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A simple method for acute gas anesthesia in mice via tracheostomy tube

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Background

Acute anesthetized preparations in mice that support neural recording and stimulation in stereotaxic coordinates are useful for real-time functional mapping of the projections of active sensory neurons in the brain using electrophysiological methods, imaging, and optogenetics. A single recording session in studies of this type can last for extended periods of up to several hours, requiring robust control over anesthetic depth and level in mice – a model species used in many areas of neuroscience albeit one that displays low tolerance for vagaries in physiological condition. To address this challenge in our acute mouse neurophysiological studies on orofacial sensory processing, we developed a method for gas anesthesia for mice where animals freely respire an isoflurane/oxygen mixture through a tracheostomy tube. To achieve this, the distal end of this tube is loosely connected to custom concentric gas exchange tubing that delivers the isoflurane mixture and scavenges exhaled gas/excess isoflurane. Under this method, mice respire under their own power, without the aid of mechanical ventilator, and can remain successfully anesthetized for hours with fast control and adjustment of anesthetic level. What is more, this preparation does not involve the use of a nose cone, which frees access to the oral cavity and whisker pad for stimulus delivery in oral sensory and somatosensory studies. Here we describe this method of anesthesia, which our group has successfully used in several publications on gustatory and trigeminal neurophysiology in mouse models (e.g., Wilson and Lemon 2014, Lemon, Kang et al. 2016, Li and Lemon 2019).

In brief, under procedures described here a tracheostomy tube is surgically secured following initial induction of mice by a short-lasting injectable anesthetic. Mice are then placed in a stereotaxic device and transferred to gas anesthesia respired through the tracheostomy tube. Importantly, these methods are for acute preparations only, where mice are not allowed to recover from anesthesia and are sacrificed at the end of the experimental session. Our procedure could be adapted for use with rat models through, in part, increasing the dimensions of the tracheostomy tube and gas exchange tubing. Prior to using these methods, investigators must consult with and receive approval from their local Institutional Animal Care and Use Committee (IACUC).

Materials

Anesthetics and chemicals (some may require a license for purchase and research use)

- ketamine
- xylazine
- atropine
- eye ointment
- isoflurane

Apparatus and supplies

- surgical tools suitable for mice
- oxygen cylinder and regulator
- various tubing and supplies, as below
- stainless steel wire, 0.011” diameter (A-M Systems, Cat No. 792100)
- vacuum system with regulator and safe exhaust discharge
- single channel isoflurane vaporizer
- Stereotaxic Alignment System (Kopf model 930)
- pulse oximeter and heart rate monitor (e.g., MouseSTAT® Jr., Kent Scientific Corporation)
- feedback controlled heating pad for mice
- acrylic mouse induction chamber

Methods

1. Concentric gas exchange tubing

The end of concentric gas exchange tubing that couples with the mouse tracheostomy tube is depicted in Figure 1 and consists of a larger outer and smaller inner tube. The inner and outer tubes supply anesthetic gas delivery and waste scavenging, respectively, to the airspace surrounding the distal tip of the mouse tracheostomy tube. The outer concentric tube is made by cutting a 3 ml Becton Dickinson (BD) syringe body to a 3-5 cm long piece. The inner concentric tube is made by taking a disposal graduated transfer pipette (Fisherbrand 13-711-9BM) and cutting a 2-3 cm long section from the shank. The inner tube is placed into the outer tube such that its endpoint is recessed by about 2 mm relative to the outer tube edge (Figure 1). Breathable foam (i.e., foam padding with large porosities, such as padding from an electrode box, etc.) is added between the inner and outer tubes to help concentrically center the inner tube. Importantly, any added foam must not block airflow through the outer tube.

