Millar Protocol: Carotid Approach

N. LaCross 2003

Additional Equipment:

- Small size/high gauge (6 or 7) silk suture
- Short 25 gauge needle with ~2mm of the tip bent at a 60° angle
- 2 custom tissue spreaders (basically paperclips bent into dull hooks to gently separate tissue)
- 2 hemostats
- Small syringe of lidocaine
- 1 set of forceps with heat-shrink tubing on the ends

Surgery: follow the main protocol until the jugular cannulation stage

- Using the forceps, bluntly dissect the connective tissue away from the mouse's right jugular (it'll be on your left). Be extremely careful when working near the jugular. The vein wall is quite thin and easy to damage. You don't need to clear everything off the jugular, just clear enough away to see what's going on. Doing this step now will save time later.
- Do not actually cannulate the jugular at this point. The IV will only get in the way.

Carotid Cannulation:

- Use the tissue spreaders to gently spread apart the tissue surrounding the right carotid (on your left). Tape them down. Be cautious, or damage that could influence the resulting data may occur.
- Use forceps to CAREFULLY dissect the carotid away from the connective tissue that covers it. Be very gentle when handling the artery; if it's punctured, the experiment is basically over. When finished, there should be 5-6+ mm of carotid free.
- Cut two lengths of high gauge (small size) suture, about 2 inches each. Pass each under the carotid, close the head and the other near the chest. Tie the beginnings of a knot in both sutures, but don't tighten them yet.
- Prepare everything else that is needed for the ensuing steps, and then tighten the knot in the suture closest to the head, thus preventing blood from flowing back out the eventual puncture.
- Lock one set of hemostats on each of the sutures, using the ends that extend away from the artery. This will allow you to put tension on the carotid. The bottommost tie should completely occlude the carotid.
- Carefully pull up on the hemostats to lift the carotid up and isolate the section you will be working on. Arrange them so that no hands are needed to keep tension on the carotid. Apply a small amount of lidocaine to the exposed artery. This should relax the muscle in the carotid, allowing for easier insertion of the catheter.

- Remove the catheter tip from the saline syringe, and move the connecters close to the head of the mouse. **CAREFULLY** grasp the catheter using the forceps with heat-shrink tubing close, but at least 5mm away from the last electrode. **Never, *EVER*** hold the catheter by the tip.
- With the catheter in one hand, use the bent 25-gauge needle to puncture the carotid. Use the tip of the needle to lift up the hole, and insert the tip of the catheter. Remove/discard the needle, and use the free hand to cautiously release tension on the lowermost tie until the catheter can be advanced. Keep enough tension on the tie so that little to no blood escapes.
- If at any time the carotid begins to stick to the catheter, apply some more lidocaine to the juncture. This should loosen it up.
- Begin acquiring data to keep a record of the insertion. Keep checking biobench to view the waveforms.
- Slowly and gently advance the catheter down the carotid. If there seems to be a lot of resistance, pull back slightly and try again. Avoid pushing to hard or too fast, as this can damage the catheter or puncture the aorta, or both.
- To insert the catheter fully into the LV, it must be advanced past the aortic valve. The catheter will meet with some resistance here, but if care is taken no harm will occur. By observing the pressure tracings, it is easy to tell when the catheter has entered the LV. They will go from the low amplitude, high average pressure typical of the arterial system to what is normally seen during catheterization.
- Once in the LV, advance the catheter just until good volume tracings are seen (meaning that the last electrode is in the LV). Damage to the heart can occur if the catheter is pushed too far.
- When a good position has been found, tighten the last tie around the catheter, holding it in place and preventing leakage of blood. Keep the loose wire in place with spools of suture if necessary.

Jugular Cannulation:

- Turn on the albumin syringe pump to eliminate all air bubbles. Insert in into the jugular and secure as per the normal protocol.
- Inserting the catheter via the carotid usually results in less blood loss, so replace volume accordingly. It shouldn't be necessary to add more than 100uL of albumin before turning the pump down to 6uL/min.

Thoracotomy:

- Cut away the skin from the lower, right part of the chest (your left).
- Use the cautery to burn a small incision in the chest wall to your left of the sternum and just above the diaphragm. Be cautious of causing too much damage to the lungs or hitting the IVC or heart.
- The object is to create enough room to manipulate the IVC while minimizing disruption of the chest cavity.

The rest of the procedure is identical to the normal method, so refer to that protocol for further guidance.

Final Notes:

While obtaining the blood sample, do not hit the catheter with the needle.

When removing the catheter, be sure to undo the tie that holds it in place.

Additional cleaning time may be necessary if parts of the carotid were removed with the catheter.