**Carotid Artery and LV Catheterization**

\**protocol created originally by Charles(cchung@med.wayne.edu) in 2015*

**Before you Begin**

First, you need to pass the appropriate animal care training for sterile and surgical procedures. Second, you'll need to be trained. If no one in the lab currently performs these procedures, Charles recommends the NEOMED Mouse Microsurgery and Small Animal Echocardiography Workshop (alternate programs exist, for example Inside Scientific just started one in 2014). Or ask a collaborator to help train a student. The good part of NEOMED workshop is that its a full week where you can sit and focus on surgeries.

**Tools for the Procedure**

|  |  |  |
| --- | --- | --- |
| Item | Use | Likely Location |
| Laptop/Computer w LabChart Software Installed | Storage of data. Labchart software stored in MN507 Drawer 18A | MN507 Shelf |
| Powerlab 8 w T-type temperature Probe | A/D Converter | MN507 shelf. Pod in Tupperware container. Extra pod and probe in MN507 Drawer 18A |
| MPVS 300 Box | Catheter Amplifier | MN507 Shelf |
| Catheter |  | MN507 Drawer 8A, or shelf |
| Catheter Cables |  | MN507 Drawer 8A |
| Light Source |  | MN507 Shelf |
| Stereoscope |  | MN507 Floor |
| Heating Pad |  | MN507 shelf, typically on Tupperware container |
| Powerstrip |  | MN507 Shelf |

**Tools**(should be stored in large tupperware box with disposables, on the shelf in MN507)

|  |  |  |
| --- | --- | --- |
| Quantity | Item | Use |
| 2 | #5/45 forceps | isolating vessel |
| 1 | #5/45 forceps with tube covering tips | handling catheter (prevents damage) |
| 1 | large/rough forceps | handling skin |
| 1 | small scissors | cutting skin, cutting heart at end of study |
| 2 | locking needle holders or hemostats | holding suture ends for easy handling |
| 3 | hooks on elastic straps | holding back suture |
| 1 | microscissors | cutting sutures at end of study |
| 1 | large scissors | cutting chest at end of study |
| 1 | Bovie Cauterizer w H101 tip |  |

**Disposables**(Most of these tools are stored in the large tupperware container. Isoflurane ddH20, and Terg are not.

|  |  |  |  |
| --- | --- | --- | --- |
| Quantity | Item | Extras | Replacement Source |
|  | 4-0 Silk Suture |  | esuture.com part #A303 |
|  | 5-0 Vycril Suture (if need to close the skin) |  | esuture.com |
|  | 2"x2" gauze | MN507 Drawer 17A | fisher |
|  | Big Cotton tipped swabs (Puritan) | MN508 Shelf 13E | fisher |
|  | Sharp Cotton Tipped Swabs (Puritan 872-PC DBL) |  | amazon |
| 5+ | 1 mL Syringe | MN508 Drawer 9A or Shelf 5E | fisher |
| 2+ | 5mL Syringe | MN507 Shelf | fisher |
| 3+ | 25g needle | MN508 Drawer 8A | fisher |
|  | Betadine | MN507 Drawer 17A | Walgreens |
|  | Heparin (1000U sufficient) | MN507 Drawer 17B | DLAR |
|  | Normal Saline (0.9% NaCl in ddH20) |  | Fisher |
|  | Depilatory Cream (Nail) |  | Walgreens |
|  | Artificial Tears |  | DLAR |
|  | Isoflurane |  | DLAR |
|  | Lab Tape | MN508 | fisher |
|  | gloves, masks, other PPE | MN507 Drawer 8A or MN508 | Fisher |
|  | Benchpads/sterile sheets | MN507 30C/30D | FIsher |
|  | ddH2O | Millipure System |  |
|  | Tergezyme (for cleaning tools 1g/100mL) | MN508 bench or 11J |  |
|  | Papertowels |  |  |

**Procedure**

If you are going to do the procedure in the lab, go to setup. If you are going to perform the procedure in the MRISC or MS641, gather all of your materials. Weigh ~4g Tergezyme and put it into a bottle.

**Setup**

Setup the equipment first. The catheter needs time to stabilize.

Plug in the computer, Powerlab and MPVS box, turn everything on. Unwrap the catheter and plug in the pressure (and volume if needed). Take a 1 mL syringe, fill it with Heparin. Remove any needles you used to fill the syringe and insert the tip of the catheter into the syringe. Calibrate the signals using the 0/25/100 mmHg and 5/25/50 RVU switches and calibration points in LabChart.

Setup the heating pad. 1/4 of a benchpad fits well to cover the area you need. Setup the stereoscope so that it reaches the working area on the heating pad.

