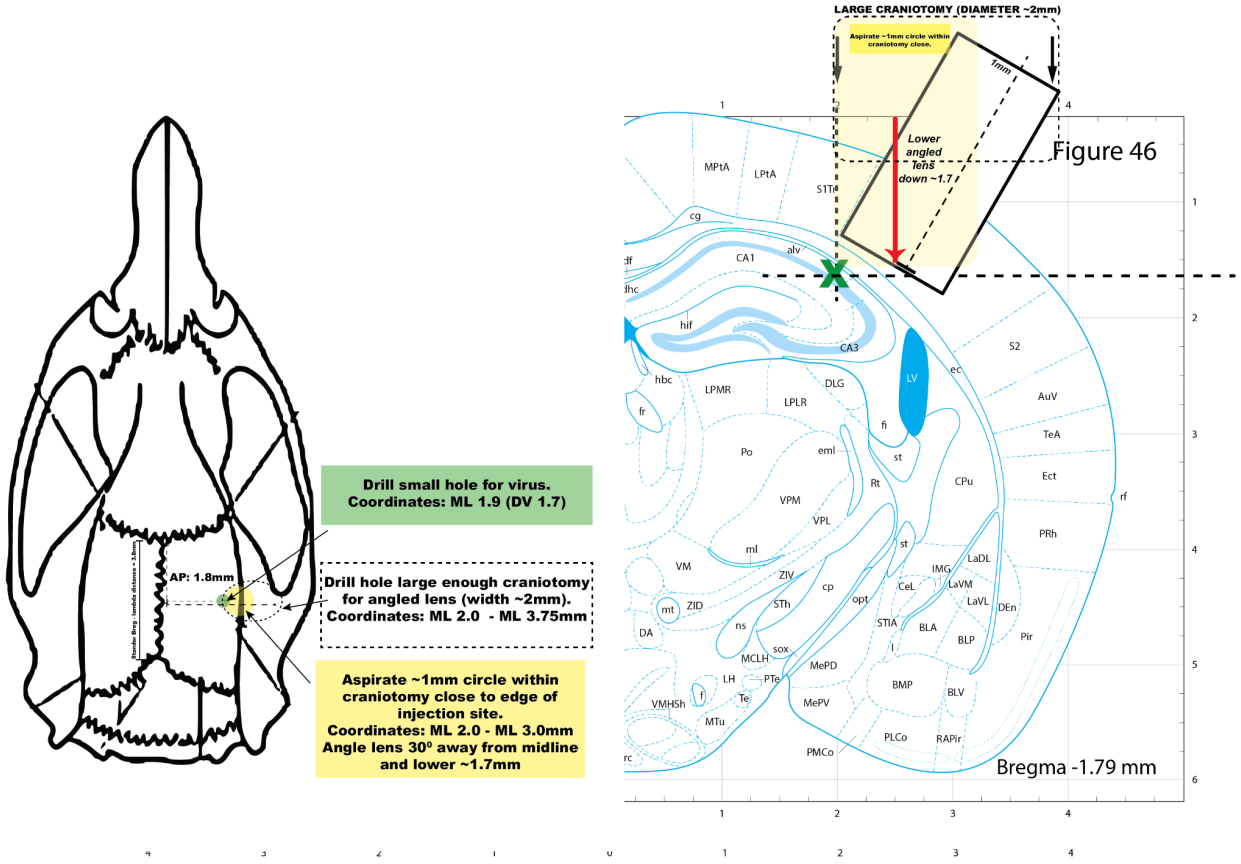
dCA1	-2.1	1.8	-1.25
DG			

Hipp. CA2 + Lens implant coordinates



More details on lens implant:

From working with Pingping

- Wait min 40 after virus injection before doing aspiration/lens implant
- For deeper brain regions, use a flattened syringe needle (gauge maybe 27?...gray base looks like) to make a track. Flattened= use hammer to pound entire needle so it is not a cylinder but a blade
    - Not good to aspirate off too much brain tissue if targeting a deep structure, so better to aspirate a little and then just make track

- *For **non-**angled implants only:* Make a slightly bigger drill hole at start of surgery so accommodate lens (but not too big because then the lens will be hard to center)
- *For angled implants only (ex. CA2 lens implant):* Make a drill hole that is approx. twice as big as lens diameter. For 1mm lens, this is a 2mm diameter craniotomy. You won't need to aspirate all of the brain within the craniotomy, but the lens will need a larger hole to accommodate angle.
- Use one set screw and score skull
- Mark Z axis "0" at bregma when lowering the needle track...measure the height difference between bregma and drill hole (because head is naturally tilted)
- Just lower needle and lift up to make track
- If no bleeding after making track, implant lens
- One layer of super glue with glue accelerator... remove lens holder
- Another layer of super glue that doesn't dry completely
- Add minimal layer of dental cement
- Built up scaffolding of dental cement to prepare for base plating
- Generous application of kwikseal

The Conor Technique:

- Set screw
- Seal lens/ craniotomy with vetbond
- Full layer of super glue on skull + accelerant
- Second layer of wet super glue + dental cement
- Note: I think I need to let the skull dry more and have cleaner hairline/skull surface