

Rat Cardiovascular and Respiration Lab

Purpose:

These directions are for students in Mammalian Physiology Lab (BSCI441) who are about to perform the Rat Cardiovascular and Respiration lab. This document is meant to supplement the surgery video and lab manual with more detailed instructions and troubleshooting. This guide aims to help students avoid the common mistakes that cause the rat to die before the data collection is complete. This document will only explain the surgery component, not the recording calibration, series dilution, or data collection.

How to use this guide:

Before reading this guide, you should:

- read the lab manual to become familiar with the equipment
- watch the surgery video to understand the basic procedure

You may find it helpful to:

- watch the video again as you read this guide
- have this document and the video handy during surgery

Directions:

General strategy:

Break up your team into a surgery subteam and a calibration and series dilution subteam. The surgery team should be two people familiar with all surgical procedures. The team should designate one person as the **surgeon**, who will be performing most of the procedure, and the other as the **attending**, who will assist and prepare equipment. You may opt to switch positions between surgeries or to have **both** subteam members work on certain parts together. The calibration and series dilution subteam should be one to two people who calibrate the blood pressure transducer and prepare the drugs and rebreathing setup. These two teams should work in parallel. Below are instructions for the surgery subteam.

The time checks reflect how much time the team should spend performing the section before it, allowing time to reinsert catheters if necessary. The surgery video does not reflect how long the surgery will take. The time between obtaining the rat and starting data collection should be 1 to 1.5 hours. The faster you complete the surgery, the more likely that your rat will survive data collection. If you take longer than these times due to reinserting catheters, you may lose the rat from blood loss. Be efficient but cautious, and remember that you have three chances to complete data collection.

Part 1: Setting Up

Attending: Getting the Equipment

Before you begin, obtain all of the following equipment if it is not already on your table:

- 6 sutures (12" long)
- 2 catheters
- trachea tube
- lab tape
- 3 1mL syringes
- 4 larger gauge (green) needles
- permanent marker
- 2 surgical scissors
- hemostat
- sharp curved forceps

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- blunt curved forceps
- sharp straight forceps
- plastic pipet
- 2 cylinders of heparinized saline
- Kim wipes
- hooked needle
- 1 suture (18" long)
- centrifuge tube tray
- 100mL plastic centrifuge tube

1. Place all materials on paper towels next to the tail end of the animal so that the surgeon will not bump them during surgery.
2. Designate one cylinder of heparinized saline as dirty and the other as clean. Label with the marker.
3. Remove the blue cap from the centrifuge tube and place it horizontally on the table parallel to the table edge with the open end towards the surgeon's dominant hand. Tape the tube to the table. This will allow the surgeon to dispose of needles easily.
4. The surgical bed (heating pad, surgical cloth, metal pan, and lamp) should already be on the table.

Surgeon: Preparing the Area

1. Plug in the heating pad. Place it in the blue surgical cloth and place the metal pan on top of it. Be sure that your set up is on the side of the table with the computer.
2. Pick up the rat. Test the anesthesia by poking gently on the sides and on the eye. *If the eye blinks, tell the TA* and he/she will give another injection to make sure the rat is completely unconscious. You can also test the anesthesia with a toe pinch, but the eye method is more sensitive.
3. Once you have an unconscious rat, place the animal on its back with its head towards the computer so that the surgery area will be near the recording apparatus. Tape all four legs to the metal plate. Turn on the lamp and turn it towards the upper chest of the animal.
4. Lift up the skin with blunt curved forceps and cut a patch of skin off with surgical scissors. Clear away the area between the shoulders, from the jaw to two inches down the chest. Then, use the two sharp forceps (curved and straight) to tear apart the clear membrane over the same area. Don't worry about nicking anything up until this point, as all the blood vessels here are superficial.