Prepare a clean area to your left and right with all of the tools, the swabs, 2x2's a syringe full of heparin, a syringe full of saline, and a syringe full of ddH2O.

Keep Nair and Betadine nearby.

**Animal Prep**

Anesthetize the animal. Isoflurane vaporizer is preferred. Urethane can be difficult to titrate. Isoflurane also provides a nosecone for you to secure suture later.

Place the animal on the heating pad, maintaining anesthesia. The nose should face you, with its right side at your right. Check for a response by pinching the paws or tail. Do not start your procedure until you get no response.

Using depilatory cream, remove the hair from the ventral side of the neck, from the jawbone to the sternum, at the width of the jawbone. Using a cotton swab, you can quickly see the hair being released. Do not leave the cream on for more than a few minutes or it will burn the skin. If you are more experienced, you can probably remove less hair, but this will give you a large working area.

Wipe off the loosened hair and cream using ddH2O and 2x2s. Do not leave the cream on for more than a few minutes or it will burn the skin.

Disinfect the area by wiping with betadine. Wipe remaining betadine with 2x2s. Wipe away from the center of the neck in all directions with a new 2x2 each time to keep the area clean.

**Surgery**

1. Make a cut/incision down the center of the neck, below the jawbone to short of the sternum.
2. Cut through the superficial layer and the fascia. You will see the salivary gland.
3. Cut or separate the salivary gland down the center. Below this, you will see muscles covering the esophagus.
4. Separate the area to the right of the esophagus until you see the inverted 'Y' where muscles intersect.
5. Clear and separate any fascia down into that Y. Down the left side, along the length
6. Isolate the vessel and separate the white nerve. Be careful not to break that nerve or your animal will die. Isolate the vessel from where it begins to branch near the jaw, down as far as you have the chest open.
7. Insert 3 cut pieces of 4-0 suture. These should be ~4 inches long. You can 'cheat' and pass 1.4" and 1 folded 8" segment of suture, then cut the 8" piece after you have passed it through.
8. Loosely knot the piece of suture closest to the chest, then secure it with a needle holder. This will be your blood stopper. As you pull the needle holder towards the feet, you will stop blood flow and you can see the vessel stop shaking.
9. Loosely knot the next piece of suture, then secure it with a needle holder. This will be your securing suture, that will seal the hole that you will make to insert the catheter.
10. Knot the final piece of suture, as close as possible to the position where it branches. Knot this tightly (3x) to stop flow. Pull the loose ends towards the nosecone and secure it with tape. This will slightly lift the vessel.
11. Start date acquisition and make sure your catheter is zeroed
12. Prepare a needle on a cotton swab or syringe. Bend it into a 'z' pattern so that you can just adjust the tip to insert into the vessel
13. Take your blood stopper needle holder (the one closest to the feet) and pull it back to secure it, stopping blood flow.
14. Use your bent needle to puncture the artery. A drop of blood will come out. If blood flows rapidly, you don't have enough tension on either end.
15. Insert the catheter under the needle or into the hole the needle made. Advance it until it hits the suture holding back blood.
16. Tie the middle suture onto the catheter (2x) to stop flow. If the end of the catheter (electrodes) are not fully into the artery, tie this loosely 1x.
17. Release the suture stopping the blood flow and advance the catheter. Quickly make sure no blood is coming out of the middle suture. You should see an aortic pressure signal.
18. At this point, you may, but do not have to, tie the blood stopping suture to give you an added seal. But be careful, because if you go to tight, it'll make it hard to advance the catheter.
19. Advance the catheter until it stops. You will probably see it shaking vigorously (it wasn't before). This means that the catheter is sitting at the heart and is shaking on each beat.
20. Advance the catheter into the LV. You might need to add tension to the catheter or spin it slightly. You might also need to push the chest of the animal around to get it in the right spot. You will need to monitor the pressure signal to see if you are in the LV.
21. Acquire data as needed. If you need Ao pressure, slowing withdraw the catheter (~5mm) until you see a clear aortic pressure trace and require data as needed.

**End of Care**

1. Withdraw the catheter until you see it against the suture tied on the vessel. Use the 3rd suture or create a new loop to again stop blood flow below the catheter.
2. Using microscissors, cut the suture that holds the catheter in place, being careful not to cut the catheter!
3. Withdraw the catheter. If needed, close off the suture stopping blood flow.
4. Open the chest of the animal, and sacrifice via exanguination (cut out the heart). Do not allow the animal to recover from anesthesia.
5. Turn off the anesthesia

**Clean Up**

1. Mix 1g/100mL Tergezyme. Soak all tools. If possible soak catheter and wipe away any blood
2. Clean up bench area, removing traces of blood, etc.
3. Return to MN507 and wash all tools. Restock disposables if needed.