

A Simple and Reliable Method of Endotracheal Intubation in Mice: Advantages of Exposing the Trachea

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Summary

Mice are popular models in experimental studies because they can be genetically modified and have a short gestation period. In long-term studies requiring recovery from anesthesia or repeated measurements of pulmonary function, endotracheal intubation is advantageous. However, this is difficult because of the small anatomic structures involved. Various methods have been previously reported, the majority of them calling for expensive devices or techniques which require special training. Therefore, we have created a simple method of endotracheal intubation in mice using a light-emitting diode (LED) light, a metal laryngoscope, a 22-gauge plastic cannula and a stylet made of a 0.3mm piano wire, which can all be easily prepared. Transillumination after exposure of the trachea makes it possible to illuminate the oropharynx with a low-priced LED light and a metal laryngoscope provides good visualization of the tracheal opening. With direct vision, a 22-gauge plastic cannula can easily be inserted into the trachea. The custom-made stylet is suitably flexible for performing tracheal intubation without tissue injury. In a series of 42 mice, the success rate with the procedure was 97.6% (n=41). Only in one case was it necessary for the procedure to be repeated. There were no airway complications. Though exposure of the trachea is invasive, it does have advantages. We conclude that this method is simple, safe and inexpensive, and could be used by any researchers interested in it, no matter how small their budget.

Introduction

Airway management is important for various types of experimental studies, such as those utilizing mechanical ventilation (*Brown et al., 1999*), measurement of pulmonary function, and the administration of drugs (*Epperly et al., 1999*). Tracheostomy is an invasive method, and thus it is not appropriate for experiments aiming for survival and follow-up. Thus endotracheal intubation is used in long-term studies, though this is hard to do in mice because their small anatomic structures make intubation difficult.

Since a method of endotracheal intubation in mice

was first reported by Ho and Furst in 1973 (*Ho and Furst, 1973*), several methods have been described (*MacDonald et al., 2009; Rivera et al., 2005; Spoelstra et al., 2007; Vergari et al., 2003*). However, many of these required hard-to-use and expensive devices such as a 150-W halogen light source (*Brown et al., 1999; Spoelstra et al., 2007*), a small fiber-optic arthroscope (*Vergari et al., 2003*) or a custom-designed fiber-optic light guide (*MacDonald et al., 2009; Rivera et al., 2005*).

In addition, there have been only a few reports to confirm the position of the cannula inserted into the trachea, though all methods of endotracheal intubation pose the risk of esophageal intubation, which may cause death (*MacDonald et al., 2009; Watanabe et al., 2009*).

Therefore, we have established a simple and reliable method of direct endotracheal intubation in mice by employing easy-to-use, inexpensive devices. Our method involves the exposure of the trachea, which

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is slightly invasive, but this can also bring advantages, including the confirmation of the proper position of the cannula.

Materials and Methods

Animals

The committee on Animal Research at the University of Tsukuba approved the experimental protocols. The mice were cared for in accordance with the Guiding Principles for the Care and Use of Animals based on the Helsinki Declaration of 1964.

The C57BL/6J mice (Japan SLC, Inc., Shizuoka, Japan) used were 8-10 weeks old and weighed 22-28 g at the time of the study. The mice were kept in standard mouse cages filled with woodchips, in groups of a maximum of 7 animals. The animals were subjected to a 14 h light/10 h dark cycle with a light intensity of 200 lux. Tap water and pelleted feed were given *ad libitum* in a room at 21-26 degrees C and 40-65% relative humidity. The mice were part of another experiment involving coronary artery ligation followed by examination of angiogenesis at the site of myocardial infarction.

Intubation procedure

Prior to anesthesia, in order to prevent bradyarrhythmia which could be induced by xylazine hydrochloride, the mice were administered atropine sulfate (ATROPINE SULFATE Injection 0.5mg, Mitsubishi Tanabe Pharma Corp., Osaka, Japan) (0.5 mg/kg) by an intraperitoneal injection. The induction of anesthesia was obtained by administration of a mixture of ketamine (500 mg/ml, Sankyo, Tokyo, Japan) (100 mg/kg i.p.) and xylazine hydrochloride (Sigma-Aldrich Co., Tokyo, Japan) (10 mg/kg i.p.). After the absence of the righting reflex was confirmed, mice were placed in a supine position and fixed with pins to a cork board by the extremities. These pins were also used as electrocardiogram (ECG) monitoring electrodes throughout the procedure. A rubber band was then placed behind the front upper teeth in order to extend the neck slightly. At first, a longitudinal incision was made on the neck. The submandibular glands and sternothyroid

muscles were separated, and then the trachea and larynx were exposed. Next, the cork board was tilted

