

A Simple, Inexpensive, and Effective Light-Carrying Laryngoscopic Blade for Orotracheal Intubation of Rats

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The research paradigm of using large laboratory animals, in which oroendotracheal intubations are relatively easy, is shifting toward the use of small animals, such as rodents, in which oropharyngeal access is limited, the arytenoid cartilage cycles are faster, and the glottis is much smaller. The considerable growth recently seen in preclinical imaging studies is accompanied by an increased number of rats and mice requiring *in vivo* intubation for airway management. Tracheal access is important for ventilation, administration of inhaled anesthetics, instillation of drugs or imaging agents, and maintenance of airway patency to reduce mortality during and after operations. I fashioned a light-carrying laryngoscopic blade (laryngoscope) from readily available acrylic–polymethyl methacrylate tubing and used it to perform rapid, effective tracheal intubation in rats. The laryngoscope design and intubation techniques are presented.

Abbreviations: IP, intraperitoneally

The administration of anesthetics, drugs, or imaging agents via the trachea is important to many biomedical studies. In addition, controlled ventilation can be the key to animal health and survival or required for an experimental protocol. Unlike nose cones or masks, proper tracheal intubation ensures a completely closed ventilation system; avoids loss of gases, aerosols, or volatile anesthetics to the surroundings; and allows precise control of ventilation. Tracheal intubation in small animals also can be used to study many other clinically relevant problems, including adverse responses caused by the mechanical stimulation of intubation itself,⁷ the proper catheter depth for endotracheal suctioning,³ infection of the airway caused by intubation,¹⁰ the effectiveness of various methods used to introduce oxygen to the lungs,² differences in injury due to mouth breathing versus nose breathing,¹⁸ and reducing the muscular rigidity that interferes with intubation.^{16,19} Intubation after tracheostomy is a reliable but invasive method of airway management that is not appropriate or recommended for experiments that include survival and follow-up.

To support experimental protocols that require airway management in rodent models, simple and efficient methods for oroendotracheal intubation are needed. A number of different methods for oro-tracheal intubation of rats have been suggested.^{1,5,6,9,11,13,17,20} Many of these techniques are cumbersome or require specialized instruments or both. I have fabricated a laryngoscope from commonly available laboratory supplies and developed an effective, reliable technique of oro-tracheal intubation in light of our experience with other published methodologies. The laryngoscope presented here was designed out of necessity during development of a surgical protocol that involved access to the main, left pulmonary artery.^{4,21} In this work, a closed ventilation system was required to reinflate and minimize atelectasis in the left lung after it was collapsed

during dissection and ligation of the left pulmonary artery. In addition, this intubation technique assisted in reestablishing negative thoracic pressure when the chest was closed and reduced postoperative mortality. My laboratory has since used the technique successfully in a number of studies that required airway control in intact rats, including *in vivo* respiratory-gated imaging and surfactant instillation studies.

Reports on rat intubation begin to appear in the literature approximately 30 y ago.^{6,8,14} Many of the basic techniques have survived as good practices, however new tools for improved access and visualization of the larynx continue to be introduced. Successful procedures usually include 1) a dorsally recumbent rat mounted on an inclined plane, with the upper incisors secured by a cord; 2) a variation of the Seldinger technique,¹⁴ in which a stylet or blunted wire is used as an introducer for the larger tracheal catheter tubing; and 3) good visualization of the pharyngoepiglottic region. Stark and coworkers¹⁷ proposed a blind oro-tracheal rat intubation, but this method is complicated by the number of attempts required and the death of 1 of every 10 rats studied. Many other techniques presented in the literature^{1,5,13,15,20} require fairly elaborate equipment that is not readily available or requires substantial fabrication and considerable practice for proficiency. In this paper, I introduce a method for making a light-carrying laryngoscope from a simple piece of translucent plastic tubing and present the accompanying methods needed to quickly perform effective oro-tracheal intubations in the rat.

