

Lickometer Filling and Testing SOP

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Box Cleaning/Preparation

- Take out metal floor and underneath collection tray in operant box
- Spray acetic acid onto paper towel and wipe down both parts thoroughly
 - Make sure to go between the bars of the floor as well as the back ends that contact the far side of the wall -- these make need to be clear to complete the circuit and count licks appropriately
 - ***NOTE***: Spray onto paper towel and not into box since the aerosolized particles could interfere with the electronics within the box and cause a short circuit
- Wipe down inside walls and ports with acetic acid and paper towel as described above
- When putting floor back, make sure the screws are firmly in place (not dying strain, but snug -- these pull the floor into the back wall of the box, which makes the contact loop. However, over-tightening can strip the screws, causing poor connections. If one particular bar doesn't work, these not being fully tightened is often the reason)

Making of Lickometers

Background

We found that the sipper tubes used in the retractable sipper (ENV-352AW, Med Associates, St. Albans, VT) worked well. However, the use of ethanol in the tubes eroded the adhesive holding the metal tip to the plastic tube over the course of a few sessions. We took the metal tips provided with the sipper tubes and adhered them into a cut down 10mL serologic pipette (13-678-11E, Fisher Scientific) using a 2-part epoxy solution (LOC-1365868, Locitite) as the adhesive. We found this stayed much more stable throughout the duration and for months after the experiment.

Step-by-Step Guide

- Using a Dremel or other rotating saw bit, cut the ends off a 10mL serologic pipette tip so just the cylindrical body remains (we cut at 0 and 8mL marks, about 15cm long)
- Check if the metal tip originally provided by Med Associates fits inside the tube
 - **Critical Step**: The metal tip should fit snug, but not tight. Forcing it into the tube will crack the plastic, making it leak during the experiment. Any cracks you see in the tubes anytime in the experiment should be addressed immediately
- If the metal tip is too large, use the Dremel to grind down the top 1-2cm of the metal. Repeat above step to see if fit is better
 - **Important Note**: How much of the tip is exposed from the pipette will determine how far into the box the sipper protrudes. We found that having it minimally extend into the cage, such that only the round end of the sipper is present when extended, leads to best results. If too exposed, animals can (and will) put their paw on the sipper causing the completion of the circuit (and thus counting as a lick), until the paw is removed or spout retracted
- Once metal tip sits comfortably within the pipette, cover the tip with epoxy and push into place

- Wipe up and epoxy that gets onto the outside of the metal tip. This is where the electrical connection is made, and so residue on the outside of the tubes can prevent contacts from being counted correctly
- Let dry at least 24 hours before use

Filling and Testing Sipper Tubes

- Place a gloved thumb over the rounded end of the sipper tube and fill tube ~90% full of solution
 - If using different sippers for each solution type, make sure solutions go into proper tube to avoid cross-contamination
- Slowly release thumb, allowing ~1mL to flow out of tip, then stop flow again. Firmly push rubber stopper into place, twisting into place as you push
 - **Critical Step:** The stopper should fit snug, but not tight. Forcing it into the tube will crack the plastic, making it leak during the experiment. Any cracks you see in the tubes anytime in the experiment should be addressed immediately
- Releasing thumb after stopper in place should result in no drips from the tip of the sipper tube
- Rubbing spout gently on finger/palm of hand or tapping with fingertip should release bubbles up the spout and wet the glove, but should not allow for free-flow of the solution out of the tube
- To place in operant box, turn sipper tube ~45° downwards and check for dripping. If dripping continues for more than a few seconds, refill the tube and check the tube for cracks. Gently passing over the tip with a gloved finger should release a bit of solution, but not drip or freely flow out of the tube
- Tighten screw firmly to hold the tube in place
 - If screw is difficult to move, try unscrewing, and wiping down threading of screw with acetic acid. Slightly damp cotton swabs with acetic acid can be used to clean out corresponding threads of port. If swab is too wet it could drip down into electronics and short circuit the lickometer

Testing Lickometers in the Operant Boxes

Lickometers work by having the animal complete an electrical circuit when licking the spout of solution, so the purpose of this test is 2-fold: 1. to test for false positives/negatives and 2. make sure solution is flowing and animals would receive corresponding reward/punishment.