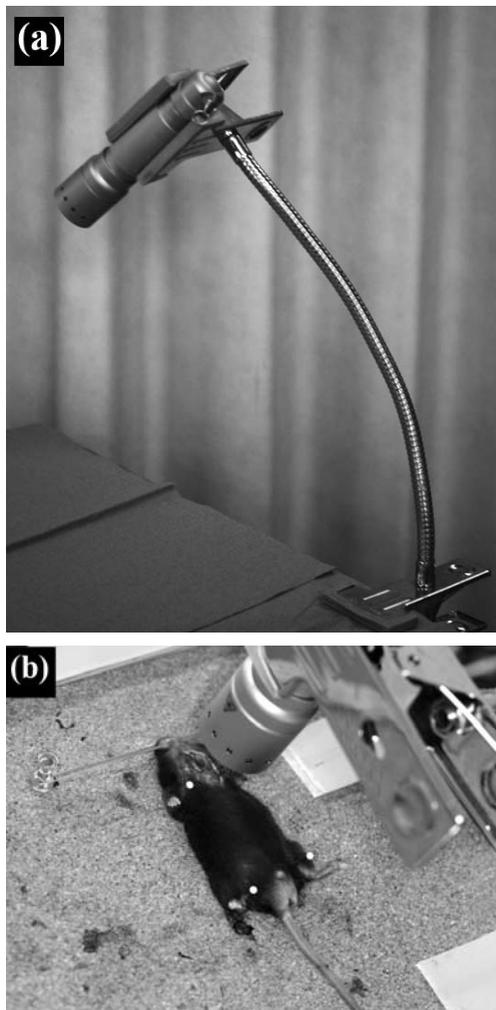


Figure 1. Positioning of mouse, LED light and flexible arm clip.

(a) An LED light is held in place by a flexible arm clip, which is a flexible metal stick with clips at both ends. (b) A mouse is fixed with pins to a cork board by the extremities and a rubber band is placed behind the front upper teeth in order to extend the neck slightly. The LED light directly illuminates the exposed trachea and larynx.

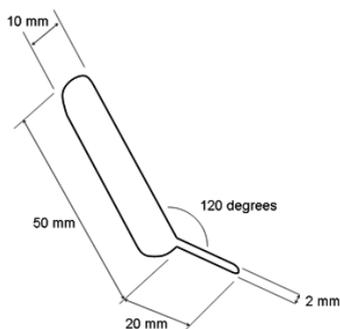


Figure 2. Custom-made laryngoscope. Made of stainless steel; blade 2×20 mm, handle 10×50 mm, bent at an angle of 120 degrees.

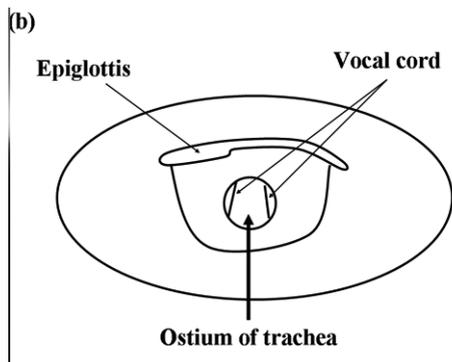


Figure 3. Typical view of the illuminated trachea seen through the oropharynx. (a) Direct transillumination of the larynx using an LED light provides adequate brightness and a custom-made laryngoscope facilitates the visualization of the tracheal opening. The arrow indicates the epiglottis (dark area) of the mouse. The ostium of the trachea is illuminated by the external LED light. (b) Schema of the photograph. The positions of the epiglottis and the ostium of the trachea are demonstrated.

ed up to 75 degrees so that the mouse's head was elevated. The LED light (LED LENSER® Hokus Focus, Zweibrüder Optoelectronics GmbH, Solingen, Germany) which was held by a flexible arm clip (arm length 320mm, Yazawa Corporation Co., Tokyo, Japan) directly illuminated the trachea and larynx (Figure 1). This facilitated visualization of the entrance to the trachea as transillumination when the operator looked at it through the oropharynx. Then, the tongue was pulled forward and a metal laryngoscope (Fig. 2), made of stainless steel, was used to lift the upper jaw of the mouse. At this point, a clear view of the tracheal opening was achieved (Fig. 3). Under direct visualization, a 22-gauge plastic cannula (Introcan Safety® 0.9 × 25 mm, B. Braun Melsungen AG, Melsungen, Germany) with a stylet (Fig. 4) inside was inserted into the trachea without difficulty. This stylet, which was composed of an introducer needle hub of a plastic cannula and a 0.3 mm piano wire, added appropriate stiffness to the plastic cannula. In addition, to prevent tissue injury, the length of the piano wire was formed so that it did not stick out from the cannula when the stylet was inserted into it. After orotracheal intubation, the stylet was removed.