Materials and Methods

Animals. Animals reported in this work are from a study on bronchial angiogenesis in which intubation was required for forced ventilation and lung reinflation during a surgery that involved occlusion of the left pulmonary artery. Approximately 70 VAF/Plus (Virus Antibody Free) male Sprague-Dawley rats (*Rattus norvegicus*, Charles River Laboratories, Wilmington, MA) weighing 250 to 300 g were intubated for surgery or study using the methods described in a later section. The rats were group-

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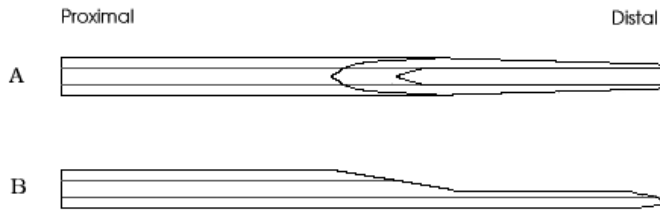


Figure 1. Two schematic drawings of a laryngoscope, indicating the general shaping needed at the distal end of the acrylic tubing. (A) Top view. (B) Side view.

housed (3 animals per cage) in conventional polycarbonate, high-temperature cages (10.5 in. × 19 in. × 8 in.) with wire-bar lids (PC10198HT, Allentown Caging Equipment, Allentown, NJ) and containing heat-treated hardwood laboratory bedding (Beta Chips, Northeastern Products, Warrensburg, NY). Rodent chow (no. 5001, Purina Mills, St. Louis, MO) and water was provided ad libitum, and cages and bedding were changed at least twice weekly. Animals were housed in a temperature (20.6 to 22.2 °C)- and humidity (50% to 60%)-controlled room on a 12:12-h light:dark cycle (lights on, 07:00 to 19:00). Once the left pulmonary artery occlusion surgery was completed and after a short (5 to 10 min) postoperative recovery, the tracheal tube was removed, and the rat allowed to recover. All protocols were approved by the Institutional Animal Care and Use Committee of the Zablocki VA Medical Center, and animals were treated in accordance with the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals*.¹²

Laryngoscope construction. I constructed rat laryngoscopes from rigid acrylic tubing (outer diameter, approximately 6 mm; inner diameter, approximately 3 mm; United States Plastic, Lima, OH). The length of the tubing can be varied to accommodate the user; my laboratory has found that making the laryngoscope approximately 11 cm in length works very well. Once cut to size, 5 to 6 cm of the distal end of the tubing was reduced and given the general shape shown in Figure 1. This shaping can be done using a Dremel tool (Dremel, Mount Prospect, IL), a file, or coarse sand paper. Figure 2 shows 3 different working versions of the laryngoscope; panels 2A and 2B show the top view of laryngoscopes that use a straight configuration, and panel 2C shows the side view of one for which the proximal end was heated and bent approximately 20°. The addition of a finger grip (Cables for Less, Mooresville, IN; shown toward the left of Figure 2B) and bending the shaft (Figure 2C) aided in preventing the operator's hand from obscuring visualization. Polishing the surfaces where light will enter and exit the laryngoscope was important and helped to transmit the light into the laryngopharynx and consequently aid in good visualization of the larynx. Fine sandpaper (400 grit or higher) was used to smooth and polish the flat proximal end and the surfaces near the distal end. As a result, a light source incident on the proximal end of the laryngoscope transmits light to the proximal end (Figure 3). Investigators in my laboratory have used both surgical and examination lights during the intubation procedure, but the hollow bore of the laryngoscope also allows for the optional insertion of a common flashlight (MAGLite, Ontario, CA) fiber optic adapter attachment (Nitelze, Boulder, CO; Figure 4). The fiber-optic light source can be used when sufficient ambient light or other light sources are unavailable. Much less light was carried by the laryngoscope when using the fiber-optic adapter inserted into the scope's bore (see Figure 4) compared to sources incident on the scope's proximal end (see Figure 3). Therefore, under these conditions, a straight version

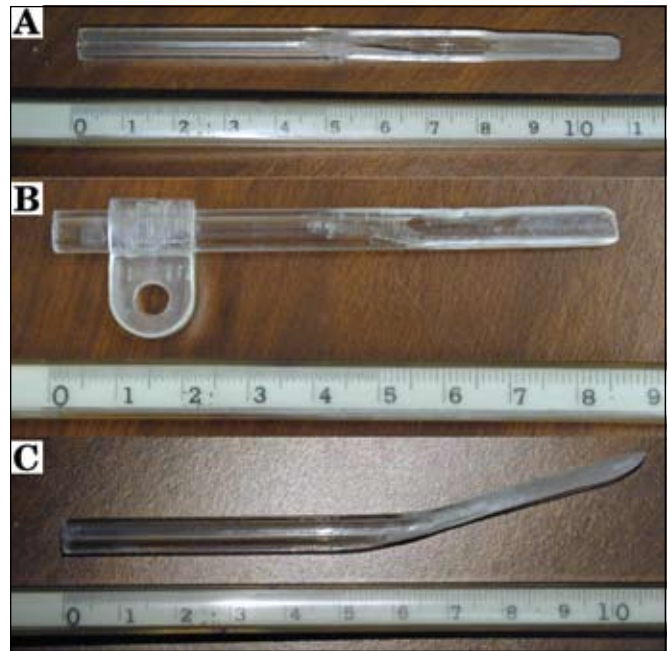


Figure 2. Three variations in laryngoscope construction. (A) Straight shaft. (B) Straight shaft with additional finger grip. (C) Bent shaft. All scales in centimeters.

