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Title: A Structured Approach to Extubation in Mechanically Ventilated Rats

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar?

No

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length
Number of Steps: 10
Number of Shots: 24

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Zhonghua Shi:** Mechanical ventilation is life-saving but can cause brain and diaphragm injury. We aim to develop an extubation model in rats. This model simulates clinical weaning to study these injuries better.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: Figure 3*

What technologies are currently used to advance research in your field?

1.2. **Xiang Qi:** Currently, most mechanical ventilation models only look at short-term effects and mainly use tracheostomy, which limits clinical relevance.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

What are the current experimental challenges?

1.3. **Xiang Qi:** Tracheostomy makes ventilation easier but bypasses the upper airway, raising the risk of lung infections and disrupting brain signaling. This makes it hard to study injury and recovery accurately.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

What research gap are you addressing with your protocol?

1.4. **Fenqin Xue:** We are filling the gap in rat models by creating a clear, step-by-step extubation protocol. It follows clinical practice, lowers complications, and helps rats recover more safely after ventilation.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 3.4*

What research questions will your laboratory focus on in the future?

1.5. **Jingyi Li:** Using this extubation model, we aim to investigate the molecular mechanisms underlying ventilator-induced injuries to the brain and diaphragm, as well as the biological pathways involved in their potential recovery.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Animal Experiments and Experimental Animal Welfare Committee of Capital Medical University

Protocol

2. Video Laryngoscopy-Assisted Oral Intubation

Demonstrator: Xiang Qi

2.1. To begin, prepare the ventilator **[1]** and adjust the oxygen concentration **[2].** Get the intubation device ready and position the anesthetized rat for intubation **[3-TXT]**.

2.1.1. WIDE: Talent attaching the tubing from the oxygen concentrator to the ventilator inlet.

2.1.2. Talent adjusting the oxygen concentration.

2.1.3. Talent at the working bench with the intubation device and rat positioned appropriately for the procedure. **TXT: Anaesthesia: Propofol (2 mg/100 g)**

2.2. Using forceps, gently pull the rat's tongue toward the right oral corner **[1]**. Insert the intubation device at the base of the tongue and advance it toward the epiglottis **[2]**. Then, lift the device tip to expose the glottis **[3]** and insert the endotracheal tube into the trachea during inhalation **[4]**. Once the tube is in place, connect the rat to the ventilator **[5]**.

2.2.1. Talent pulling the tongue to the side with forceps.

2.2.2. Talent inserting the intubation device and guiding it toward the epiglottis.

2.2.3. Close-up of the device tip lifting to reveal the glottis.

2.2.4. Talent inserting the endotracheal tube during the rat's inhalation.

2.2.5. Talent connecting the endotracheal tube to a mechanical ventilator.

2.3. Now, set the ventilator parameters based on the rat's weight and experimental requirements **[1]**. Infuse propofol continuously using a syringe pump to maintain anaesthesia **[2]**.

2.3.1. Show settings being entered on the ventilator, including respiratory rate and tidal volume.

2.3.2. Talent connecting the syringe pump to deliver propofol intravenously.

2.4. Monitor the rat's blood gas levels throughout the procedure to ensure respiratory stability **[1]**.

2.4.1. Display a blood gas monitoring system showing partial pressure of oxygen and carbon dioxide levels.

3. APEC Extubation Procedure

3.1. For weaning, reduce the propofol infusion rate by 50 percent using the syringe pump settings and waiting for approximately 10 minutes for the sedation level to decrease **[1]**. Disconnect the rat from the ventilator for 1–2 minutes to assess readiness for weaning **[2]**.

3.1.1. Talent reducing the infusion rate of propofol by 50 percent using the syringe pump controls.

3.1.2. Talent disconnecting the rat from the ventilator.

3.2. During this period, ensure the respiratory rate is between 60–70 breaths per minute **[1],** confirm a chest displacement of at least 1 millimeter **[2],** and verify that arterial blood gas values and pH levels are within acceptable ranges **[3].** If all parameters are met, disconnect the ventilator **[4]**.

3.2.1. Talent observing and counting respiratory rate.

3.2.2. Close-up of a steel needle placed on the xiphoid process to measure chest displacement.

3.2.3. Display of blood gas analysis results showing $PaCO_2$ and pH values. **TXT: $PaCO_2$: 35-45 mmHg; pH: 7.35-7.45**

3.2.4. Talent disconnecting the rat from the ventilator.

3.3. To prepare the rat for extubation, use a custom-made face mask to ensure adequate oxygenation **[1]**. Then, observe the rat's skin color to assess oxygenation **[2]**.

3.3.1. Talent placing a custom face mask on the rat.

3.3.2. Close-up of the rat's skin color to assess oxygenation status.

3.4. Using a self-made suction device, perform suctioning at a rate of approximately 2 milliliters per second for 3 to 4 seconds per session to clear airway secretions **[1-TXT]**.

3.4.1. Talent performing airway suctioning with controlled speed and duration. **TXT: Maintain sedation during extubation**

3.5. Next, using a two-handed technique, stabilize the rat's head with the left index finger and thumb, then gently remove the endotracheal tube with the right index finger and thumb **[1]**. Discontinue the propofol infusion once the tube is removed **[2]**.

3.5.1. Talent stabilizing the rat's head and carefully removing the endotracheal tube using both hands.

3.5.2. Talent turning off the syringe pump delivering propofol.

3.6. Monitor the rat's vital signs post-extubation **[1]** and continue providing oxygenation as needed using the custom face mask **[2-TXT]**.

3.6.1. Talent checking the respiratory rate and heart rate.

3.6.2. Talent positioning the oxygen mask over the rat's face for continued oxygen delivery. **TXT: Prepare for re-intubation if respiratory distress occurs**

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Results

4. Results

4.1. In the structured protocol applied to 20 rats, all extubations were successful, with full recovery achieved in approximately 48.5 minutes post-extubation **[1]**.

4.1.1. LAB MEDIA: Table 2. *Video editor: Highlight the row under "Caring post-extubation" that reads "Return of the righting reflex after extubation, min: 48.5 ± 10.4."*

4.2. During the "Approaching Weaning" step, average oxygen partial pressure was 87.6 millimeters of mercury **[1]**, carbon dioxide partial pressure was 43.2 millimeters of mercury **[2]**, and pH was 7.421 **[3]**.

4.2.1. LAB MEDIA: Table 2. *Video editor: Highlight the "PaO_2, mmHg" value in the "Approaching Weaning" section.*

4.2.2. LAB MEDIA: Table 2. *Video editor: Highlight the "$PaCO_2$, mmHg" value in the "Approaching Weaning" section.*

4.2.3. LAB MEDIA: Table 2. *Video editor: Highlight the "pH" value in the "Approaching Weaning" section*.

4.3. No complications were reported during either the extubation phase **[1]** or the post-extubation care period **[2]**.

4.3.1. LAB MEDIA: Table 2. *Video editor: Highlight the entry "None" next to "Extubation related complications" under "Executing Extubation."*

4.3.2. LAB MEDIA: Table 2. *Video editor: Highlight the entry "None" next to "Post-extubation complications" under "Caring post-extubation."*