Flexible tubing is used to connect the outer concentric tube to the base port a Y-type polypropylene tube fitting (e.g., VWR, 89076-794). A length of smaller diameter flexible tubing, smaller than the inner diameter of the Y fitting, is coupled to the inner concentric tube and routed through one of the open ports of the Y fitting. Silicone is used to form an airtight seal between the smaller diameter flexible tubing and fitting port. The smaller diameter tubing is coupled to the output channel of the vaporizer supplying a mixture of isoflurane and oxygen, which in turn is delivered to the inner concentric tube. Light vacuum applied to the remaining open port of the Y fitting induces negative pressure in the outer concentric tube to scavenge excess isoflurane emanating from the inner tube and also exhalation gas.

2. Adult mouse tracheostomy tube

The tracheostomy tube consists of a short length of bent polyethylene (PE) tubing with a small, lightweight circular semi-seal (see below) fitted over the distal end of the tube (Figure 1). The semi-seal must be made of very light material and helps “trap” the tracheostomy tube to the airspace at the tip of

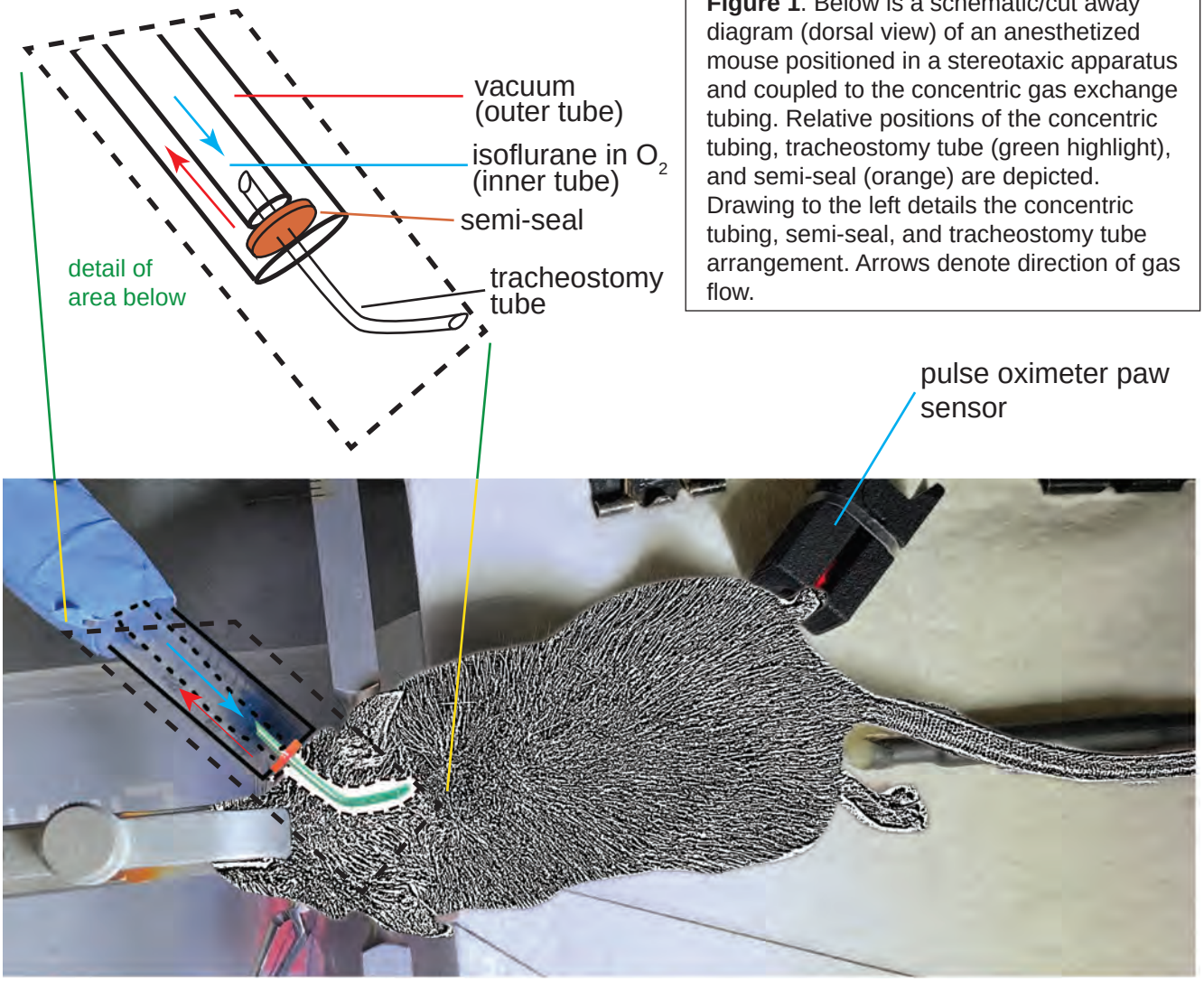

Figure 1. Below is a schematic/cut away diagram (dorsal view) of an anesthetized mouse positioned in a stereotaxic apparatus and coupled to the concentric gas exchange tubing. Relative positions of the concentric tubing, tracheostomy tube (green highlight), and semi-seal (orange) are depicted. Drawing to the left details the concentric tubing, semi-seal, and tracheostomy tube arrangement. Arrows denote direction of gas flow.

the concentric anesthetic gas exchange tubing without forming an airtight seal. Under the present methods involving free respiration by mice, the tracheostomy tube cannot be attached to the vaporizer output in an airtight manner, as constant positive pressure would not allow for exhalation. If needed, an airtight seal may require use of a ventilator and a more complex vacuum/scavenging system not discussed here.