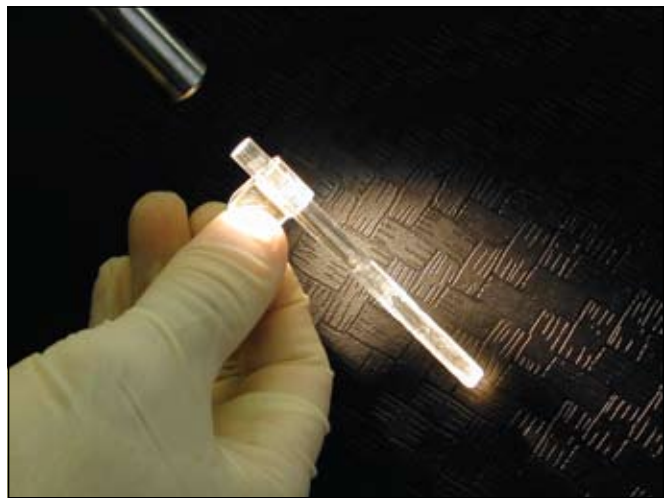


Figure 3. This photograph of a laryngoscope with a light source incident on the proximal end illustrates how light is transmitted to the distal surfaces.

of the laryngoscope provides the geometry that best takes advantage of the light emitted from the fiber-optic adapter.

Equipment and preparation for tracheal intubation. Items used in the intubation procedure included: 1) a general anesthetic, 2) an inclined plane, 3) 2-0 suture of any type, 4) a local topical anesthetic, 5) cotton-tipped applicators, 6) a laryngoscope (constructed as described earlier), 7) a blunt-ended, rigid guidewire or stylet, 8) a tracheal tube or catheter, and 9) a light source. Our work used a number of injectable anesthetics, such as sodium pentobarbital (50 mg/kg intraperitoneally [IP]) or a mixture of ketamine (75 mg/kg IP) and medetomidine hydrochloride (0.5 mg/kg IP), but inhalation anesthetics also are used frequently to anesthetize and restrain the rat prior to intubation. The inclined plane (at an angle of 15° to 20° with respect to the bench top) keeps the rat in the proper orientation (Figure 5). Several centimeters of the 2-0 suture wrapped



Figure 4. Laryngoscope with an optional fiber-optic light source attached. Scale in centimeters.

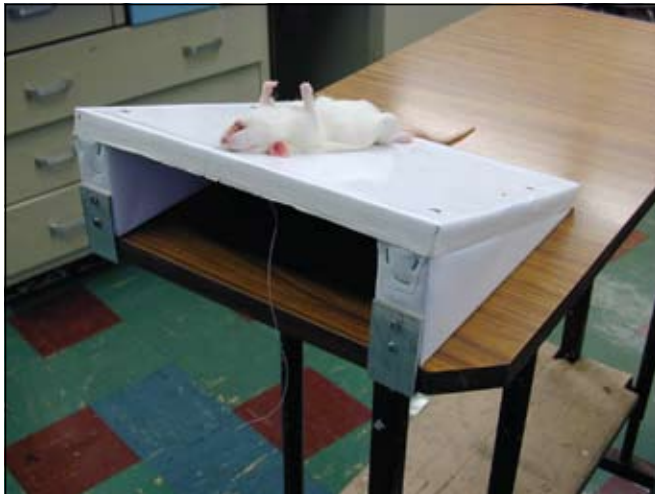


Figure 5. An anesthetized rat positioned and restrained on inclined plane.

around the upper incisors was used to secure the rat to the inclined plane. The inclined plane can easily be made from a flat plate or board with a block underneath. The plane used (Figure 5) was made from a pine board measuring $27 \times 27 \times 2$ cm for the top and another of $7 \times 26 \times 4$ cm cut diagonally into 2 wedges for legs. The surface of the boards was covered with Bytac surface protector sheeting (Cole-Palmer, Vernon Hills, IL) to allow sterilization and easy clean up.

