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Day before surgery

Have your intra-oral cannula (IOC) and colder coupler ready and place it, along with your choice of tall or short skull screws, into cetylcide in a metal tray overnight. If you’re extra wary toss in your dental drill bits.

Double check that your electrode drive is ready. It might be a pain, but it truly helps to know which electrode is which when you’re comparing either side or seeing dead channels.

Finally, make sure your communal and definitely communistic surgery tray has been autoclaved and isn’t missing any doodads. Speaking of communism, make sure the Ketamine/Xylazine (KX) cocktail bottle has enough for your surgery, and as a courtesy to others, for any other activities involving ketamine that day.

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1. Print two brand new surgery sheets (one for your records and one for the labs).
2. Fill out red surgery card from the vivarium, weigh your rat, and bring it into the surgery suite.
3. Grab a surgery coat, a surgical mask, and some of the single use gloves.
4. Let your rat chill in the surgery suite while you pull up KX, alloxate, lidocaine, ringer’s solution, and penicillin.
5. Now let the isoflurane gas induction chamber warmup by pushing down the green plunger on the syringe tip, then pressing induction and deliver on the screen.
6. While that’s going on get out 2 x iodine wipes and 2 x alcohol wipes, 2 x yellow needles, and the prepackaged scalpel blade on the shelf.
7. If you’re feeling ready and relaxed, now you can put some gloves on and scoop up your rat and place it in the iso-chamber. Be sure to keep a few fingers on the lid to stop your rat from escaping and your Zen from being ruined.
8. Now wait until your rat stops writhing and slumps down. Give it a good 10 seconds and tilt the box a bit. If she’s down, take her out and inject her intraperitonially (IP) with the KX induction does. (Hint! Be quick with this!)
9. Put her back in the home cage without the cage part but with the lid. (Now is a good time to go get coffee and brag about how well your surgery is going).
10. After you come back, check to see if your rat is down for the count (asleep) by taking it out of the home cage and putting it onto a brand-new diaper pad on the surgery table and giving her toe a pinch.
11. If 30 minutes have passed since the induction dose of KX, she’s withdrawing her leg to the toe-pinch, go on and give one of the KX boosters IP. If it’s still not working after 15 minutes give another boost. (Something is wrong if this isn’t working.)
12. Now you can get on to the real bits of the surgery.

The real bits of the surgery

1. Now using the Wahl **clippers** in the drawer shave a football-shaped pattern onto the top of the skull. This will point up right between the eyes and to the bony bump on the back of the head. I like to do this over the trashcan and shave the hairs directly into it. AVOID getting hair in the eye. This is a big no-no.
2. Take your shaved rat and place it gently yet firmly into the stereotaxic stage, this is a good time to give some **saline**, **alloxate**, and check for a **toe pinch**.
3. If your rat is like my rat, then you’ll want to place the **ear bars** at one or two deviations from the 10 on the ear bar meeting the 5 on the stereotax marker. Then place the animal into that one and fix the second one to match. (Make sure this is stable, level, and something no one would be suspicious about.)
4. Secure the two front teeth on the **head holder** and gently pull away and secure the screw.
5. **Clean the incision site** with iodine and alcohol. THEN put eye gel onto the rat.
6. Inject **lidocaine** either into two sites on the scalp or just one and wait for ~5 min. (Good time for a sip of coffee!)
7. Using a scalpel, create a clean **incision** (please not multiple incisions), rostral to caudal, that is large enough to expose the area of operation.
8. Use the head wound **retractors** to pull the skin to the sides and expose the skull. Make sure you’re actually getting under everything and not just the top layer. You should be able to see the sutures of the skull.
9. Now get your lights ready. Make sure the **eye gel** is on, so you don’t roast the eyeballs. (Ring light available. To use as well as gooseneck lights.)
10. Use the head bar to make the **skull level**. Align the front spike with bregma, and the middle with lambda as shown below:
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12. Attach one of the needle tips (yellow) to bar mount to discern position of bregma. Mark it in the surgery sheet and calculate the **GCL and GCR coordinates**. Lower the needle tip over the GC coordinate and use the cauterizing tool to mark the location on the skull. Repeat with the other side.
13. Use the small drill bit LA¼ to create 4-5 shallow holes for **skull screws** as depicted below. About half the size of the small drill bit tip. Screw in each screw using a screwdriver and forceps, just until stable in the skull.
14. Now **RECHECK** your coordinates. Grab the big burr LA2 and get to making small donuts around the coordinate marker. Notice the GC is on a slope of the skull demarcated by the ridge on the skull the carriers the jaw muscles.
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16. Go slowly until you get the hang of it. Faster **drill** speeds = less harsh bite. So really slam the pedal down and lightly tap away with the drill bit.
17. Use a von Graefe knife to slice up the **dura** and the curettes to pick out any bone.
18. Now carefully **repeat this** on the other side. I recommend using a fine digital caliper to measure the distance from your craniotomy to the marker for the other side as well as the distance between your electrodes.
19. Make sure to **soak** up any liquid on the brain or around the craniotomy at this point.
20. Now prepare your **electrodes** for going into the brain.

Electrode Implants

1. Using either a brush or 0.5mL syringe put the tiniest drop of **Vybrant Dil** onto the tips of the electrode bundles. This ensures you can find out what the heck you recorded later on.
2. Using the alligator clip adjustable holder or the 3D-Printed implant tool **attach** your drive to the stereotax.
3. **Center** your bundles over the implant locations.
4. **Drop down** to the height of the brain such that the tips of the electrodes are touching the brain.
5. Record the D/V measurement and now begin **lowering** your electrode to -4.4mm from the surface of the brain over the period of 30 minutes.
6. Using **Kwik-Cast** mixed in a weighboat or with the applicator tip apply the green goo to the craniotomy. The skull around this area NEEDS to be dry.
7. Now using fine and medium curved forceps tie the **ground** wire around the ground screws.
8. Using **dental cement** build up a central pillar to the drive body and let it cure for a few minutes (coffee time).
9. Gently add another layer of dental cement around the drive and wait a little longer. Now unscrew or unclip the drive and **lift** of the stereotaxic holder.

IOC Implants

1. Remove **IOC supplies** from cetylcide into a saline jar.
2. Use bead sterilized forceps to insert a cut section of **silicone tubing** from the acetone container and affix it onto the IOC connector mount. Use a drop of vetbond on the window of the connector to secure the tubing in place.
3. Now put a **yellow needle**, tip to the washer, into the IOC and place it on a clean kimwipe.
4. Use the mouth **retractors** and small wound retractors to make the mouth open up wide.
5. Using the needle as a guide, **insert** it with the bevel facing towards the side of the second molar up into the flesh aiming towards your IOC pocket above.

A drawing of a cat's face

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A drawing of a cat's head

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1. Connect the silicone tubing and connector onto the IOC and begin the process of **dental cementing** the IOC in place and affixed to the drive body.
2. Once cured **check** around for any places that need filling in.
3. Now give all remaining saline, **penicillin**, and love.

Post-Surgery

1. Prepare a new cage with soft bedding, wet food, water, and an enrichment packet as a pillow. Make sure your surgery cage card is filled out and return the animal to the vivarium.
2. Clean up after yourself and autoclave the trash and surgery tray.
3. Weigh your animal everyday post-surgery for a week and regularly clean cage, flush IOC and keep an eye on health.
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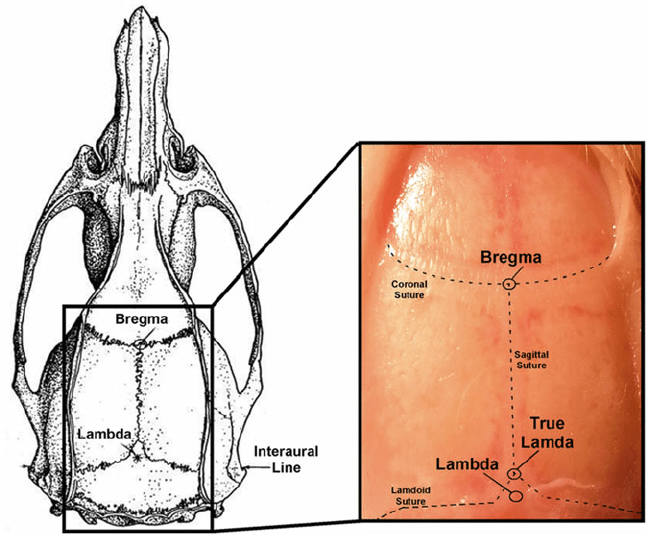
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A long metal pole with a white clip