To make the tracheostomy tube, start by cutting an approximately 6 cm length of PE-60 tubing; we have found PE-90 works well for mice with body weights greater than 35g. Insert a length of 0.011" diameter stainless steel support wire through the PE tubing such that it protrudes from both ends of the cut PE tube. Bend the PE tubing/wire at a point near the middle of the tubing such that an obtuse angle is formed (approximately 120°). The support wire helps the tubing conform to this angle, which is permanently shaped into the tubing by placing the bent PE tubing/wire into ~80°C water for about 2 hours. After this period, the steel wire is then pulled from the PE tubing. Use sharp scissors to bevel-cut both ends of the now-bent tracheostomy tubing to achieve a "short" (~6 mm) and "long" (~15 mm) length on either side of the bend (Figure 1).

Note that a heat gun can also be used to facilitate the tubing bend, albeit with an increased chance of melting the tubing to an unusable state. Nevertheless, if a heat gun is

used, it helps to fill the PE tubing with water (via syringe) prior to heating to mitigate melting/destroying the tubing.

The lightweight semi-seal for the tracheostomy tube can be made by first filling a small length of 3/8" diameter Tygon tubing with liquid silicone. Ensure the 3/8" tube is tightly packed with silicone fluid, void of air, and then allow the fluid to dry for about one week. Once dry, use a sharp razor blade to cut a cylindrical slice (<1 mm thick) from the tube embedded with dried silicone. Push the smaller silicone cylinder out the 3/8" tube, and then puncture the circular silicone cross section with the long end of the tracheostomy tube, as in Figure 1. Ensure that the tracheostomy tube fits through the center of the circle and that the silicone removed by the puncture does not clog the tube. Save the remaining 3/8" tube of dried silicone to cut additional semi-seals, as needed.

3. Surgery

Our approach is to first induct mice with a short-acting injectable anesthetic to enable surgical preparation and insertion of the tracheostomy tube. Mice are then transferred to the stereotaxic device and the distal end of the tracheostomy tube coupled to the anesthetic gas exchange tubing. Anesthesia is then maintained by mice breathing, under their own power, a mixture of isoflurane and oxygen.