The investigator put several drops of 2% lidocaine (20 mg/ml) on a cotton-tipped applicator, and used it to desensitize structures in the oropharynx and clear the region of excess mucus. This treatment minimized laryngospasms, dilated the pharyngeal cavity and helped to visualize the larynx. Hyper-salivation can be addressed by fitting a small tube on a syringe and using suction. Alternatively, a preanesthetic adjuvant, such as atropine (1.0 mg/kg intramuscularly), can be used to reduce salivary and bronchial secretions that can occlude the airway. The guidewire or stylet (approximately 15 cm long and with an outer diameter of approximately 0.8 mm; Figure 6) can be made from rigid stainless steel tubing or wire. I used 21-gauge heavy-walled stainless steel tubing (Small Parts, Miami Lakes, FL) with the distal (introductory) end filled with epoxy resin (Devcon, Riviera Beach, FL) and then blunted to protect the animal.

Tracheal tubes can be made from a piece of material of the appropriate diameter (depending on the size of the rat), with the introductory end cut at an angle to promote penetration of the glottis. Investigators in my laboratory typically used a straightened 10-cm length of polyethylene tubing (PE 190–240, Becton Dickinson, Franklin Lakes, NJ) or polytetrafluoroethylene thin-wall tubing (12- to 18-gauge, Small Parts, Miami Lakes, FL). This range in tubing sizes will accommodate the opening of the glottis, which is approximately 1.5 to 2.5 mm in the adult rat. PE 240 with an outer diameter of 2.42 mm will fix snugly



Figure 6. Cannula-tracheal tube (bottom) and stainless-steel stylet (top). Scale in centimeters.

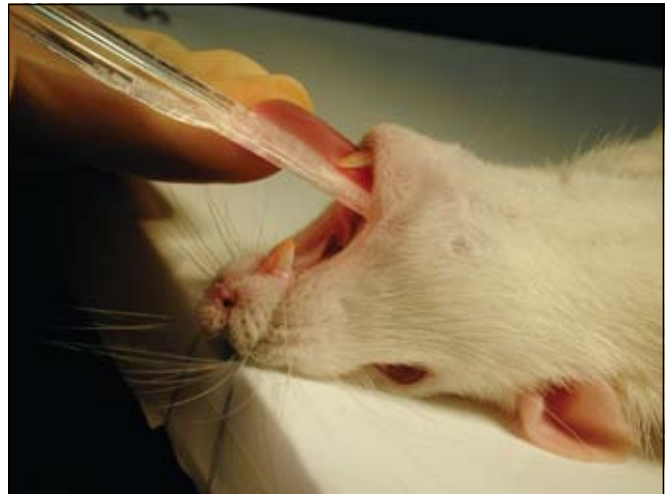


Figure 7. Anesthetized rat restrained on inclined plane with tongue grasped against laryngoscope. This initial positioning allows oropharyngeal access for application of local anesthetic and clearing of excess mucus.

in a 300-g rat. To avoid overinsertion of the tracheal tube and inadvertent intubation of a bronchus, the tracheal tube can be positioned along the outside of the neck and marked prior to intubation. The investigator positioned the proximal end of the tube at the height of the clavicle and marked the tube near the upper incisors. The distance from the upper incisors to the main carina is 5.5 to 6.5 cm in the adult rat. Alternatively, a short length of rubber tubing or an O-ring can be pushed onto the tracheal tube such that it will abut the larynx and prevent the tube from being inserted too far. The distance from the larynx to the main carina is 3.5 to 4.0 cm in the adult rat. Illumination of the pharyngoepiglottic region was needed; with the laryngoscope properly positioned, light provided by most surgical or laboratory sources was sufficient.

Method of intubation. After the rat was anesthetized sufficiently, it was placed in dorsal recumbency on the inclined plane with the suture hooked around the upper incisors (Figure 5). The tongue was extended and moved laterally. The distal end of the laryngoscope was partially inserted into the mouth, and the tongue was extended and grasped securely against the shaft of the laryngoscope (Figure 7). A drawing of the regional anatomy as viewed through the mouth of a rat in dorsal recumbency (Figure 8) is provided for reference.

A cotton-tipped applicator, which had been saturated with a topical anesthetic, was inserted into the oral cavity and momentarily placed in general contact with the base of the tongue, epiglottis, arytenoid cartilage, and the palate to desensitize the area and clear it of excess mucus. I recommend using topical anesthetic, because my laboratory has found that placing pressure on the back of the tongue or soft palate without local anesthetic can cause respiratory arrest in some rats.