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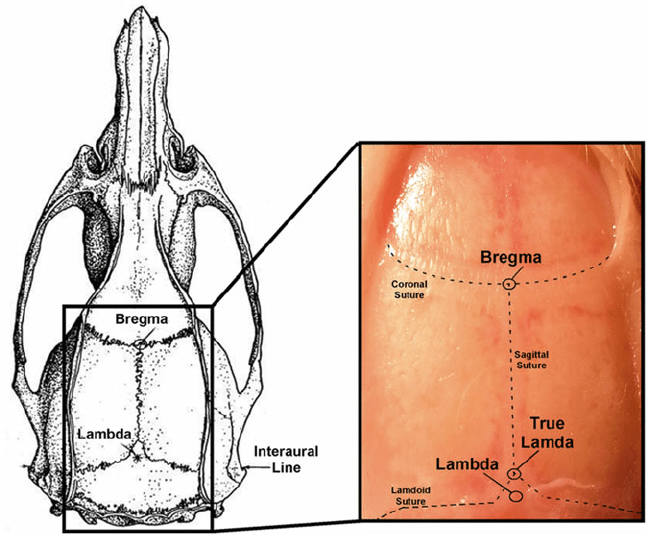
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EMG Implants

1. For the EMG wire implant, keep the rat off of the stereotax and flip her so that her head is back and in one of the Altoid tins with gauze in it as a pillow.
2. Carefully shave a patch of hair around the middle of the throat and rub it with iodine and alcohol wipes.
3. There is a small bump in the skin that is hard to shave and when shave can irritate the rat post-surgery. Avoid it!
4. There are several useful muscles for analyzing gaping, jaw-movements, and swallowing; the anterior digastric, masseter, and the suprahyoid.
5. For the anterior digastric, use a brand-new scalpel blade and do a small incision in the skin of the throat.
6. Using round tip scissor cut up along the epidermis keeping close to the skin until you get back up to your primary incision at the top of the head.
7. Using forceps pull a small hollow metal tube through the top of the head to your EMG incision site. This tube will help you ferry the EMG wires towards the anterior digastric.
8. Once pulled through carefully extract the tube and open up your incision site with the mini retractors.
9. Use 6-0 sutures and fine hemostats to make a loop with suture and tie to the end of the EMG wire and stab into the belly of the muscle pulling it through leaving a half-inch of slack.
10. Using a cauterizing pen, melt the protective coating around 1mm of the wire that will go into the belly of the muscle.
11. Pull the rest of the half-inch of slack through so that the expose bit of the wire is in the muscle.
12. Use vetbond on a small needle to tap the entrance and exit of the wire through the muscle. Cut off any excess wire. Repeat the process up until this point for any other muscles you want to record from.
13. Suture up the wound and dab some vetbond on it.
14. Finally, dental cement up any expose wire near the head cap so the animal doesn’t yank it out.
15. Now give all remaining saline, **penicillin**, and love.

Post-Surgery

1. Prepare a new cage with soft bedding, wet food, water, and an enrichment packet as a pillow. Make sure your surgery cage card is filled out and return the animal to the vivarium.
2. Clean up after yourself and autoclave the trash and surgery tray.
3. Weigh your animal everyday post-surgery for a week and regularly clean cage, flush IOC and keep an eye on health.
4. For 2 days after surgery give meloxicam. The day after surgery give penicillin.
5. If your animals weight drops below 85% pre-surgery weight you cannot water deprive them and must nurse them back to above this threshold.
   1. **GC-PC Implant with Nasal Cannula**

Day before surgery

Have your intra-oral cannula (IOC), colder coupler, and nasal cannula with cap ready and place it, along with your choice of tall or short skull screws, into cetylcide in a metal tray overnight. If you’re extra wary toss in your dental drill bits.

Double check that your electrode drive is ready. It might be a pain, but it truly helps to know which electrode is which when you’re comparing either side or seeing dead channels.

Finally, make sure your communal and definitely communistic surgery tray has been autoclaved and isn’t missing any doodads. Speaking of communism, make sure the Ketamine/Xylazine (KX) cocktail bottle has enough for your surgery, and as a courtesy to others, for any other activities involving ketamine that day.

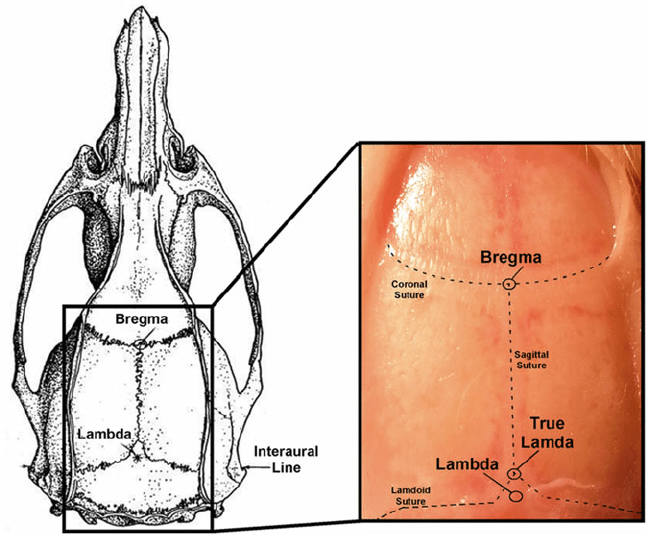
Day of the surgery

1. Print two brand new surgery sheets (one for your records and one for the labs).
2. Fill out red surgery card from the vivarium, weigh your rat, and bring it into the surgery suite.
3. Grab a surgery coat, a surgical mask, and some of the single use gloves.
4. Let your rat chill in the surgery suite while you pull up KX, alloxate, lidocaine, ringer’s solution, and penicillin.
5. Now let the isoflurane gas induction chamber warmup by pushing down the green plunger on the syringe tip, then pressing induction and deliver on the screen.
6. While that’s going on get out 2 x iodine wipes and 2 x alcohol wipes, 2 x yellow needles, and the prepackaged scalpel blade on the shelf.
7. If you’re feeling ready and relaxed, now you can put some gloves on and scoop up your rat and place it in the iso-chamber. Be sure to keep a few fingers on the lid to stop your rat from escaping and your Zen from being ruined.
8. Now wait until your rat stops writhing and slumps down. Give it a good 10 seconds and tilt the box a bit. If she’s down, take her out and inject her intraperitonially (IP) with the KX induction does. (Hint! Be quick with this!)
9. Put her back in the home cage without the cage part but with the lid. (Now is a good time to go get coffee and brag about how well your surgery is going).
10. After you come back, check to see if your rat is down for the count (asleep) by taking it out of the home cage and putting it onto a brand-new diaper pad on the surgery table and giving her toe a pinch.
11. If 30 minutes have passed since the induction dose of KX, she’s withdrawing her leg to the toe-pinch, go on and give one of the KX boosters IP. If it’s still not working after 15 minutes give another boost. (Something is wrong if this isn’t working.)
12. Now you can get on to the real bits of the surgery.

The real bits of the surgery

1. Now using the Wahl **clippers** in the drawer shave a football-shaped pattern onto the top of the skull. This will point up right between the eyes and to the bony bump on the back of the head. I like to do this over the trashcan and shave the hairs directly into it. AVOID getting hair in the eye. This is a big no-no.
2. Take your shaved rat and place it gently yet firmly into the stereotaxic stage, this is a good time to give some **saline**, **alloxate**, and check for a **toe pinch**.
3. If your rat is like my rat, then you’ll want to place the **ear bars** at one or two deviations from the 10 on the ear bar meeting the 5 on the stereotax marker. Then place the animal into that one and fix the second one to match. (Make sure this is stable, level, and something no one would be suspicious about.)
4. Secure the two front teeth on the **head holder** and gently pull away and secure the screw.
5. **Clean the incision site** with iodine and alcohol. THEN put eye gel onto the rat.
6. Inject **lidocaine** either into two sites on the scalp or just one and wait for ~5 min. (Good time for a sip of coffee!)
7. Using a scalpel, create a clean **incision** (please not multiple incisions), rostral to caudal, that is large enough to expose the area of operation.
8. Use the head wound **retractors** to pull the skin to the sides and expose the skull. Make sure you’re actually getting under everything and not just the top layer. You should be able to see the sutures of the skull.
9. Now get your lights ready. Make sure the **eye gel** is on, so you don’t roast the eyeballs. (Ring light available. To use as well as gooseneck lights.)
10. Use the head bar to make the **skull level**. Align the front spike with bregma, and the middle with lambda as shown below:

