

Lee's Lab Notes-Rodent Handling and Surgery

- A. Becoming comfortable handling rats and mice – Most folks have received thousands of messages while growing up that rats and mice are dirty creatures that carry disease, want to bite you, etc. We may not even remember hearing or seeing these messages, but they can strongly affect us and make us uncomfortable or down right frightened to handle mice and/or rats. Cartoons showing women jumping up on chairs to get away from a mouse on the floor, scary movies showing people be eaten or attacked by rats, seeing someone we know scream when a mouse or rat runs across the room, etc., ALL leave messages in our brain that mice and rats are to be avoided or FEARED. Like any other message that makes us “pre-judge” a type of animal, a type of person, etc., those thousands of “little” messages make us PREJUDICE against rats and mice. Just like any other prejudice, you cannot use intellect to overcome a fear of rats and mice. You cannot talk, or read, or listen yourself into feeling comfortable handling mice and rats. You must EXPERIENCE the TRUTH that they are OK, that they can be handled safely, that they don't really want to attack and bite you, etc. Only by SHOWING your body and brain that they are OK, predictable, safe, can you dispel the untruths you have believed up to now. If you are afraid or uncomfortable reaching into a cage and handling a rat or mouse, or maybe even getting into a room with them, or maybe even entering a building you know houses rodents, KNOW that you can change that! And it's not HARD! It would be really unfortunate for YOU and the MICE AND RATS to go through a career not being COMFORTABLE handling them. So how do you get comfortable handling mice and rats? Don't get UNCOMFORTABLE! WHAT????? You have to give your body and brain enough “good” experiences to overcome the “bad” messages you've believed in the past. Just like using “progressive desensitization” to teach someone not to be afraid of getting in water at the beach or teaching a pet not to be afraid of thunder, you can teach yourself not to be afraid of rats and mice. First get in touch with your body, notice your breathing, notice your heart beat, and notice any tension in your muscles (especially in your neck and shoulders). The goal is to give your body and brain enough “good” experiences with rats and mice that you begin to feel comfortable in situations where you used to feel uncomfortable. If you are afraid or uncomfortable even being in the same room with rodents, start out by just spending 3-5 minutes at a time at a distance far enough away from a cage of rats or mice that you FEEL OK! NO increase in your breathing, no increase in your heart beat, feeling NO tension. IF you do FEEL anything negative, back up until you feel OK. If you try to “push it” or go too fast and your body feels something bad, you are teaching your body and brain that mice and rats are BAD. When you can spend 3-5 minutes at some distance from the cage without feeling any tension, repeat that 4 or 5 more times over the next day or two. You MUST have repeated “good” experiences at that distance to PROVE to your body that at least that much is OK! Only after you feel absolutely comfortable at one level should you move to the next level. Go from being OK seeing the cage, to being close to the cage, to watching someone else just leave their hand in cage (mice and rats may be a little afraid at first, but

then they will become curious.) Watch as the rats or mice come over to the other person's hand in the cage and just sniff it. Then watch as one of them eventually gets close enough to "feel" the person's finger with their teeth. Humans have thumbs, we usually "explore" new objects by touching, pinching, handling them; rats and mice "explore" by using their teeth to "pinch" something to see if it is hard or soft, they are NOT biting! Once you are comfortable watching someone else leave their hand in cage (this may take 5-10 times of watching someone else do it over 2-3 days) try leaving your hand near the cage, then on top of cage, then in the cage, then in the cage while the rat or mouse smells your finger, and finally until they "feel" you with their teeth. Remember SUCCESS is feeling...nothing! If you begin to feel uncomfortable at any stage, BACK UP and repeat the previous step several times until you feel... bored! Then progress to the next step again. Next steps: grasp the animal by the tail and just follow it around the cage holding onto the tail (eventually it will go to a corner and just sit there), pick the animal up by the base of the tail and put in on your opposite arm folded against your belly so the animal is well supported (the animal will most likely just sit still or may try to "hide" its head between your elbow and belly), put the animal on a table or cage top and just hold onto the tail while it walks around a little (if it starts to struggle to get away just put it back in the cage, let it calm down for 3-5 minutes, and repeat, repeat, repeat until it is clear YOU and the animal are OK with this "bonding time". Next, using the same 5-10 repetitions of 3-5 minutes at a time, pick the animal out of the cage, place it on a cage top or table top and grasp the animal in one of the "restraint" holds (one or two handed method for mice, behind the shoulders for the rat. See the videos on restraining mice and rats at: <https://oacu.oir.nih.gov/training-resources>) Now CONGRADULATE yourself!! YOU deserve it! Now watch the other 17 training videos at the OACU web site and start practicing.