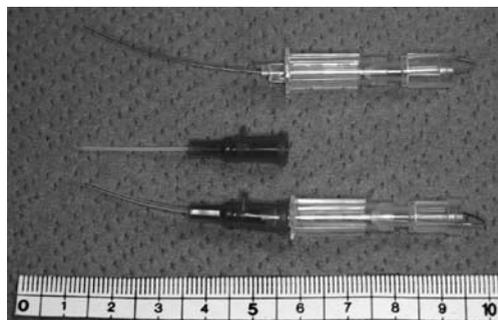


Figure 4. Custom-made stylet and the 22-gauge plastic cannula.

The stylet (top) is composed of an introducer needle hub of a plastic cannula and a short length of 0.3 mm piano wire. To prevent tissue injury, the length of the piano wire is designed so that it does not stick out from the cannula when the stylet is inserted into it.

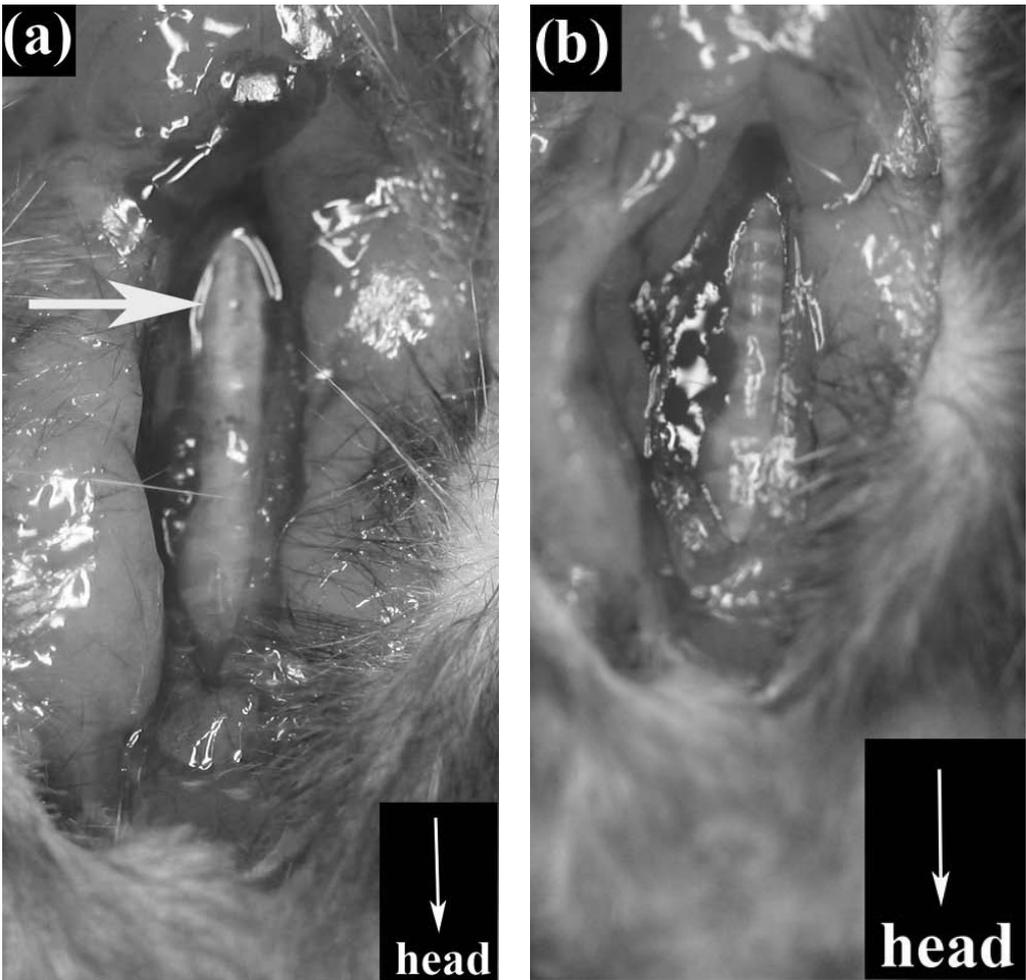


Figure 5. (a) Exposed trachea after intubation. (b) before intubation. The inserted cannula can be confirmed with direct vision through the exposed trachea. The arrows indicate the inserted cannula.