A long metal pole with a white clip

Description automatically generated 

1. Using the spatula, make **IOC pocket** by finding the ridge on the lateral part of the skull and gently pushing the tissue off on the other side. Remove the ligament connecting the muscle off the ridge and below the bone to create a small pocket about ½ the length of the spatula.
2. Attach one of the needle tips (yellow) to bar mount to discern position of bregma. Mark it in the surgery sheet and calculate the **GC and PC coordinates**. Lower the needle tip over the GC coordinate and use the cauterizing tool to mark the location on the skull. Repeat with PC. The distance between these two areas should be 2.8443mm using the caliper.
3. Use the small drill bit LA¼ to create 4-5 shallow holes for **skull screws** as depicted below. About half the size of the small drill bit tip. Screw in each screw using a screwdriver and forceps, just until stable in the skull.
4. Now **RECHECK** your coordinates. Grab the big burr LA2 and get to making small donuts around the coordinate marker. Notice the GC AND PC is on a slope of the skull demarcated by the ridge on the skull that carries the jaw muscles.
5. You’ll need to **move** those muscles away like you did with the IOC pocket, or you can nudge it and cauterize away a little bit of the tissue.
6. Go slowly until you get the hang of it. Faster **drill** speeds = less harsh bite. So really slam the pedal down and lightly tap away with the drill bit.
7. Use a von Graefe knife to slice up the **dura** and the curettes to pick out any bone.
8. Now carefully **repeat this** with PC. I again recommend using a fine digital caliper to measure the distance from your craniotomy to the marker for the other side as well as the distance between your electrodes.
9. Make sure to **soak** up any liquid on the brain or around the craniotomy at this point.
10. Now prepare your **electrodes** for going into the brain.

Electrode Implants

1. Using either a brush or 0.5mL syringe put the tiniest drop of **Vybrant Dil** onto the tips of the electrode bundles. This ensures you can find out what the heck you recorded later on.
2. Using the alligator clip adjustable holder or the 3D-Printed implant tool **attach** your drive to the stereotax.
3. **Center** your bundles over the implant locations.
4. **Drop down** to the height of the brain such that the tips of the electrodes are touching the brain.
5. Record the D/V measurement and now begin **lowering** your electrode to -4.4mm from the surface of the brain over the period of 30 minutes. Now you will need to turn the screw for PC. Each full turn of the half-moon screw (M1.2 X 16MM) to the left will cause the bundle to lower 0.25mm per turn. So you will need to turn the PC screw 10 complete turns.
6. Using **Kwik-Cast** mixed in a weighboat or with the applicator tip apply the green goo to the craniotomy. The skull around this area NEEDS to be dry.
7. Now using fine and medium curved forceps tie the **ground** wire around the ground screws.
8. Using **dental cement** build up a central pillar to the drive body and let it cure for a few minutes (coffee time).
9. Gently add another layer of dental cement around the drive and wait a little longer. Now unscrew or unclip the drive and **lift** of the stereotaxic holder.

Diagram of a skull with different types of bones

Description automatically generated with medium confidenceNasal Cannula Implant

1. Ready an extra tall screw that you will insert right behind the nasal suture line to the right.

Nasal Cannula Site

Screw Site

1. Begin burring directly down with the LA2 1-2mm above and 1mm to the right of the center of the nasal suture line. Try to go straight down.
2. Once close to the top of the nasal cavity use the 1mm tip of a 30-gauge needle to gently cut open the tissue. When done right this should not bleed too much. Provides direct access to the nasal olfactory epithelium.
3. Using a layer of kwik-cast on the bottom part of the white thread of the nasal cannula. Insert the cannula into the hole above the nasal cavity and let dry in place.
4. Now using dental cement join the bottom of the thread to the supporting screw behind it. Carefully avoid dental cementing the cap of the nasal cannula.

IOC Implants

1. Remove **IOC supplies** from cetylcide into a saline jar.
2. Use bead sterilized forceps to insert a cut section of **silicone tubing** from the acetone container and affix it onto the IOC connector mount. Use a drop of vetbond on the window of the connector to secure the tubing in place.
3. Now put a **yellow needle**, tip to the washer, into the IOC and place it on a clean kimwipe.
4. Use the mouth **retractors** and small wound retractors to make the mouth open up wide.
5. Using the needle as a guide, **insert** it with the bevel facing towards the side of the second molar up into the flesh aiming towards your IOC pocket above.
6. Connect the silicone tubing and connector onto the IOC and begin the process of **dental cementing** the IOC in place and affixed to the drive body.
7. Once cured **check** around for any places that need filling in.
8. Now give all remaining saline, **penicillin**, and love.

Post-Surgery

1. Prepare a new cage with soft bedding, wet food, water, and an enrichment packet as a pillow. Make sure your surgery cage card is filled out and return the animal to the vivarium.
2. Clean up after yourself and autoclave the trash and surgery tray.
3. Weigh your animal everyday post-surgery for a week and regularly clean cage, flush IOC and keep an eye on health.
4. For 2 days after surgery give meloxicam. The day after surgery give penicillin.
5. If your animals weight drops below 85% pre-surgery weight you cannot water deprive them and must nurse them back to above this threshold.

2. Experimental Protocols

2.1. BAT Taste-Induced Odor Preference

**Table of Contents:**

* Pages 1-4 Relevant Protocol from 22007
* Page 5 Calendar for Protocol (Skip to Here if Familiar)
* Pages 6-10 Day to Day Protocol

22007 IACUC Protocol

**Surgical procedures are as follows:**

• Adult rats will be anesthetized by intraperitoneal injections of a ketamine/xylazine mix (25 mg/mL ketamine, 1.33 mg/ml Xylazine in a 7.1/.4/22.5 Ket/Xyl/Saline mixture, [dosage titrated by weight to 100 and 5 mg/kg respectively](https://docs.google.com/spreadsheets/d/1VCzmohddc2-16-rrBvW4RZAsH7gLgklSeU_WLBJ-hE4/edit?usp=sharing)).

Isoflurane gas will be administered through a nose cone with oxygen so that the animal remains deeply sedated (2.5-3% for maintenance following induction). Depth of anesthesia will be monitored using frequent (4/hr) toe pinch—if any response is observed, isoflurane concentration will be increased. Temperature will be monitored (via rectal thermometer); any substantial changes will be compensated for via update of anesthesia or change of temperature of a heating pad placed under the animal through the duration of the surgery. Temperature will be kept at approximately 37C.

• During the surgery, sterile normal saline will be administered subcutaneously (2.75 ml/kg for each hour of surgery) as follows: 1) estimation of surgery length, on the basis of individual surgeon’s experience, 2) calculation of overall fluid dose; 3) delivery of 1/3 total dose at the start of surgery; 4) delivery of 2nd third of dose at the approximate halfway point; and 5) delivery of last third of dose, plus or minus correction, at surgery’s end.

• Alloxate (MELOXICAM) (0.05mL) will be administered subcutaneously to the anesthetized rat prior to incision. The animal will be placed on a standard stereotaxic frame.

• Its scalp will then be shaved and aseptically prepared (3 sequences of a betadine scrub followed by an isopropyl alcohol scrub) for surgery.

• Line blocks at the surgical incision sites will be done with approximately 0.2 mL lidocaine (0.5%) 5 minutes before making the incision. The scalp will then be incised.

• Up to 8 holes (1 mm diameter) will be bored in the skull for ground screws

• Screws will be placed in the skull, a fiber optic probe for delivery of light for optogenetics experiments, which will then be lowered into the brain, guided by stereotaxic measurements. Once in position, the assemblies will be cemented to the skull with dental acrylic.

• The entire surgical procedure takes 4-6 hours. [A log is kept of each surgery- rat #, anesthesia used (type and amount), length of procedure, etc. A separate log devoted to tracking of drug use (type and amount) is also kept with the drugs themselves (i.e., in a locked drawer within a 24-hour locked room).](https://drive.google.com/file/d/1elps5iF3SjYiAKCNg0H3o7N2rdJuNW9W/view?usp=drive_link)

**Virus surgery (optogenetics) for Rats**

Replication-incompetent virus (lentivirus or AAV) will be injected into gustatory cortex, by CITI-trained, Brandeis biosafety- trained lab personnel wearing lab coats, sterile gloves, and masks.

Surgeries will proceed identically to those described above, with the following exceptions. Two small, bilateral craniotomies will be made in the skull over the target region using a dental drill (location identified using cranial markers and stereotaxic measurements), and a glass micropipette containing 500 nL of virus will be then lowered into the brain (depth again guided by stereotaxic measurements). Ejection of virus will be made over the course of 2 min using a microinjector, which will then remain in place for 2 additional minutes (to ensure diffusion away from the pipette tip). Once the pipette is removed, sterile silicone will be used to fill the craniotomies. 5ul of virus over 3 injection sites in GC: D/V: -4.8, 4.6 and 4.4mm

Waste material from the procedure will be immediately placed in a biohazard bag and disposed of. Only persons trained to handle viral constructs or injected animals will be allowed to come in contact with substances or animals. If a cage change is performed in the 3 days during which an animal is considered a BSL-1, then the cage will be autoclaved or disinfected by laboratory personnel when they are empty, and contaminated bedding will be treated as biohazardous waste. Decontaminated cages will be removed immediately from the autoclave when the cycle is finished by trained laboratory personnel.