Now FULL disclosure and a somewhat mixed message. Not ALL rats and mice are nice all the time. Some will want to bite you if you hurt them. A few may want to bite you even if you don't hurt them. Signs that a rat or mouse is having a bad day or just doesn't like you anymore: standing on their feet with their belly lifted off the floor/bedding, standing with their tail lifted and straight, shoulders up with head lowered and ears flat, showing their teeth or screeching. If you see these signs, usually just backing off for a few minutes will let them calm back down. If not, consider using a leather glove and/or some type of restrainer (decap-a cone, small towel wrap, tunnel, etc.).

OK worst case: YOU get bit. TRY to remain calm, wait until the animal releases its bite, and then put it back in its cage. Most of the time you can just wait a few minutes and the animal will calm back down. If it still seems upset (see the signs above) consider that leather glove again! Really try NOT to pull the animal away when it is biting you and teach it "fly". Pulling on a biting animal just scares it more, usually makes it bite harder, and may convert a small puncture wound to a rather jagged tear! If the experience makes your heart beat faster, your breathing increase, and your muscles tense up: GOOD! You are normal. If it makes you run from the room screaming; go back, read this whole procedure again and start where you feel OK! OK? OK!

B. Basic restraint and visual examination: See videos at <https://oacu.oir.nih.gov/training-resources> One technique not shown in the OACU videos for handling and calming rats is the “Rat Swing”. This is performed by grasping the rat with one hand with your fingers just behind the front legs. If the rat is “friendly” you can do this in the cage. If the animal is a little “shy”, you can remove it from the cage using one hand at the base of the tail and place it on your opposite forearm tucked against your belly, It will likely try to hide its head between your arm and belly especially near your elbow. When it does, you can slide your hand forward from its tail to just behind its shoulders. Then with your arm extended, swing the rat in a large arc as if your arm is a swing at a kid’s playground. Lift your arm up level with your shoulder and let it “fall” down past your waist then up behind you. Each “swing” should take about one second. You will soon feel the rat relax and almost become limp in your hand. Once it has relaxed you can do a quick exam, give it a oral-gastric gavage, etc. To KEEP the rat relaxed you can just “bob” your hand up and down 20-30 cm once every second. Unfortunately this procedure does not work to relax mice.

C. Animal Identification:

- Ear tags- be sure you place the ear tag with the numbers/letters UP! Place the tag far enough in so it penetrates the cartilage in the ear, but not so far in that it makes the ear fold up inside the tag.
- Ear notches: Using an ear punch you can create up to 3 “notches” (half a hole at the edge of the ear) or 3 complete holes (just inside the edge of the ear) per ear, i.e., top, middle, and/or bottom of ear. See charts on the internet for several different “numbering” systems. Be aware that holes can close up and disappear if the punch is too small, and holes can get ripped during fights and become notches.
- Tattoo- Most “human” tattoo machines are designed for much thicker skin than that on rat and mouse tails, be sure you can adjust the depth of needle penetration. Black ink on black colored tails can be a real challenge to read.
- Subcutaneous Microchip injection- Be sure needle is inserted at least 2-3 times the length of the chip under the skin so the chip doesn’t just migrate back out of the needle hole when the needle is withdrawn. Always check each chip with a chip reader BEFORE placing it in an animal.