Finally, the position of the inserted cannula was confirmed with direct vision through the exposed trachea (Fig. 5) and by sense of touch with the forceps. At this time, the tip of the inserted cannula was adjusted at the level of the sternal incision. If the inserted cannula could not be detected in the trachea, the same intubation procedure was re-performed.

Mechanical ventilation

The mice were connected to a rodent miniventilator (Rodent ventilator model 683, Harvard Apparatus, Holliston, MA, USA) and ventilated at a rate of 120 breaths per minute with a tidal volume of 1 ml. During mechanical ventilation, surgery for coronary artery ligation was performed. The skin incision on the neck was extended to the xiphoid

process and the thoracic wall was opened through the left third inter-costal space. After the coronary artery ligation, the thoracic wall was closed by one or two interrupted stitches and the skin incision was closed by running a stitch between the xiphoid process and the neck, so that the exposed trachea was closed only at the skin layer.

Extubation procedure

After the skin incision was closed, the rate of mechanical ventilation was reduced to 40 breaths per minute. The mice then immediately started breathing and the ventilator was disconnected. The inserted cannula was extubated when adequate spontaneous breathing and heart rate were confirmed.

ECG monitoring

Continuous ECG monitoring was performed by using computer software (Chart 5 Pro®, ADInstruments, Australia) and the heart rate before and after intubation was analyzed thereafter.

Results

Forty-two mice underwent tracheal intubation and coronary artery ligation. The mean execution time between making the neck skin incision and the end of the cannula insertion was about 3 minutes. The ECG analysis showed that the heart rates before and after intubations were 466 ± 42 and 389 ± 46 beats per minute, respectively (mean \pm standard deviation).

The success rate of endotracheal intubation was 97.6% (n=41). In only one case, there was esophageal intubation at first, but the mistake was detected with direct vision through the exposed trachea before the onset of mechanical ventilation and a second procedure was successful. The heart rate after the second intubation was maintained (340 beats per minute) and there was no arrhythmia.

No airway complications were brought about by this method and the mice did not show any signs of respiratory distress.

Discussion

We have described a simple method for endotracheal intubation in mice which utilizes an LED light, a metal laryngoscope, an IV catheter and a stylet.

Although similar methods for endotracheal intubation in mice have been previously reported (*Brown et al., 1999*), our method introduces two new features that contribute to the safety and cost-effectiveness of the procedure.

The first is a modified transillumination technique, which is the direct illumination of the larynx after its surgical exposure. The transillumination technique, which has been reported by several authors, including Brown et al. (*Brown et al., 1999*), requires an expensive 150-W halogen light source with fiberoptic arms (US\$400-500). Exposure of the trachea is not technically difficult and makes it possible to adequately illuminate the oropharynx externally using a low-priced LED light (US\$40-50). In addition, direct visualization of the trachea makes it possible to confirm proper placement of the intubated tube in the trachea before implementing mechanical ventilation. This is an important advantage, as esophageal intubation can be a cause of death in mice when mechanical ventilation is started without noticing this. Vergari et al. stated that surgical exposure of the larynx leads to the development of scar tissue that disturbs repeated intubations (*Vergari et al., 2003*). However, in our experience at sacrifice after four weeks, re-exposure of the trachea itself did not lead to any problems, such as bleeding, because the submandibular glands are avascular tissue. Therefore, we believe it is possible to perform our intubation procedure repeatedly.

The second feature is the use of a custom-made stylet. We use a 22-gauge plastic cannula as an intubated tube because thicker cannulas can injure oropharyngeal structures. However, it is difficult to successfully insert the thin 22-gauge plastic cannula alone into the trachea of mice because of insufficient stiffness in the cannula. This stylet, which is made from a 0.3 mm piano wire, adds appropriate stiffness to the 22-gauge plastic cannula without leading tissue injury.

In conclusion, our method for endotracheal intubation of mice is a simple and reliable procedure and the devices involved are inexpensive. Although exposure of the trachea is slightly invasive, it brings with it advantages, especially in that the proper position of the cannula can be confirmed before mechanical ventilation or administration of drugs is initiated.

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