Trained  personnel will wear appropriate PPE when handling cages or animal bedding and hands will be washed after these procedures. Glass capillaries used for the injection procedure will be placed in a biohazard sharps container immediately after inoculation. Sharps containers will then be autoclaved once two-thirds filled. Gloves and other supplies used during injection will be disposed of into an autoclavable bag within a leak-proof, hard-sided autoclavable container and autoclaved. Laboratory personnel will be responsible for autoclaving all waste, and waste will be removed from the autoclave by personnel as soon as the autoclave cycle is finished.

**Post-operative care/recovery**

Immediately following surgery, adult animals (both rats and mice) will be carefully monitored for signs of postoperative pain (lethargy, ruffled fur, hypersensitivity to touch or noise, hunched back, failure to eat). The Alloxate dose will be repeated once a day for 48 hours. Following surgery, animals will be placed in a recovery cage heated from underneath by a heating pad, with access to water.

The animal will be returned to its home cage only when the anesthesia has worn off (i.e., the animal is awake and has begun moving around the cage). The adult rat will be housed singly: this is an absolute necessity for the chemosensory studies related below, both because (as described below) social interactions can cause confounds in any experiment designed to test taste preferences (as ALL of our studies do), and because group housing would make it nearly impossible to confidently relate our results to those of other labs (all of which single-house their adult rats). Veterinarian- approved environmental enrichment will be placed in home cages.

The animal will be monitored and weighed each of the following 6 days.

**Water Restriction**

Rats will be placed on a water restriction regime while undergoing behavior procedures (both during training and testing phases), to ensure they are motivated to drink during the experiment. In addition to the fluid they receive during daily experimental sessions (between 5 and 8 ml), rats will also receive access to an additional 15 ml of water per day in their home cage.

Note, this restriction will never amount to an entire day without water. This restriction is absolutely standard in behavioral neuroscience studies, daily evaluation and recording of weight will allow monitoring of health; in the unlikely event of weight loss that is larger than 15% normal body weight (as determined by Charles River, the breeder), that particular rat will be removed from the protocol.

**Preference Test**

This paradigm allows us to determine our animals’ preferences to different tastes and concentration ranges, which helps us in accessing the palatability relationship between tastants.

Length of training/ testing: Approximately 7 days.

Animals will be adapted to a variant of the “Davis Rig,” which allows an examination of taste preferences for a small set of odors in a single session. The rig is nominally a 1-lick spout chamber, but the spout is behind a sliding panel in the chamber. A computer- controlled conveyor belt allows any one of 12 actual lick spouts to be positioned behind the panel, which then opens for a period of 10 sec at a time (one “trial”). In adaptation sessions, the animal will learn to wait for the panel to slide out of the way, and then to drink from the proffered spout. Once the animal has learned to approach and lick water, sessions will ensue in which each trial is a randomly selected 1 of 2 odors is offered. An infrared detector, very similar to the beams that signal approach to the doors of many stores, will allow us to know exactly when the animal’s tongue extends to reach the lick spout. At the end of a 60- 90 min session, the animal will have consumed between 5 and 10 ml of fluid, and the resultant data- number of licks for each fluid- will provide us with more information as to the animal’s particular odor preferences.

**Taste-potentiated odor association (TPOA)**

This test investigates how taste and odors interact with one another, which is important when looking at the mechanisms behind taste preferences.

Length of training/ testing: Approximately 6 days.

1) in training sessions, tastes will be presented accompanied by the smell of either Carvone or Cis-3-hexenal diluted to 0.01% in water, a specialized lick spout will be used that emerges from a variant of the “Davis Rig” in a pseudorandom order and 2) a second testing session, in which water is available in the odorized lick spouts, will evaluate any learned aversions for the odor itself.

In a subset of these animals for 3 days prior to the BAT preference test the animal will receive 0.01% of Ethyl Butyrate and Methyl Valerate in their home cage water (50mL of solution per 36 hours).

Handling and care

Per protocol animals must be in vivarium for 1 week prior to any experimental procedures/surgeries.

These can be done days in a row, or with a couple days in between.

Animals should be handled for ~10 min each unless otherwise stated 3-4 times a week.

At least once a week (during cage changes if in early stages of handling) rats should be weighed and logged on the cage card.

If you are not logging your handling sessions by weighing them, you should be writing them in your notebook (good to do both)

After surgery or when on water dep animals must be weighed daily

* For the the first couple days once they arrive don’t handle them
* Handling day 1: first day place them in room they will be used in for 30 min with cage top off, but metal grate on to acclimate to sounds and smells.
* Handling day 2: After adjusting to the room with the lid off you can take off the cage and wait in the room with them. Go on phone, clean up around, do work on laptop etc.
* Handling day 3: Next day can start putting your hand in the cage, near the edge, just letting them get used to you. I like to stroke my thumb along my fingers to make a sound. I always make that sound when I enter the cage and before I pick them up from then on to help them know it’s me and what’s happening/cue them
* If they are receptive to your hand (sniffing, letting you touch them in the home cage) you can start petting them and interacting with them the same day. If not, just leave your hand in there as a benign object that doesn't cause startle/is aversive.
* Handling day 4: Next day repeat the slow hand introduction, interacting with the hand in the home cage. Skittish rats may just be allowed out onto the cart, more ‘sociable rats’ can be pet more and even start to be picked up onto the lap, picked up out of the cage. But they should be given plenty of freedom to return to the cage, leave your lap, or avoid your hand. Important that the rats learn that you and your hand are a positive or neutral stimulus and NOT an aversive one.
* Handling day 5: All rats should be picked up and be spending time on your lap (may be less time for certain rats, but they should still be getting used to the lap, give more skittish ones the option to escape onto the cart)
* Handling day 6+: Final stage of handling! Introduce your hand into the cage to let them know you are there, then pick up and weigh, and pour out into your lap and let them roam/practice injection pinches/practice head touching/give them pets!

I usually start picking up rats with the scoop method then transition into under the shoulders. NEVER pick up by the tail. You may HOLD the tail while you scoop when a rat is hard to corral, but do not pick them up solely by their tail.

Always clean everything with 70% EtOH between animals. Try to do this with some time to air out the ethanol smell before the next animal

Be very careful to look for startle responses like freezing (Whiskers stop moving when they’re startled)

Never interrupt grooming (it’s a coping mechanism for them)

**Introducing touch**

* Touch rats in ways they touch each other.
* Right below the shoulder blades is a good spot to touch.
* Really only use fingers because hands are too big.
* Faster rat like strokes of touching are better.

Be careful of sudden transitions - move slowly when handling them.

You can manipulate them by using your hand to get them comfortable with your hands and maneuvering them. You can make them turn right by using your hand etc use it as a platform they have to touch to get down from or up to some place.

There is a certain amount of pressure you can figure out to put on them to make them immobile without startling them so experiment with how much pressure that is.

Start touching them in ways you might use to pick them up.

Mess with the parts of the body that you want to touch and pick up from

Get them used to the sounds of gloves, any clicking, hand motions that loom, and changes of light in front of them.

Use short pick-ups with the immediate option of them being able to escape/get down.

