**Ligation Injury in Mice**

**Rationale:**

**Hypothesis:**

**Vascular Injury via R Common Carotid Ligation Injury**

1. Sign out and take ketamine and xylazine down to the procedure room or can perform the ligation injuries on the mouse bench in 1226. Make up sterile saline/ketamine/xylazine in 6:3:1 ratio. Inject 3 uL/g (or 3 mL/kg) ip per weighed mouse. I normally inject .4 CC for a 4-6 week old mouse and .6 CC for an 7-9 week old mouse. I do not like to injure them at they get older than 10 weeks because they are difficult to get to anesthetized and do not wake up well.
2. Warm up Germinator, turn on electrical heating pad and place sterile cover over dissection scope and around surgery area. Set up three working stations: 1) a station to remove hair from thorax and neck of mouse; 2) surgery station to perform the actual surgery with iodine and 70% ethanol, eye lube, surgical glue and dissection scope, also have 6 n sterile silk for suturing(I have found that silk works the best, nylon does not tie well and tends to come undone while still in the mouse); 3) heating pad for mice after surgery. Before each surgery place surgical instruments in germination for sterilization.
3. Put on sterile surgical gloves and place sterile blue pad under microscope eye piece. Place sterile instruments on glove paper.
4. After sufficient anesthetic induction (assessed via toe pinch), remove hair from neck and part of thorax of mouse with Nair hair remover. Add Nair and allow it to sit for three minutes. Remove Nair and hair with a gauze pad wetted down with 70% ethanol. The Nair needs to be fully removed because it bothers the mouse’s skin.
5. Tape anesthetized mouse via the front appendages to cleaned, sterilized plexiglass plate. Also tape down the mouth of the mouse by folding a piece of tape in half and then placing the folded tape into the mouse’s mouth. This allows for the throat of the mouse to be exposed and it also properly aligns the carotid so that it is easy to access for surgery. Add betadine/iodine to shaved portion of neck and wipe off with clean Q-tip soaked in 70% ethanol and throw the Q-tip away. This swiping of the neck with the betadine/iodine and then wiping with a q-tip makes the neck somewhat sterile to perform the surgery.
6. Inject .25 cc bupivicane as local anesthetic for recovery into neck of mouse where the surgery will be performed.
7. Make mid-lateral incision on mouse neck just to the right side of the midline. Separate fascial tissue, spread apart fat pads to the right of the trachea and use a dull tweezer to hold the fat and tissue away from the surgery area. Look to the right of the trachea for the pulsing carotid artery. It is normally set back a little bit from the front of the mouse and will sit next to a vein and a nerve.
8. Clean and isolate the common carotid artery (CCA) just distal to the bifurcation. Use forceps to remove neck tissues surrounding the common carotid artery. I do not like right angle forceps; I prefer 45 degree forceps (right angle forceps I feel don’t separate the tissue as easily as the 45 degree forceps).
9. Once cleaned and isolated, run a 6/0 silk suture under the CCA and tie two consecutive, flat square knots tight enough to restrict blood flow. Do not tie the flat square knots so tight that you completely cut off blood flow; just enough to restrict blood flow is optimal. Also make sure that the nerve is not tied off with the common carotid when surgery is being performed or that the vein is tied with the common carotid. Cut ends of the ligature.
10. Flush site with saline. Remove with a Q-tip if necessary (I don’t do this unless I nick something inside and the mouse is bleeding in the area surrounding the ligation).
11. Replace fat pad over the ligature, bring skin back together, add skin glue to the site of the open wound to close the skin. Oppose skin flaps with wooden ends of two Q-tips to close the wound. Add eye lube to the eyes of the mouse.
12. Place mouse back in cage on 37 degree Celsius heating pad.
13. Wait for mouse recovery before placing back in the vivarium mouse room. They normally have to go into quarantine because I like to perform injuries upstairs in the lab.
14. Wipe with ethanol and re-sterilize surgical instruments, replace blue pad, and don new surgical gloves before next surgery( I just make sure I stay sterile from mouse to mouse- I perform too many surgeries to wear new gloves for each surgery).

After injections, allow mice to remain in quarantine for the desired period of time for the study requirements.