D. Compound/drug administration:

- Subcutaneous, Intramuscular, Intravenous, and Intraperitoneal injections (see videos at <https://oacu.oir.nih.gov/training-resources> Confirming placement of injections: Intradermal injections will cause the swell and “blanch”. With subcutaneous injections slight lifting of the needle will clearly outline the needle below the skin and injecting fluid will cause a swelling under the skin. When giving intraperitoneal injection lifting the needle will broadly raise the skin and there will be no clear outline of the needle. Injecting fluid IP will not cause a localized swelling near the needle. The effects of “IP” injections that get delivered in the gut lumen or fat bodies will be delayed, e.g., pentobarbital given in a fat pad may take 10-15 minutes to take effect compared to 2-3 minutes if given in the gut lumen. The effects of “IP” injections that get delivered in the gut lumen or fat bodies will be delayed, e.g., pentobarbital given in a fat pad may take 10-15 minutes to take effect compared to 2-3 minutes if given outside the fat (be VERY

careful about giving additional doses of anesthetic if it seems to take a long time to take effect, it WILL eventually take effect and if you've give extra doses during the wait, you can SERIOUSLY or FATALLY overdose the animal. Most common cause of death and delayed recovery from anesthesia in rodents is HYPOTHERMIA. ALWAYS provide external heat for anesthetized rodents and monitor their body temperature!! If you are using "hand warmers" for supplemental heat, be sure to wrap in a few layers of paper towel before placing the animal on it to avoid skin burns. If using "heat lamps" to provide heat check the intensity of the heat source frequently by placing your hand directly over/next to the animal. If it is uncomfortably hot on your hand it will likely BURN the animal's skin!

- Gastric gavage See Lee's or any of the many YOU TUBE videos (Instech has a very good video for gavage of mice.) Be sure to note where the animals nose/teeth will be on the gavage needle when the tip of the needle is in the animal's stomach (just past the last rib). Don't begin gavage if the needle isn't in as far as expected. Draw back on the syringe before giving the gavage- no air, or one small bubble is OK. If you draw back a lot of air the needle is probably in the trachea-pull out and start over!

E. Sample collections:

- Urine, Feces- You will almost always get a "free" sample when you restrain a mouse. If you need to collect it just have a small weigh boat or container ready to collect it when you pick the mouse up. When I'm collecting urine from mice, I favor the one hand technique for restraint because it leaves the other hand free to position the small weight boat. Rats- not so much. Pick up feces from cage. If you need urine, consider a raised floor or metabolic cage. Placing a rat or mouse on a clean expensive white shirt sleeve or on your head in front of your girlfriend almost guarantees a sample deposit. Remember rats and mice like rabbits are coprophagic and produce two types of feces. One is the lighter colored, moist, "sticky" feces that comes from emptying the cecum. It is high in bacteria, and thus higher in protein and vitamins and is usually ingested directly from the anus as it is expelled. It is frequently referred to a "night feces" in rabbits. The harder darker drier fecal pellets are the "recycled" 2nd time through feces and is usually left in the bedding. If you are asked to collect fecal samples from rats, mice, etc., be sure to ask which type of sample is needed.
- Mandibular and tail vein blood collection: see videos at <https://oacu.oir.nih.gov/training-resources> and You Tube (Golden Rod Lancet). When performing a mandibular stick, I use 4.5-5.5 mm lancet (Goldenrod, Inc.) like those used by diabetics for blood sugar testing. Sticking the "dry skin and hair" over the mandibular vein will result in very quick clotting and a small or nil blood collection. Wiping the area with an alcohol swab before puncturing will delay clotting and increase blood volume collected. Coating the area with petroleum jelly will greatly increase clotting time and blood volume collected. Once enough blood has been collected the flow can be stopped by wiping the cheek clean and applying slight temporary pressure It can be a little tricky at first finding the

mandibular vein. Just like people have different shaped heads with correspondingly small differences in where one structure is located relative to another, there are small differences between different size and strains of mice. Fortunately once you've located the vein on one animal it is in the same spot on all the rest (assuming you are using the same size and strain of animal in your study. As a default, I start about 1mm behind the small hairless point on the mouse's cheek. You do not have to “bury” the lancet all the way to hilt! They are made for puncturing human finger tip skin which is MUCH thicker than mouse cheek skin!! The vein lies just under the skin and on top of the masseter muscle so the puncture only needs to be 1-2 mm deep.