- Lickometer tubes will be held at an angle in the operant boxes, making reading the start and end volumes more challenging. Make sure the tubes are oriented the same direction each time and that however you decide to read the volume, you remain consistent with the reading method. With the graduated sippers provided by Med Associates ([see here for more information on lickometers](#)) the resolution that could be confidently read was to 0.05mL.

Floor/Lickometer Testing

Note: in this situation, wall with ports is on RIGHT of box and LEFT hand is being used to test contact

- With an ungloved hand (must be ungloved, otherwise will not complete the circuit) place index finger on bar 1 (closest the spout/bottles) and put thumb by the spout. Make sure you are not

touching any other part of the box such as the larger metal box that is enclosing the chamber as that can make it seem as though the contacts are sound when in fact they are not

- Touch spout ONCE with thumb while watching MED-PC screen to see if lick is counted quickly and correctly (on correct solution side, only 1 contact, etc.)
- Then flick thumb 5-10 times *quickly* over the spout to simulate fast licking (mice will often lick dozens of times in a row quickly) while watching MED-PC screen to see if those contacts are correctly counted
- Then take thumb and place on spout for at least 3 seconds. Contact should initiate the start of a lick, but the lick shouldn't be counted until the contact ends. Sometimes the counter gets stuck from air bubbles or other things, so prolonged contact like this can lead to the count rapidly escalating without any animal contact
 - The program records contact onset and offset, so these should correspond to when the thumb started/stopped touching the spout
- Repeat these steps with other bars near the spout and around the cage as well as in the second port/spout (if used)
 - ***NOTE***: Be sure you are only touching one bar at a time. If you are even slightly touching multiple bars at once a bar that is not working can seem like it is
- After testing the bars, make sure the lickometer still works if you have one finger on a bar and are also touching the metal port as the mice sometimes stand on one of the bars and put their paws on the port when licking

Floor/Lickometer False Positives Testing

The mice like to explore and touch everything so it's also important to make sure that when they're doing so, the lickometer isn't recording false positive contacts

- Make sure that if you're touching a bar and some other metal part of the box (such as the walls or any part of the port that is *not the lickometer*) the lick counter is not increasing
- After testing all bars close to spout for accuracy, clean out port with tip of a twisted up paper towel. Try to avoid touching the spouts and just get any extra liquid that's fallen from them
- If using a triggered laser, make sure the laser is firing as expected upon making contact. It is always important that the lickometer is working and there aren't any false positives or negatives, but it is especially important if the licks are triggering something (like a laser) because not only will the data recorded be inaccurate, but the behavior of the animal may be affected: if you have any false positives, you'll get stimulation when not intended and if you have false negatives, the animal could be licking without receiving the intended stimulation

Troubleshooting

There are many potential reasons for the lickometer not to work or not to work as well as expected, but below are some common things to check:

- Make sure the bottles are screwed in tightly. Even having the screws loose by a quarter turn can prevent the lickometers from working properly as it all depends on the circuit being closed

- Make sure the floor is fully in contact with the back wall of the box and that it's screwed tightly (but not too tightly because you don't want to strip the screws). The floor can sometimes be screwed in, but if the back wires that stick out of the floor are not in contact with the holes in the back, the lickometer will not work well as the bars won't be fully closing the circuit
- If the boxes are old, sometimes you will find that particular bars are not working when you are testing the lickometer. If that is the case, it may be possible to "connect" the dead bar to a bar in the back (which the animal cannot possibly stand on and reach the lick spout at the same time) by connecting the bars with a paperclip or wire on the back of the floor before placing the floor into the wall. Note that this will only work if you do not need the shock floor and you should still keep an eye on the lickometer because this is a temporary fix and eventually the wire may need to be replaced
- Sometimes, the lickometer will randomly count licks when no contacts were made. A potential reason for that is the lickometer, port, or any of the equipment or wires connecting the wire being wet, so make sure everything is dry