Time Check: 15 minutes (15 min total)

Attending: Throughout the Surgery

1. Monitor the animal's breathing. If breaths have become shallow or less frequent, locate the respirator and have it on hand in case breathing ceases.
2. *If the animal stops breathing*, get the respirator and turn it on to 3:1 ratio. Push the end of the respirator tube into the trachea tube until a good seal is formed. Allow the respirator to breathe for the animal for 10 seconds, and then remove it. Wait for 10 seconds to see if breathing has resumed. If not, repeat respiration. Breathing should resume within 3 minutes. If it does not, the animal will likely not breathe on its own again.
3. Before each time the pipet is needed, wash it with the dirty saline by dipping your fingers into the saline and running them up and down the length of the pipet.
4. When the surgeon has inserted the glass pipet, remove a new needle from the outer packaging. Remove the cap and place the needle sharp side down into a tube tray next to the surgeon.

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5. Monitor the trachea tube after it is inserted. If you cannot see condensation in the trachea tube, check every ten minutes with scissors to make sure it is still secure. Watch the exposed end of the tube to make sure it is free of fluid. This is especially important when the pipet is in, as the pipet can push down on the trachea tube and dislodge it.
6. Every ten minutes, apply dirty saline to the exposed chest muscle with your finger to keep the area moisturized.
7. *If you see blood pooling in the body cavity*, dry it with a Kim wipe. Don't apply saline until the bleeding has stopped because the heparin in the saline will prevent clotting. Watch the area for the source of the bleed: it is likely that a catheter is loose.
8. *If the catheter comes out of the blood vessel*, apply the brake for the carotid artery and use Kim wipes to dry up any blood. Reinsert the pipet. Allow some blood to exit the nick to find it again. Pick up the top wall of the blood vessel with the sharp curved forceps and insert the catheter as before.
9. After each of sutures is tied off, label a piece of tape with the name of the structure (e.g. "trachea" or "cephalic vagus") and use each piece of tape to attach both ends of the suture to the board away from the surgical area. This will clear the area for the surgeon and will allow you to find the correct suture in case one of the catheters or the trachea tube slips out.
10. *Be careful with exposed needles*. Unless you are nicking a structure, extracting drugs, or removing air bubbles, needles should be covered at all times. You can cover a needle by placing it sharp-side down into the tube tray, sharp-side inwards into the centrifuge tube, or pushed into the cap when the cap is resting in the tube tray. Never leave exposed needles lying on the table, and never reapply the cap with your hand.

Part 2: Trachea Tube

Attending: Preparing the Trachea Tube & Verifying Insertion

1. Taper the trachea tube with surgical scissors. The more acute the angle of the tube, the better it will insert. Make sure that your tube is at least two inches long so that it won't fall into the body cavity. Find a new one if it is too short.
2. Wash the outside of the tapered end of the trachea tube with saline. Be sure not to get saline into the trachea tube.
3. Watch the trachea during the tube insertion. If there is any blood on or near the trachea, dry it with a Kim wipe to ensure that no blood enters the trachea.
4. To make sure your tube is inserted properly, hold surgical scissors at the exposed end of the tube. If the metal shows a small circle condensation appearing and disappearing, the tube is inserted correctly.

Surgeon: Finding the Trachea & Inserting the Tube

1. Look up to the area below the jaw. You will find two yellowish sacks just below it; these are the salivary glands. Just below that area are two parallel muscles. Use the two sharp forceps to tear the muscles. You should see a white tube that looks like a bendy straw running down the middle of the body below these muscles. This is the trachea.
2. Lift the trachea with the sharp curved forceps. Push the sharp straight forceps between the two arms of the sharp curved forceps to tear the underlying cartilage. Push the sharp curved forceps through the hole and spread the arms to enlarge it.
3. Push the pipet under the trachea between the arms of the forceps. Continue pushing until both ends clear the body cavity, yet neither end is dangling in an area where it could be bumped. Insert the

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suture by pushing the sharp curved forceps under the trachea again, grabbing the end of the suture, and pulling it under the trachea along the caudal side of the pipet.

4. Take a needle and push it gently into the trachea between two cartilaginous (white) rings to nick it. Drag the needles from side to side along the incision to widen it.
5. Push the tapered end of the tube into the trachea with the pointed part of the tip towards the body. Hold the trachea below the incision with the blunt curved forceps. Twist the tube as you insert it, pushing until the open end of the tube is completely inside the trachea. If the tube does not insert easily, widen the incision or pull the pipet upwards away from the body.
6. Before removing the glass pipet, tie the suture around the base of the tube.