Include chew block, enviro-pak, and cardboard tube in the cage

Prep

Order animal 1 week before (**7-9 weeks old**)

Handle

Need 2 fibers per animal made, plus extra

Calibrate fibers to laser

Book BAT rig

Have virus needles ready

Put screws in cetylcide

Virus and implant surgery (day 0)

5ul of virus over 3 injection sites

D/V: -4.8, 4.6 and 4.4mm

See [virus\_injection\_surgery\_KM2023.docx](https://docs.google.com/document/d/1leBkRjZqMWSSJCdhcRFKi8UpUYFw1hak/edit?usp=sharing&ouid=101010380107635377469&rtpof=true&sd=true)

Implant fibers in same surgery

1 week daily weighing

2 days post-operative care (melox, bacitracin)

Pre-Exposure Day 12, BAT habituation Day 15, Preference test Day 20, Conditioning Day + laser 21-26, test day 27,28

See: [Isaac Stuff](https://docs.google.com/presentation/d/1ejXYtA3JkyTGgH7K3ABn0VasRurC-oOAu1GH9TW5mQ0/edit?usp=sharing)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| -14  Order rats | -13 | -12 | -11 | -10 | -9 | -8 |
| -7  Rat handling 1 | -6  Rat handling 2 | -5  Rat handling 3 | -4 | -3  Rat handling 4 | -2 | -1 |
| 0  Virus  + implant | 1  post-ops | 2  post-ops | 3 | 4 | 5 | 6 |
| 7 | 8 | 9 | 10 | 11 | 12  pre-exposure | 13  pre-exposure |
| 14  pre-exposure | 15  BAT hab 1  pre-exposure | 16  BAT hab 2  pre-exposure  **Start water dep** | 17  BAT hab 3 | 18  BAT hab 4 | 19  BAT hab 5 | 20  Pre-preference test  60 trials |
| 21  Conditioning  48 trials  LASER | 22  Conditioning  LASER | 23  Conditioning  LASER | 22  Conditioning  LASER | 25  Conditioning  LASER | 26  Conditioning  LASER | 27  Condition Preference Test  60 trials |
| 28  Condition Preference Test  60 trials |  |  |  |  |  |  |

Odor paired with saccharin counterbalanced (mint v grass)

Unpaired (water+odor) and paired order (sacc+odor) start counterbalanced (AB v BA)

1 Animal - Enriched - Paired Odor = Carvone - Order = AB

1 Animal - Enriched - Paired Odor = Cis-3-hexen-1-ol - Order = BA

1 Animal - Enriched - Paired Odor = Carvone - Order = BA

1 Animal - Enriched - Paired Odor = Cis-3-hexen-1-ol - Order = AB

1 Animal - Unenriched - Paired Odor = Carvone - Order = AB

1 Animal - Unenriched - Paired Odor = Cis-3-hexen-1-ol - Order = BA

1 Animal - Unenriched - Paired Odor = Carvone - Order = BA

1 Animal - Unenriched - Paired Odor = Cis-3-hexen-1-ol - Order = AB

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Animal** | **Enriched (E) or Unenriched (U)** | **Enriched Odor** | **Paired Odor** | **AB or BA** |
| GW05 | E | Methyl valerate | Carvone | AB |
| GW06 | U | - | Carvone | AB |
|  |  |  |  |  |

Pre Exposure Begins - Day 12

Make fresh daily.

Use graduated cylinder to measure 100mL of DI/miliQ water. Add to amber 100mL bottle. In fume hood, select odorant. Use p10 to carefully pipette 10ul of odorant into 100mL bottle (pipette up and down to mix). Top 100mL bottle and shake. Label with animal and odor. Remove ad lib water bottle. Place lick spout with ball into amber bottle and into animal cage at same time daily (within the same hour timepoint). Measure how much water is consumed by animal.

Methyl valerate

Ethyl butyrate

Switch which odor is pre-exposed after 2-3 days (e.g. if started with MV, switch to EB)

Add infused water (0.01% concentration of each odor) into its home cage.

50ml of cis-3-hex in 100mL in conical tube with spout, remove ad lib water.

Unenriched also get conical tube and spout.

Remains until water dep starts day 16, end of BAT hab 2.

Habituation (hab) in BAT - Days 15-19

Weigh animal daily

Clean rig thoroughly with 70% EtOH, with 10min to air out in between animals

Fresh lick spouts used for each animal

MiliQ water fresh on hab1

**Hab 1**

Rig habituation,

no shutter or tastants. 30min

**Fan on**

**Hab 2**

Rig habituation,

no shutter or tastants. 30min

**Fan on**

Water restriction starts

**Hab 3**

Licking Habituation-

shutter open with 1 water bottle (stink\_hab3), 30 min

**Fan on**

9am 10-15ml water

**Hab 4**

2 bottles water - 30 trials, 15 each bottle (stink\_hab4)

**Fan on**

9am 10-15ml water

**Hab 5**

2 bottles water - 30 trials, 15 each bottle (stink\_hab5)

**Fan on**

9am 10-15ml water

Make Tastants

For Preference tests (pre and post):

Bottles:

1. 20mL MiliQ water
2. 20mL MiliQ water
3. 20mL 0.01% Carvone - **2μL Carvone and 20mL distilled water**
4. 20mL 0.01% Carvone -  **2μL Carvone and 20mL distilled water**
5. 20mL 0.01% cis-3-hexen-1-ol - **2μL cis-3-hexen-1-ol and 20mL distilled water**
6. 20mL 0.01% cis-3-hexen-1-ol - **2μL cis-3-hexen-1-ol and 20mL distilled water**

→ 48 presentations of 6 bottles (10 presentations each; hab4/5 = 2 bottles presented 15 times)

Number of presentations: 48

Licktime: 5s

IPI: 30s

Maxwaittime: 60s

Session time limit: 100min

Store in glass bottles

\*\* FOR CONDITIONING DAYS, 2 BOTTLE PROTOCOL DESCRIBED BELOW\*\*

Paired odor: in 20mL miliQ (DI water) (2ul paired odor) + 1.37g sucrose

In 50mL miliQ, 5ul paired odor + 3.423g sucrose

Unpaired odor: in 20mL miliQ (DI water) 2ul odor

In 50mL miliQ, 5ul odor

Con 1/3/5 (**paired odor**), bottles:

1. 0.2M sucrose + paired odor
2. 0.2M sucrose+ paired odor

Con 2/4/6 (**unpaired odor**), bottles:

1. 2μL unpaired odor in 20mL distilled water
2. 2μL unpaired odor in 20mL distilled water

→ 30 presentations of 2 bottles (15 presentations each)

Number of presentations: 30

Licktime: 5s

IPI: 30s

Maxwaittime: 60s

Session time limit: 100min

Pre-preference test - Day 20

6 bottles: 2 bottles water, 2 bottles Carvone, 2 bottles cis-3-hexen-1-ol - 48 trials, 10 each bottle (stink\_prepref\_test)

**Fan on**

9am 10-15ml water

Conditioning (con) - Days 21-26 (AB)

**Con 1**

2 bottles **paired odor** - 30 trials, 24 each bottle (stink\_con\_paired1)

**Fan on**

9am 10-15ml water

**Con 2**

2 bottles **unpaired odor** - 48 trials, 24 each bottle (stink\_con\_un1)

**Fan on**

9am 10-15ml water

**Con 3**

2 bottles **paired odor** - 48 trials, 24 each bottle (stink\_con\_paired2)

**Fan on**

9am 10-15ml water

**Con 4**

2 bottles **unpaired odor** -48 trials, 24 each bottle (stink\_con\_un2)

**Fan on**

9am 10-15ml water

**Con 5**

2 bottles **paired odor** - 48 trials, 24 each bottle (stink\_con\_paired3)

**Fan on**

9am 10-15ml water

**Con 6**

2 bottles **unpaired odor** - 48 trials, 24 each bottle (stink\_con\_un3)

**Fan on**

9am 10-15ml water

Post-preference tests - Day 27-28

6 bottles: 2 bottles water, 2 bottles Carvone, 2 bottles cis-3-hexen-1-ol - 48 trials, 10 each bottle (stink\_prepref\_test1)

**Fan on**

9am 10-15ml water

6 bottles: 2 bottles water, 2 bottles Carvone, 2 bottles cis-3-hexen-1-ol - 48 trials, 10 each bottle (stink\_prepref\_test2)

**Fan on**

9am 10-15ml water

Run Experiment

⚠️ See [LASER use SOP](https://docs.google.com/document/d/1wnVBU3T7piEQ9_zCmP_KmQEwbspqm-1_Xjjfc_p7vQs/edit?usp=sharing) for detailed laser use instructions

Prep

1. Make odors daily (paired or unpaired), Use miliQ (di-H2O).
2. Turn on laser, but do not turn key to “on” yet. Let it warm up for at least 15min.
3. Fill bottles appropriately labeled with tastant at least ½ up (bottle 1- MiliQ, bottle 3-Carvone, etc.).
4. Make sure air bubble is out of the spout and liquid comes out of the spout when you dot it on your hand.
5. **Weigh bottles (on side so air bubble stays out of spout) before experiment and note weight in notebook.**
6. Double check bottle numbers and clear and place in appropriate door (1 is left most slot).
7. Turn the fan on by plugging the orange pin into any port by the red line (check it is on by placing your hand below the fan).