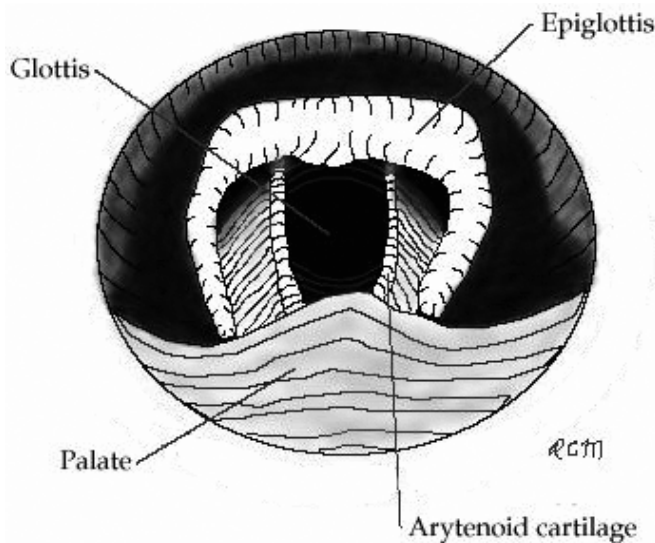


Figure 8. Schematic drawing of the rat larynx as viewed through the mouth, with the animal in dorsal recumbency.

After the area was anesthetized, the laryngoscope was positioned to further depress the base of the tongue ventrally in order to visualize the larynx. With the stylet inside the tracheal tube and extending no more than 1 cm beyond the distal end (Figure 9), the operator's free hand grasped the stylet and tube while maintaining their relative position. While the arytenoid cartilage and glottis were visible, the stylet tip was positioned near the glottis. The arytenoid cartilage was observed for several cycles of opening and closing in order to appreciate the timing required to effectively introduce the stylet.

The stylet was inserted into the glottis by anticipating when it was open. It was important that the stylet was inserted only several millimeters into the glottis, to prevent rupture or trauma to the tracheal wall. The laryngoscope was retracted while care was taken to maintain the depth of penetration of the stylet. The tracheal tube was released and allowed to slide along the stylet. The hand previously holding the laryngoscope was used to advance the tracheal tube past the arytenoid cartilage and into the trachea, with gentle twisting during the insertion. The tracheal tube was inserted to a proper depth by using the methods described earlier. Finally, the position of the tracheal tube was verified by: 1) visualizing condensation of water vapor, evident on the inside surface of the tube (easily seen if a transparent cannula was being used), 2) placing a mirror near the proximal end of the cannula and identifying the condensation of water vapor on the mirror surface, or 3) attaching the cannula to a ventilator and identifying proper expansion and collapse of the chest.

Results

The laryngoscope and methods presented here provide a very efficient means for quick, effective intubation of rats. The laryngoscope design permits securing and manipulating the tongue, while illuminating the pharyngoepiglottic region to yield good visualization of the arytenoid cartilage and glottis. Once the rat reached a sufficient plane of anesthesia and was secured to the inclined plane, the procedure of intubation easily could be performed in a minute or less. The intubation technique worked successfully for all 70 rats in the study, although in a few animals, a 2nd or 3rd intubation attempt was required. Tracheal intubation allowed us to efficiently ventilate the rat, fully reinflate the lungs prior to chest closure, and easily instill



Figure 9. Anesthetized rat restrained on inclined plane. The laryngoscope is positioned in the oral cavity to provide visualization of the larynx. The tongue is grasped against the shaft of the laryngoscope. The stylet and tracheal tube are shown before being inserted into the oral cavity. Note the relative position of stylet within tracheal tube.

compounds into the lungs when necessary.

The technique was quickly learned. A technician and a doctoral candidate were taught the technique. Both trainees required several attempts with the 1st rat. Each failed attempt involved inadvertent intubation of the esophagus due to incorrect initial placement of the stylet or pulling the stylet out of the glottis before the cannula was advanced. However, both trainees achieved a 100% success rate after the 1st rat, although some subsequent rats required more than 1 attempt.