Time Check: 10 minutes (25 min total)

Part 3: Jugular Vein, Carotid Artery, & Vagus Nerve

Attending: Preparing & Inserting the Catheters

1. Taper both catheters with surgical scissors. The more acute the angle of the tube, the better it will insert. Trim the end of the tube so that it is blunt. Sharp ends can puncture the blood vessel walls.
2. Fill two 1mL syringes with clean saline. Attach each one to a catheter and push the plunger down until saline spills out the end of the catheter.
3. Once the needle is inserted into either blood vessel, take the tapered end of catheter and align it with the needle. *As soon as the surgeon removes the needle*, push the catheter horizontally into the blood vessel with the pointed end towards the animal. Continue pushing until about half an inch of the catheter is inserted. If you cannot push the catheter in, either the nick is too small or you have entered cartilage. Try to expand the blood vessel by pushing a small amount of saline as you insert the catheter. If this fails, ask the surgeon to make a new nick.
4. Once the catheter is inserted, hold it horizontally along the animal towards the head. If the catheter is angled, the tapered end could push through the blood vessel wall. Hold this until the surgeon has tied the suture and taped the catheter down.
5. After the catheter is taped down, push a small amount of saline from the syringe into the blood vessel. Watch the insertion site: if you see fluid flowing out, then the catheter is likely inserted into the cartilage or has punctured both sides of the blood vessel. If this happens, tell the surgeon to remove the catheter and make a new nick.
6. If step 6 is successful, pull back on the syringe plunger. You should see blood entering the catheter. If no blood appears or the blood is not continuous (i.e. there are bubbles), tell the surgeon to remove the catheter and make a new nick.
7. When the brake is applied, pull back gently on it. Release the brake when instructed by the surgeon.

Surgeon: Preparing the Jugular Vein

1. On the right side (from your perspective) of the animal's chest, you should see a dark blue line; this is the jugular vein. Gently pull the cartilage around the caudal end of the vein apart with the two sharp forceps until the vein is exposed.
2. Lift the vein with the sharp curved forceps and clear the cartilage under the vein as you did with the trachea. Insert the pipet beneath it and pull the suture through.
3. Use the sharp curved forceps to lift the top wall of the blood vessel. Push a needle into the vein horizontally. Aim to nick the vein as close to the head as possible. If you lose the hole and have to make another nick, you can make another insertion towards the tail of the animal, but not in the other direction. *Do not remove the needle or release the forceps yet!*

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4. Remove the needle when the attending is ready to insert the catheter. Dispose of it into the open centrifuge tube.
5. Once the attending is holding the catheter in place, tie the suture as before.
6. Take two pieces of tape and attach the catheter to the table on the side towards the computer. Tape will prevent the catheter from falling out when the syringes are moved.
7. If the vein has been punctured too many times, it may collapse. If this happens, try again with the left jugular vein.

Time Check: 15 minutes (40 min total)

Surgeon: Preparing the Carotid Artery & Vagus Nerve

1. On the left side (from your perspective) of the animal's chest, you should see a pink muscle along the trachea. Pull this apart using the two sharp forceps until you can see under the trachea. You will find there the carotid artery and vagus nerve attached to each other running parallel to the trachea. The vagus nerve is white and the carotid artery is pink with blood clearly running through it.
2. Lift the vein with the sharp curved forceps and clear the cartilage under the artery and nerve. There should be less cartilage here than there was around the jugular vein. Insert the pipet beneath both structures.
3. Separate the nerve and the artery by running the sharp curved forceps up and down the space between the two structures. Pull the vagus nerve up with the sharp curved forceps and reinsert the pipet so that it is under the nerve but over the artery.
4. Pull a suture through on the caudal side of the pipet as before. Pull another suture through on the cephalic side of the pipet. Tie off both sutures and label them “cephalic” and “caudal” with tape.
5. Pull the carotid artery up with the sharp curved forceps and reinsert the pipet so that it is under the artery but over the nerve. Pull two sutures through on the caudal side of the pipet. Attach a hemostat to both ends of one suture and pull the hemostat down along the animal until the suture is taut. This hemostat will act as a brake to occlude arterial blood flow.
6. Nick the artery with a needle as before. The carotid artery will be harder to pierce than the jugular vein since it has more smooth muscle tissue. However, blood pressure is higher here, so be sure to keep the break on at all times so that the animal does not bleed out. If you have lost the artery nick, ask the attending to release the brake to allow some blood to exit the nick.
7. Insert the catheter and tie it off with a suture as before. You should see blood in the catheter moving up and down as the heart beats.