**A white mouse with black text

Description automatically generated**Computer setup

1. Use icon with ‘data collection’ to start.
2. Select .pro file (or hab4 or test or test\_1, etc.)  from drop-down menu and press ‘show’ to check everything is correct.
3. ‘Test Hardware’ and check licks are registering by touching metal (completing circuit, seeing licks are counted in the program), check it moves to the correct door#, door opens and closes.
4. ‘Exit’
5. Enter animal ID.
   1. Animal ID MUST BE 4 characters. E.g. if animal is BT5 input animal ID as ‘BT05’’

Computer setup for LASER days

1. Open VNC viewer for the BAT pi
2. In Desktop right click on “shutter\_lick\_laser\_codes” and select “open in terminal”
3. Terminal should open, enter the code as follows:
   1. Sudo ipython
   2. From Laser\_lick\_trigger  import \*
   3. laser\_trigger()
4. It will ask for the sequence of your bottle presentations. Open the \*.pro in notepad and copy the sequence into the easygui box, i.e. stink\_con\_paired1.pro sequence is: 2,1,2,1,2,2,1,1,2,2,1,2,2,2,1,1,1,2,1,1,2,1,2,1,2,2,1,2,1,1

A screenshot of a computer

Description automatically generated

1. Enter animal ID, but do not enter unless all davis rig program is set up.

Animal in

1. Retrieve the animal, weigh the animal.
2. For test days:
   1. Place the animal in your lap and connect the optic fibers to the laser lines, noting which laser line is on which side of the head.
3. Place animal in box and secure lid
4. Tape off part of the roof of box so not a t-shape, just a line
5. For test days:
   1. Adjust laser output to match the optic fiber with the lowest output (if fiber 2 needs 750 to put out 40mW, and fiber 1 needs only 600, then set it to 750). **Turn key to ON.**
6. Press ‘Run’
7. Wait until animal licks to leave. Set a timer for 25 min (alternatively, watch pi on VNC viewer)
8. Return to check 30 presentations have occurred.
9. Remove animal by simultaneously pulling out grate and animal.
10. Save data.
    1. MMDDAn##\_TEST#
    2. MMDDAn##\_HAB#
    3. Pull data from computer with a flashdrive.

a.   Just need the .txt files (not .MS8)

1. Pull laser trials off of BAT pi.
2. Turn laser key off, then laser off.
3. Weigh bottles.
4. If any bedding is in the box remove with dust blaster, careful not to send any bedding into gears.
5. Clean every surface with 70% etoh, careful around shutter.
6. Remove spouts from doors, remove from bottle and either replace with clean spouts for the next animal or clean spouts with 70% etoh then water.
7. If running a second animal, make sure to remove air bubble again.
   1. Passive Taste/Odor Delivery with Spontaneous Trials

Setting up for day’s experiments

1. Fill your taste-tubes with corresponding tastants. Close them, seal the system, and pressurize.
2. Open Desktop
   1. Open VNC session for raspberry pi (blechpi)
   2. If not open:
      1. Enter the VNC Viewer window.
      2. VNC connection window should open with raspberry pi desktop.
   3. If login menu appears
      1. Usr: realpi
      2. Pwd: blech
3. Start the taste delivery program.
   1. cd Desktop/Dan\_code
   2. sudo ipython
      1. pwd: blech
   3. from dan\_taste\_delivery import \*
   4. From pi\_rig import\*
4. Clearout the lines.
   1. clearout([31],5)
      1. [31] is the valve #, you can replace with [33], [35], [37]
      2. 5 is the open time in seconds. Replace with whatever you need. Advise to not go over 30s at a time.
      3. Repeat until the taste lines you are using are clear of air/put out a steady stream. (sometimes an air bubble gets stuck below the valve. You can’t really clear that out)
      4. You can also simultaneously open all with [31, 33, 35, 37]
5. Calibrate each taste line you will use today.
   1. calibrate([31], 0.05, 5)
      1. [31] is the valve #, you can replace with [33], [35], [37]
      2. 0.05 is the open time in seconds. Modulate this until the output of the taste line weighs 0.15gr
      3. 5 is the number of repeats. Keep this constant always.
   2. Write down the calibration value for each taste line for later. Calibration is good food the day.

# Set up an animal to run

1. Lance rat’s IOC with clean lance from the IOC container.
2. Install headstage amplifier chip, **with chipside towards the text on the EIB.**
3. Put rat in rig, plug manifold into rat’s IOC, plug intan cable into headstage, close doors.
4. Keep red lights on, white lights off inside rig box.
5. Turn on intan power strip behind computer/monitor. Intan board LED strip should turn on.
6. In the Desktop:
7. Open intan software from the terminal:
   * 1. Usually there’s a terminal that’s kept open that’s already cd’d to the directory ~RHD2000interface\_source\_code\_v1\_5/source. If that’s open, just click on that terminal
     2. If opening from scratch--open a terminal and enter:
        1. cd RHD2000interface\_source\_code\_v1\_5/source
     3. Type in or key up until you see:
        1. ./RHD2000interface
        2. hit enter, intan software should open
8. In the intan software:
   1. Select File format
      1. Menu opens, select “one file per channel”
   2. Select Base Filename
      1. Navigate to your storage folder
      2. Enter folder labeled with animal’s ID
         1. If there isn’t one, click on “new folder”, enter the animal ID, hit the enter key
      3. Base file name should be:
         1. [ANID##]\_spont\_h2o (for water days)
         2. [ANID##]\_spont\_taste (for taste days)
         3. I.e. TG44\_spont\_h2o
   3. Click on board digital inputs.
      1. Click on the squares on the right representing the digital inputs you want to open and hit space to unlock them.
      2. Open DIN 00 and 04 for water days
      3. Open DIN 00, 01, 02, 03, 04 for taste days
   4. Set amplifier sampling rate to 30.0 kS/s
   5. Check Software/DAC high pass filter, leave value at default 250Hz
   6. Set Notch filter Setting to 60 Hz
9. In the raspberrypi terminal window:
   1. For the following, under opentimes fill in the calibrated opentime for that valve instead of “##OP” i.e. “0.009”
   2. For water days:
      1. passive(outports=[31], intaninputs=[24], opentimes=[“0.009”], itimin=20, itimax=30, trials=60, sponttrls=60)
   3. For taste days:
      1. passive(outports=[hi 31,33,35,37], intaninputs=[24,26,19,21], opentimes=[31OP,33OP,35OP,37OP], itimin=20, itimax=30, trials=30, sponttrls = 60)
   4. Hit enter.
10. Go to the Intan software and hit **Record.**
11. Leave the room, turn off the lights, take your shit.

Anatomical Notes:

Joost: “GC: 1.6 mm anterior to bregma, 4.6–5.2 mm lateral from the midline, and 4.8–5.5 mm ventral from the surface of the brain”

Joost: 2.4mm nasal cannula base

Rat skull thickness measures anywhere from 0.5-1.0mm

Respiration recordings require the nasal cannula be attached to a well-sealed tube (P1 Technologies, C315CT) which connects to a pressure sensor (Honeywell, CPXL04GF). Respiration was amplified through DP-301 single-channel differential amplifier (Warner Instruments) and filtered from 0.1 Hz to 100 Hz.

To monitor the sniff signal, we implanted a thin 7-mm-long stainless cannula (gauge 23, Small Parts capillary tubing) in the nasal cavity. The cannula was capped between experimental recordings. During experiments, the cannula was connected to a pressure sensor with polyethylene tubing (801000, A-M Systems). The pressure was measured with pressure sensor (MPX5050, Freescale Semiconductor) and homemade preamplifier circuit. The signal from the preamplifier was recorded together with electrophysiological data on one of the data acquisition channels. The timing of the pressure signal was calibrated against the hot wire anemometer (mini CTA 5439, Dantec Dynamics, Denmark) and shifted back for 32 ms for all analysis (Supplementary Note and Supplementary Fig. 1).

Sniffing behavior was monitored by measuring intranasal respiratory transients in the nose via the sniff cannula. A piece

of polyethylene tubing (0.1 mm ID · 0.15 mm OD) was connected from the sniff cannula to an airtight swivel (model

375/22PS; Instech Laboratories, Plymouth Meeting, PA),

which allowed the animal to freely move within the testing

chamber. The swivel was then connected to a pressure

transducer (model CPXL04GF; Honeywell International,

Morristown, NJ) to convert pressure transients into voltage.