- While blood can be collected from the lateral tail vein using a 23-25 gauge needle on a 1 ml syringe, it is difficult to collect much blood before the needle slips out of the vein or the vein collapses. More blood can usually be collected by using just the needle shaft. Holding the shaft with hemostats break off the hub, and then insert just the needle shaft into the tail vein. Let the blood drip from the needle shaft into a blood container (e.g., 1 ml flip top Ependorph tube.). Removing the hub not only reduces the needle's mass and makes it less likely to “fall” out of the vein, it also prevents clotting because each drop of blood falls off the needle shaft before compliment is activated and forms a platelet plug. If the hub is left on the needle the surface tension of the blood across the inside of the hub will slow/stop the blood flow and a clot forms inside the hub before any blood can drip into a collection vial.
- Intra-cardiac blood collection- This is only performed as a terminal procedure with the subject under deep anesthesia. A 23 gauge 1-1 ½ inch (25-37 mm needle is attached to a 1 or 3 cc syringe for mice, 6-10 cc for rats. With the animal on its back, the needle is inserted through the notch between the manubrium and the sternum on the right side of the manubrium and guided toward the point of the left shoulder. Once the bevel of the needle tip is completely under the skin a slight vacuum is formed in the needle by pulling back on the syringe plunger. The needle and syringe are advanced SLOWLY toward the left shoulder. When blood begins to flow into the syringe the advance is stopped. In an adult 30 gram mouse you should be able to collect 1.1-1.4 ml of blood. Just before death it is quite common for the animal to urinate a few drops and then stop breathing. If blood stops flowing into the syringe before the animal dies it probably means the ventricle has collapsed. Holding the needle still and releasing the vacuum in the syringe for a moment will frequently allow the ventricle to refill and you can begin collecting blood again by withdrawing slowly on the plunger again. If blood does not begin flowing again the bevel of the needle may be up against the ventricular wall. Without moving the needle in or out, just rotate the needle slightly and see if blood begins to flow again. If one misses the heart it is quite common for the needle to puncture a lung and create a pneumothorax. This allows the heart to drop away from the sternum and fall more “forward” in the chest. If this happens it is best to withdrawal the needle until the needle is just barely in the chest and then advance the needle slowly toward the thoracic inlet.

You should keep a slight vacuum in the syringe as it is advanced SLOWLY. If you are unable to find the heart you can try repositioning the needle to the 4th or 5th left intercostal space where you see the heart beat most prominently and again advance the syringe and needle toward the heart with a slight vacuum in the syringe. If you still can't find the heart but need a blood sample, you can use a pair of small scissors or a scalpel blade (#10) to cut through the skin and muscle over the left armpit and cut the brachial arterial. You can usually collect 0.25-0.75 ml of blood from the brachial artery. Whether collecting from the ventricles or the brachial artery, it often helps to "compress" the animal's abdomen slightly with the palm of your hand to help blood return to the heart from the abdomen so it can be collected.

- Tissue collection for DNA testing- You can use tissue or cells from several sources including the tissue removed when doing ear punches to ID the animals, from removing the terminal 1-2 mm of the tail under anesthesia, buccal swabs etc. If using a single instrument on multiple animals, e.g., ear punch, scissors, or scalpel blade, be sure to clean and "denature" the instrument between animals so you don't contaminate samples from one animal with the DNA of the previous animal(s). 70% isopropyl alcohol or a hot bead sterilizer can be used to denature any DNA remaining on most instruments.