Discussion

The laryngoscope presented here was easily and inexpensively made from readily available materials (see Table 1). It was used quickly and efficiently to minimize the time required for intubation, thereby reducing possible complications such as excessive salivary and bronchial secretions, which can interfere with visualization and obstruct air flow. The laryngoscope described has been used on rats ranging from 200 to 450 g. Animals smaller than 200 g will probably need devices constructed from narrower plastic tubing to provide adequate access to the laryngopharynx. Conversely, animals larger than 450 g likely will need laryngoscopes made from wider tubing, to provide more strength and avoid breakage.

Each of the laryngoscopes constructed were still in use at the time of writing, after approximately 3 y of service. The laryngoscopes and stylets are reusable and can be cleaned in a mild detergent, such as Terg-A-Zyme (Alconox, New York, NY), between uses. Avoid cleaning the laryngoscope with more harsh chemicals, such as alcohols, as they may cause stress fractures in the plastic. One of the laryngoscopes cracked while in use and was easily repaired with Weld-On clear acrylic solvent and cement (IPS, Compton, CA). A laryngoscope that has stress fractures or has been repaired will not transmit light as efficiently as one that is undamaged and may need replacement.

As mentioned previously, if the laryngopharynx was not anesthetized with a local anesthetic, there was a risk of respiratory arrest which apparently was associated with manipulation of structures near the back of the tongue. Whether the incidents also were associated with an individual's response to general anesthetics is not clear. The phenomenon did occur prior to ap-

Table 1. List of specialized materials

Item	Description	Source	Website	Approx. cost (USD)
Laryngoscope				
Acrylic tubing	outer diameter, 1/4 in. inner diameter, 1/8 in.	United States Plastic, Lima, OH	www.usplastic.com	\$0.10/ft
Optional laryngoscope components				
Finger grip	1/4-in. cable clamp	Cables for Less, Mooresville, IN	www.cablesforless.com	\$0.17 ea
Flashlight	2 AA	MAGLite, Ontario, CA www.all-maglite-4-less.com	www.maglite.com or	\$9.00 ea
Fiber optic adapter attachment	7 in.	Nitelze, Boulder, CO	www.niteize.com or www.all-maglite-4-less.com	\$6.00 ea
Other specialized materials used during intubation				
Protective sheeting for inclined plane	Bytac, 12 1/2-in. width	Cole-Palmer, Vernon Hills, IL	www.coleparmer.com	\$8.00/ft ²
Stylet, stainless steel tubing	21-gauge, heavy wall	Small Parts, Miami Lakes, FL	www.smallparts.com	\$5.00/6 in.
Cannula, polytetrafluoroethylene tubing	12- to 18-gauge, thin-wall	Small Parts, Miami Lakes, FL	www.smallparts.com	\$0.30–0.60/ft

plication of lidocaine to 2 rats in this study, and the procedure had to be halted to allow recovery of or resuscitate the rat before continuing. Therefore, it was important to intermittently visualize the chest and verify that the rat was breathing, in particular when first positioning the laryngoscope. Once the laryngoscope was positioned, visual confirmation of the arytenoid cartilage opening and closing (approximately 50 to 70 times per min in the anesthetized rat) was sufficient indication that rat was breathing.

One factor involved with the initial tube misplacement by trainees was that they understood the risk of possible injury to the animal due to overinsertion of the stylet, therefore they tended to error on the side of drawing the stylet back rather than overinserting it. In addition, the presence of an object in the glottis, even in a well-anesthetized rat, may cause a pharyngeal reflex. Therefore although much less likely, even in experienced hands, there were cases when reflexive animal movement dislodged the stylet from the glottis and additional attempts were required to intubate the trachea.

Insertion and removal of the tracheal tube typically caused no injury to the animal, making this technique suitable for survival procedures. However, approximately 5 of the 70 animals appeared to have undergone minor injury caused by the intubation procedure. The injury was identified by an audible “popping” sound, presumably the arytenoid cartilage slapping closed, which persisted for 3 to 5 d but apparently did not cause any further complications. There was no detectable weight loss or change in food or water consumption. Although no necropsy or histology was performed on these rats, the noise likely was caused by overdilation of the glottis and minor damage to the surrounding cartilage. Therefore, any injuries caused by the technique were considered to be related to the choice of an oversized tracheal tube. When properly sized, the tracheal tube remained securely in place and was removed without injury. Depending on how frequently the animal is handled after intubation, it may be necessary to anchor the tracheal tube with 4-0 suture (any type of sterile suture can be used because it will be removed later, along with the tracheal tube) or tape to prevent it from moving. In conclusion, the presented tool and associated simple, effective method for rat intubation are nondestructive, faster, and less traumatic than tracheostomy.

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