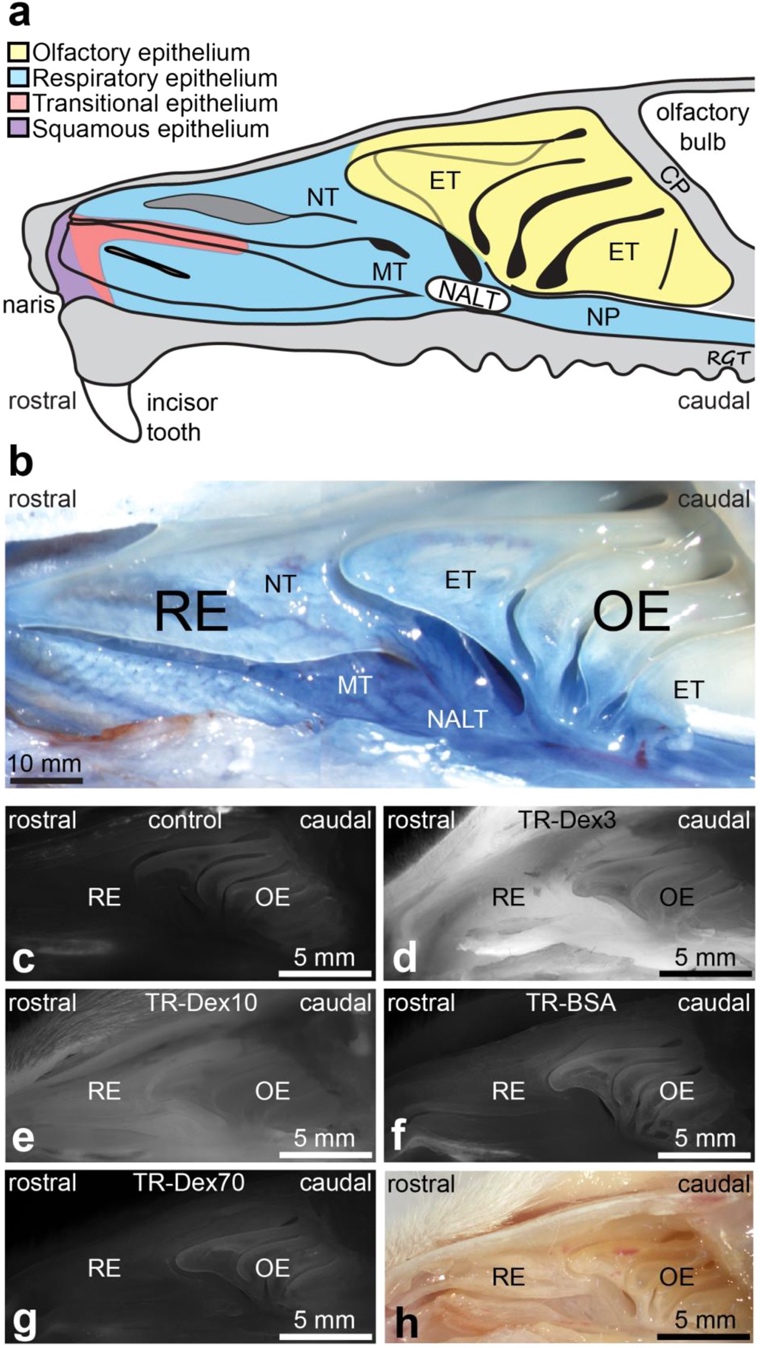
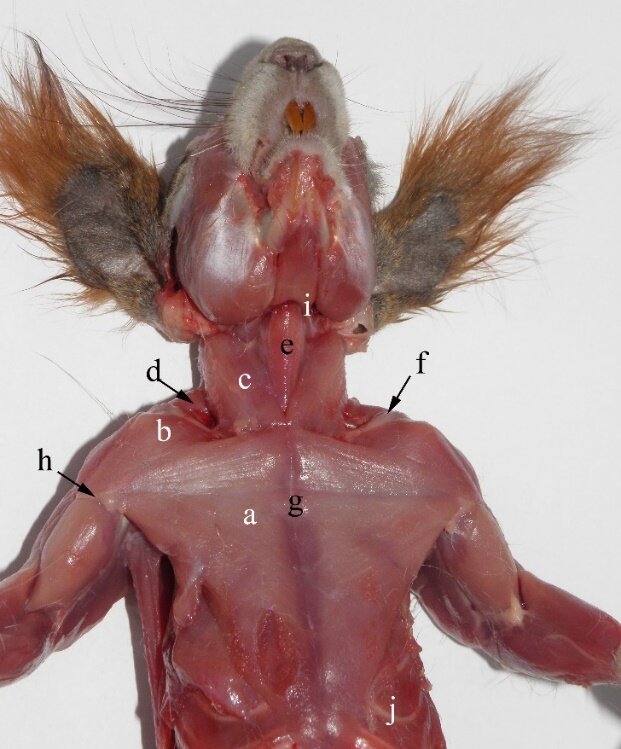
This method has been previously verified to correlate

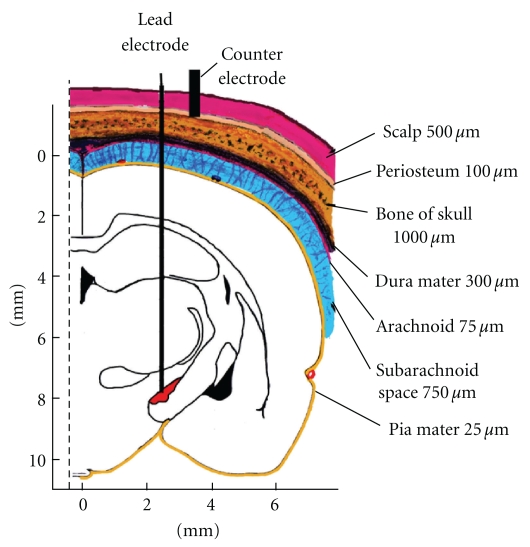
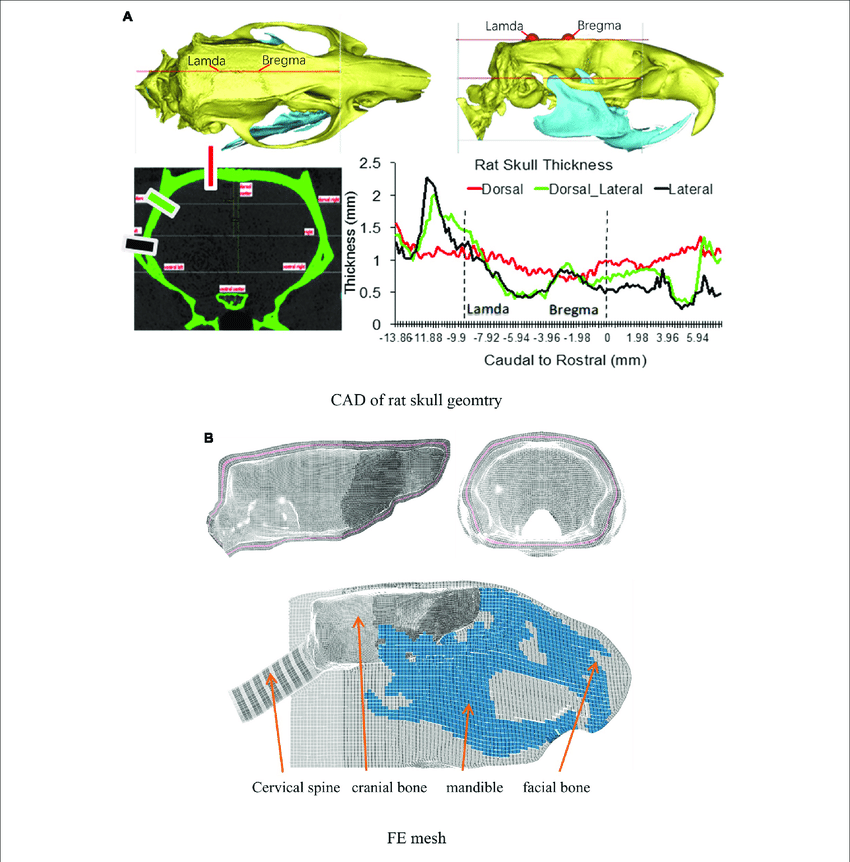
strongly with airflow (measured via intranasal thermocouple) within the nose of the rat (Verhagen et al. 2007). The

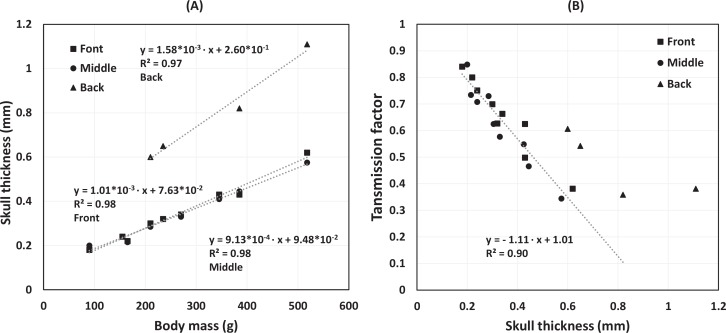
voltage was then amplified 100·, low-pass filtered at 100