F. Miscellaneous notes:

- When tunneling catheters subcutaneously you can reduce "dead space" around the catheter by using a trocar instead of blunt dissection with scissors or hemostats. The trocar inner diameter has to be bigger than the outside diameter of the catheter, in many cases a 18 or 19 gauge 1 ½-2 inch (37-50mm) hypodermic needle can be used. The sharp cutting edge and tip of the needle should be dulled using very fine sandpaper (600-2000 grit) so the tip "slides between the skin and underlying muscle and doesn't cut into either.
- Most commercial vein picks and dilators are made for humans with much bigger vessels than those frequently targeted in rodents. A stainless steel wire larger than the catheter size being used can be cut on an angle and then smoothed and polished with a file and very fine sandpaper to make a vein dilator, e.g., 0.040 inch wire for use with PE-10 catheters. A vein pick can easily be made from a 23 gauge hypodermic needle by smoothing the cutting edges and tip of the needle with very fine sandpaper (600-2000 grit). The tip of the smoothed 23 gauge needle is then inserted 0.05-1mm inside the lumen of 19-20 gauge needle and bent "backward" 90 degrees to form a triangular tip with the concave lumen portion is at the very terminus of the needle. The tip is then placed in the cut or punctured vein and the catheter slid under the needle tip and into the vein.
- To prevent the catheter from sliding out of the vein before is it secured with ligatures, place the catheter near the vessel in the approximate position you want it to stay, e.g., tip 1.5-2mm past the vein opening then lay a light instrument on the catheter to keep it from sliding out of the vein until you get the ligature tightened.

- While mouse jugular veins are smaller than rat jugular veins they are often easier to catheterize, because the dense connective tissue around the rat's vessels make it challenging to get the vein exposed without causing significant vessel contraction. Placing 1 or drops of 1% lidocaine on the vessel will HELP prevent the vessel from contracting.
- When doing a jugular catheter, the volume of the completed catheter should be carefully measured by measuring how much hep saline it takes to remove all the air from the catheter so you'll know how much "Lock solution" you need to inject when flushing the catheter especially when using a "thick" lock solution like heparin in 50% dextrose or glycerol. If you inject too much you tax the liver and may "heparinize" the whole animal. Short term heparin solutions used for initial flush during insertion or multiple same day blood withdrawals can be made with 25-50 IU heparin/ml in 0.9% saline or ringers lactate solution. Longer term lock solutions can be made "thicker" or more viscous by using heparin in 50% dextrose or glycerol. This reduces "wash out" of catheter and clotting at the tip but requires the solution be by withdrawn from the catheter at each "flush" or use, and requires you know the catheter volume so you don't "overflow" the catheter and heparinize the animal. Students should review the literature and commercial catheter maker's web sites for more info on the various flush and lock solutions, and sample collection and flush procedures used.
- I not only use the decap cones for decapitation of mice and rats (provides a good "handle" and keeps fingers away from the decap blades), but also for restraint of unruly creatures. You can hold the cone open by bending back the top "corner" of the cone (keeps the bag open and puts at least a little protection between your finger the rat/mouse's teeth on their way into the cone. Grasping the animal by the base of the tail in their cage, they can easily be "swung" into the cone in one easy swing (usually!?!?!). Once in the cone, the cone is tilted down, the rodent slides down, and the cone squeezed off at the base of the animal's tail. Most animals calm down immediately, and are easy to inspect. Holes can be cut in the bag to access injection ports, treat wounds, remove sutures, give SC, IM, IP injections, etc
- Castroviejo needle driver locks commonly get out of alignment during cleaning, autoclaving, etc., and then they either don't catch on the "half squeeze" or won't release on the "full squeeze". When (not if) that happens, it is fairly simple to 're-align" them by pressing one the lock halves in or out a little so the pieces meet, catch, and release as they should. Rarely, the "single" half has to be "twisted" a bit to get it to slide onto and then past the notched half.