3a. Anesthesia – initial induction

Record the weight of the mouse before surgery. Place the mouse in an acrylic mouse induction chamber and sedate the animal through the introduction of 2% isoflurane in oxygen. Sedation eases handling and animal stress during the injection and is evidenced by a lack of movement, slowed breathing rate, and lack of reflex response. Next, remove the mouse from the chamber and administer a mixture of ketamine (10 mg/kg) and xylazine (1 mg/kg) at a dose volume of 10 ml/kg (i.p.) using a 1.0 ml syringe with 25 gauge x 5/8'' needle. This injection results in an initial anesthesia for surgery that is rapidly achieved but will safely last only about 30 mins. With practice, this time window is sufficient to accommodate surgical placement of the tracheostomy tube and for transferring the mouse to the stereotaxic device and gas anesthesia. Atropine (0.04 mg/kg, i.p.) can also be administered to reduce bronchial secretions.

3b. Tracheostomy

Once the mouse is anesthetized to a surgical level, use a clipper (Oster Golden A5) to trim fur on the ventral neck area. Brush loose fur away from the surgical area. Lay the mouse in the supine position on a heating pad (36-37°C). Sterilize the incision area with betadine (povidone iodine) and then make a small incision along the midline with a scalpel blade (no. 15), beginning about 1 cm below the lower border of the mandible and extending caudally 1 to 1.5 cm. Use a pair of straight fine forceps (Fine Science Tools [FST], 11295-10) to retract the skin, superficial fascia, and muscles (both the anterior and posterior belly of digastric, sternomastoid and sternohyoid) to expose the rostro-ventral part of the trachea. Cut a small hole 2-3 mm below the larynx with a spring scissor (FST, 15020-15) taking care to not completely sever the trachea. Hold the proximal part (i.e., the "short" end) of the custom tracheostomy tube with small curved forceps (FST, 11052-10) and carefully insert the tube into the trachea via the cut hole. The tube should be inserted into the trachea up to a couple of millimeters before its angle, with the "long" end of the tube pointing away from the head in a lateral direction (Figure 2). At the same time, care should be taken not to block the bifurcation point between trachea and bronchus by drawing back the tip of the tubing 1-1.5mm away from the bifurcation point. Secure the tube by carefully passing a curved suture (4-0) needle around the dorsal surface of the trachea, lightly tightening the suture around the trachea/trache-

Figure 2. Schematic (ventral view) of an anesthetized mouse showing the insertion point and positioning of the tracheostomy tube (green highlight). Note that the "long" end of the tube (with semi-seal [orange]) is angled away from the head in a lateral direction.

ostomy tube, and tying a knot in the suture about 1 mm caudal of the tracheal hole. Use a cotton pack to clean and dry the area. Close the surgical area with silk suture.

3c. Stereotaxic positioning

Affix the head by placing the mouse into the stereotaxic device ensuring ear bars are directly behind the zygomatic arch such that the head can swivel up and down, but not side to side. Place the upper incisor teeth into the standard bite bar (for mice) and tighten the bar clamp until the snout is firmly fixed. Insert the distal end of the implanted endotracheal tubing into the inner tube of the concentric gas exchange tubing. The mouse and/or concentric tubing should be positioned such that tracheostomy tube tip is inserted about 1 mm into the inner tube, with the semi-seal near, but not touching, the inner tube tip. We have found that it helps to attach the concentric gas exchange tubing to a 3-dimensional micromanipulator to facilitate fine adjustments and stability in tubing position. Also ensure the semi-seal is smaller than the inner diameter of the outer concentric tube and does not create an airtight vacuum connection.

On the vaporizer, set the oxygen flow to approx. 0.5 liter/min and the concentration of isoflurane to 1.2-1.8% or higher, as needed. Apply minimal vacuum to the outer concentric tube to facilitate gas scavenging; vacuum pressure may vary depending on setup, etc. Put artificial ophthalmic ointment on both eyes. Monitor hind paw pulse oxygen level and heart rate with the pulse oximeter. Maintain rectal temperature at 36-37°C. Allow the mouse to adapt and stabilize to the gas system for several minutes before proceeding with experimentation while monitoring heart rate and blood oxygenation. A schematic/cut away diagram showing final configuration of the mouse positioned in the stereotaxic device and coupled to the gas anesthesia tubing is given in Figure 1.

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