Time Check: 30 minutes (70 min total)

Part 4: Data Collection and Clean Up

Both: Data Collection Setup

1. Tape the blood pressure transducer to the table. Quickly remove the carotid artery catheter from the syringe and attach it to the blood pressure transducer. Blood will rush up the catheter whenever it is not attached to a syringe or the transducer.
2. Tie the long suture to the hook. Insert the hook into the upper chest wall. Be sure to puncture the wall and not just the skin. Push the hook into and back out of the chest wall; this will ensure that you do not puncture the wall. Pull up on the suture; if the hook pulls up a tent of tissue, you have only punctured skin.

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3. Tie the other end of the suture to the force transducer. Start the recording. If the suture is too loose, you will not see spikes on the respiratory graph. If the suture is too tight, you will see the hook lifting the animal's chest wall.
4. Watch the blood pressure recording with a zoomed out scale for a few minutes. You should see a steady pressure. If the blood pressure changes drastically over the course of one second with no additional drugs, be certain that you have not bumped any of the equipment. If you have not, make certain that you have no bubbles in the blood pressure transducer or in the catheter. If this does not work, test the connections to the recording box. Finally, if this does not work, try using a different transducer, cable, or recording box. You will have to recalibrate if you change the equipment.
5. *If the blood pressure is slowly dropping*, your animal is likely dying. Try injecting epinephrine to increase heart rate and blood pressure.

Both: Throughout Data Collection

1. To insert the drugs, attach a needle to the end of a new 1ml syringe with the needle cap still on. Remove the cap and extract 0.2mL of clean saline. Invert the needle and tap it until most of the bubbles have reached the top. Push the plunger until all the bubbles are out of the syringe. Push the rest of the saline into the dirty saline cylinder.
2. Extract 0.2mL of the current drug. Remove the needle by place the cap into the tube tray, pushing the needle down into the cap until it is attached, and twisting the needle off. Attach the syringe to the catheter and inject it slowly, being careful not to inject any bubbles that remain. Reattach the saline syringe and push 0.05mL to flush the drug into the system.
3. Clean the syringe and apply the next drug as given in steps 5 and 6.
4. *If you see that the drugs are not inducing a response*, you may have a clot. Pull the plunger back on the jugular vein saline syringe and on the blood pressure transducer. Once the catheter is filled, push the blood back into the system. This should break up any clots. Try not to get blood into the blood pressure transducer. If you think that you may, quickly remove the catheter from the transducer and reattach the saline syringe. Use this syringe to remove the blot before reattaching the transducer.
5. *Watch the catheter for bubbles*. If you see any, remove them by attaching a saline syringe and pulling back on the plunger until the bubble is in the syringe. Quickly remove this syringe and replace it with a new saline syringe.
6. Keep the scale on the graphs zoomed out to look for drastic changes, especially directly following drug administration. Watch out for breathing cessation and significant drops in blood pressure.

Both: Clean up

1. Empty the centrifuge tube into the sharps container.
2. Dispose of any solid animal tissue and disposable materials containing blood, including lab tape and Kim wipes, into the biohazard container. All other materials, including syringes, metal tools, and plastic pipets, are not considered disposable. Wash these in the sink and return them to the table.
3. Pour all drugs down the sink followed by copious amounts of water.
4. To ensure that the animal is deceased, your TA may give you KCl. Introduce 0.5mL of KCl into the jugular vein. This should stop the heart and lungs almost immediately. Watch the recording to ensure that the heart has ceased beating.
5. Remove the catheters and trachea tube and cut off the tape. The sutures can remain on the animal. Place the needles from the catheters in the sharps container and through the rest away into the biohazard bin. Place the animal into the bag your TA gives you for disposal.