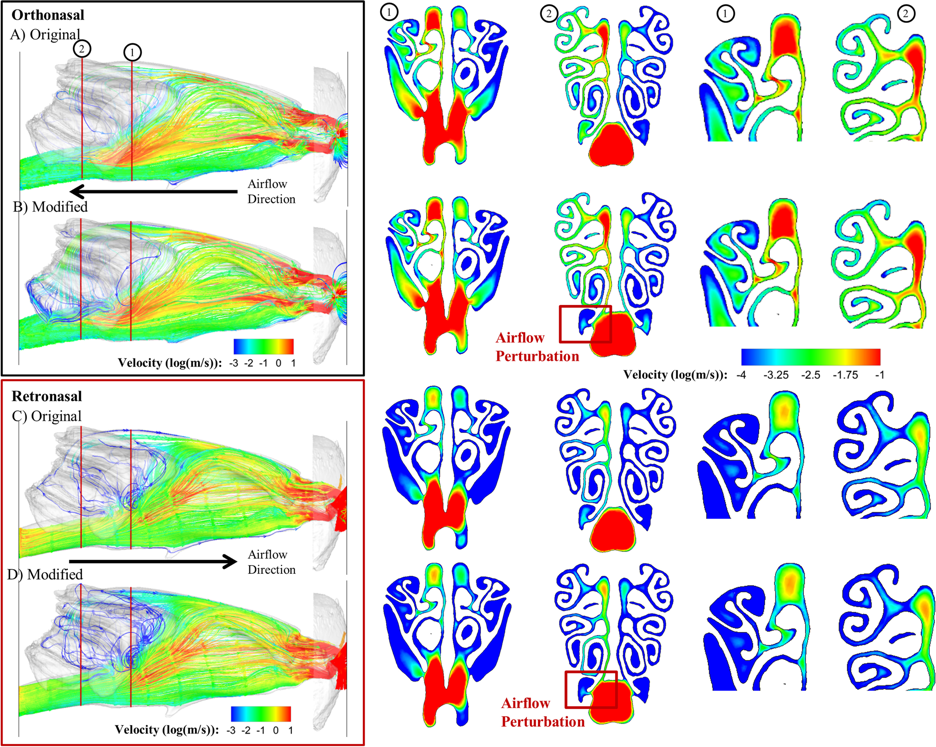
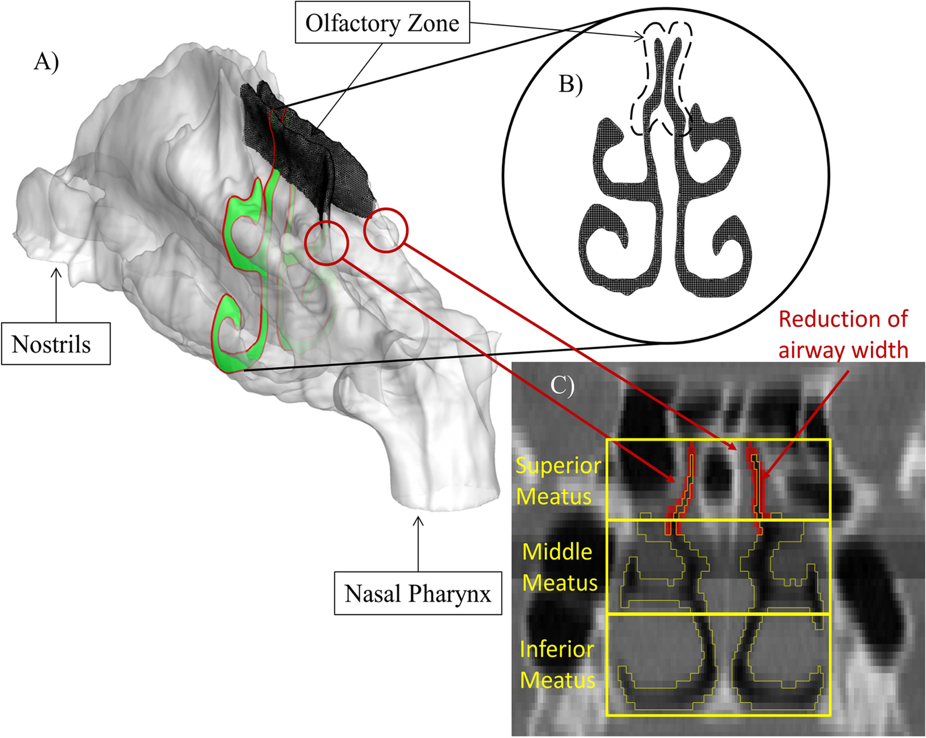
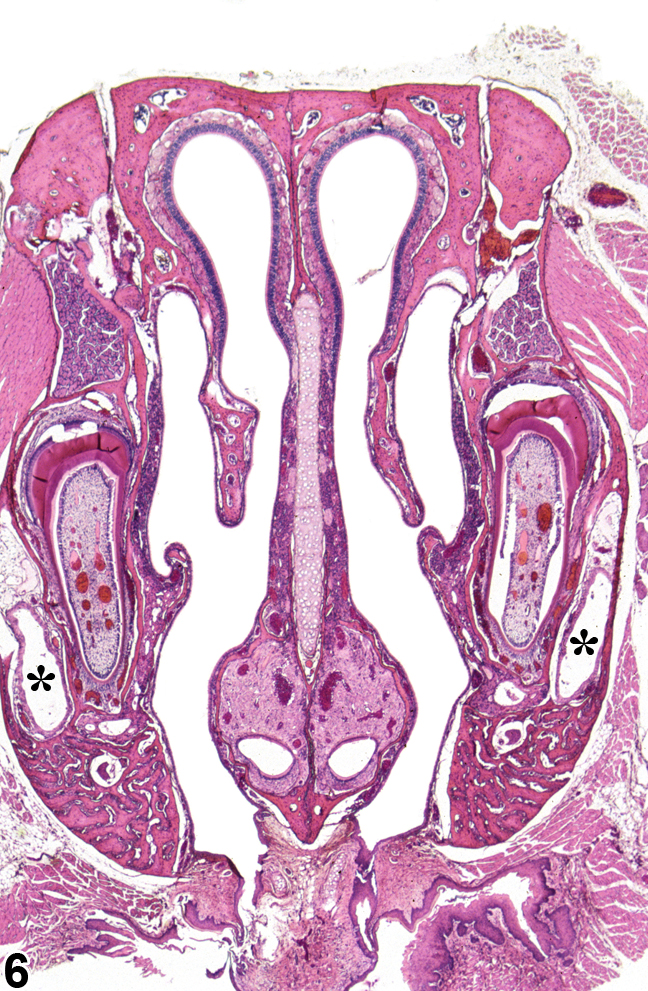
Hz, and digitized at 500 Hz using custom software written

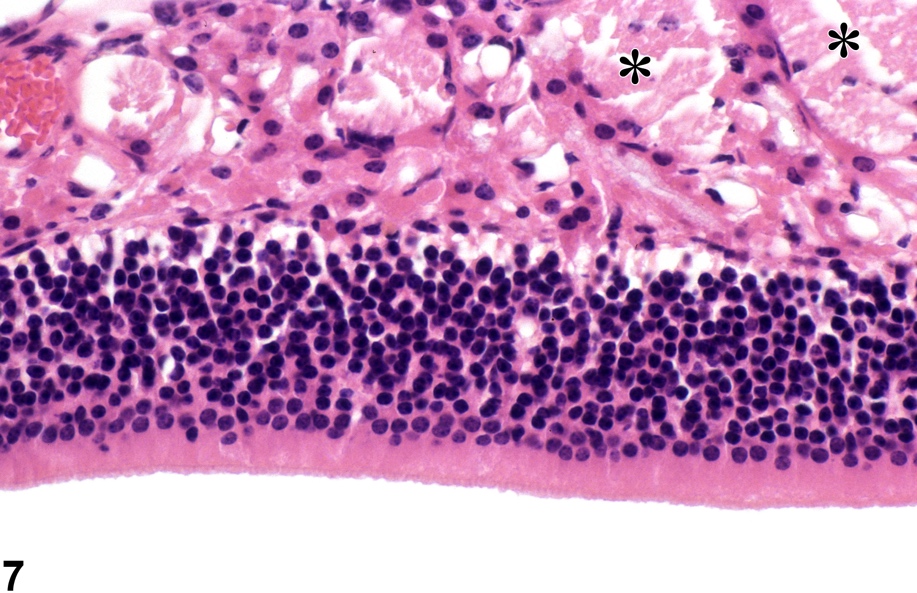
in LabVIEW (Austin, TX).











A diagram of a diagram of a variety of different types of sound waves

Description automatically generated with medium confidence