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Scriptwriter Name: Poornima G
Project Page Link: https://review.jove.com/account/file-uploader?src=21044743

Title: Precision Induction and Distinction of Coughing and Sneezing Reflexes in Mice

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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? **Yes, all done**

   If **Yes**, can you record movies/images using your own microscope camera?

   Yes

   SCOPE: 2.5.1, 2.5.2, 2.5.3, 2.6.1,2.6.3, 2.7.2, 4.3.1, 4.3.2, 4.4.1, 4.5.2, 4.5.3

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**

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

4. **Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length
Number of Steps: 25
Number of Shots: 57 (2 SC, 11 Scope)

Introduction

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

1.1. **Zhimin Ye:** This protocol focuses on respiratory reflexes in mice. We aimed to stimulate the trachea and nasal cavity to induce coughing and sneezing, and to differentiate between these two types of events based on their audio features.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll:2.3.1*

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

1.2. **Zhimin Ye:** Previous studies often use nebulized capsaicin or citric acid to stimulate the respiratory tract and elicit coughing and sneezing behaviors in mice.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll:2.9.1*

What are the current experimental challenges?

1.3. **Zhimin Ye:** However it remains unclear whether respiratory tract stimulation by nebulization induces coughing, sneezing, or both, as there is currently no reliable method for distinguishing these responses.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll:3.3.1*

What research gap are you addressing with your protocol?

1.4. **Rong Yi:** For this project, we established a method to induce coughing and sneezing by selectively stimulating the trachea or the nasal cavity. We also used acoustic analysis to identify the distinctive features between a cough and a sneeze.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll:4.3.1*

What questions will future research focus on?

1.5. **Rong Yi:** Our research in mice provides a powerful tool that will directly support the development of future drugs for coughing and sneezing disorders

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll:5.3.1*

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

Ethics Title Card

This research has been approved by the Animal Use and Care Protocol at the Guangzhou National Laboratory

Protocol

2. Establishment of Cough-Like Behavior in Mice

Demonstrator: Zhimin Ye

2.1. To begin, cut a twenty-eight-gauge needle into a cannula of approximately five millimeters in length **[1-TXT]**. Using forceps and abrasive paper, smooth both its ends **[2]** and attach one end to silicone tubing **[3-TXT]**. Connect the tube-attached cannula to a one-milliliter syringe **[4]** and flush the cannula with water to verify patency **[5]**.

2.1.1. WIDE: Talent cutting the 28-gauge needle into a short ~5 mm cannula using appropriate cutting tool and forceps. **TXT: Needle: Inner diameter: 0.19 mm; Outer diameter: 0.35 mm**

2.1.2. Talent holding the cannula with forceps and smoothing both cut ends with abrasive paper.

2.1.3. Talent attaching one end of the cannula to the silicone tubing. **TXT: Tubing: Inner diameter: 0.3 mm; Outer diameter: 0.8 mm**

2.1.4. Talent connecting the other end of the silicone tubing to a one-milliliter syringe.

2.1.5. Talent flushing water through the cannula to test patency.

2.2. Next, bend the cannula at a ninety-degree angle with a two-to-three arm ratio **[1]**. Insert the longer arm into the five-centimeter-long silicone tube **[2-TXT]**.

2.2.1. WIDE: Talent bending the cannula at 90 degrees, demonstrating arm proportions.

2.2.2. Talent inserting the longer arm of the bent cannula into the prepared silicone tube. **TXT: Prepare: Whole-body plethysmography system; Ultrasonic recorder; High-speed camera; Microinjection pump**

2.3. Randomly assign mice to three groups with six mice each **[1]**.

2.3.1. TEXT ON PLAIN BACKGROUND:

Control group: Untreated

Sham group: Implant the tracheal cannula (no stimulation)

Stimulation group: Implant the tracheal cannula + administer capsaicin to induce cough

2.4. Now, secure the anesthetized mouse in a supine position on the table **[1]**. After shaving the cranial and cervical hair, disinfect the area with iodophor **[2]**. Using surgical scissors, trim a circular section of scalp approximately five millimeters in diameter to expose the skull **[3]**.

2.4.1. Talent positioning the anesthetized mouse supine on surgical board. **TXT: Anesthesia: Tribromoethanol (125 - 250 mg/kg, i.p.)**

2.4.2. Talent applying iodophor disinfectant to the shaved area.

2.4.3. Talent trimming a 5 mm diameter scalp circle to expose the skull.

2.5. Under a microscope, incise the cervical skin for approximately one centimeter and bluntly dissect through tissues and muscles to expose the trachea **[1]**. Puncture the soft tissue between the cricoid and tracheal cartilages using a thirty-two-gauge needle **[2]**. Then, create a minimal tracheal opening with ophthalmic scissors **[3]**.

2.5.1. SCOPE: 2.5.1.mp4 00:05-00:15.

2.5.2. SCOPE: 2.5.2.mp4 00:05-00:17.

2.5.3. SCOPE: 2.5.3.mp4 00:05-00:15.

2.6. Insert the silicone-sheathed metal cannula approximately one millimeter into the tracheal lumen **[1]** and secure the cannula-trachea junction with a 7-0 *(7-oh)* suture **[2-TXT]**.

2.6.1. SCOPE: 2.6.1.mp4 00:04-00:20. **TXT: Avoid advancing insertion depth beyond 1 mm**

2.6.2. SCOPE: 2.6.2.mp4 00:10-00:20.

2.7. Now, bluntly tunnel subcutaneously from the neck up to the skull **[1]**. Route the silicone tube through the subcutaneous tunnel to the cranium and fix it onto the skull using dental cement **[2]**.

2.7.1. Talent inserting blunt tunneling tool under the skin from neck to skull.

2.7.2. SCOPE: 2.7.2.mp4 000:05-00:15.

2.8. Suture the cervical incision and let the animal recover for 5 days **[1-TXT]**.

2.8.1. Talent suturing the neck incision. **TXT: Flush the tracheal cannula and tubing daily with a 1 mm air syringe**

3. Cough Recording in Mice

3.1. Fit the ultrasonic probe with a port-compatible sealing ring **[1]** and insert the probe into the whole-body plethysmography chamber port to ensure airtight contact **[2]**.

3.1.1. Talent fitting a sealing ring onto the ultrasonic probe.

3.1.2. Talent inserting the probe into the WBP chamber port and ensuring a tight, airtight seal.

3.2. Connect the whole-body plethysmography chamber to the flow sensor and bias flow system **[1-TXT]**.

3.2.1. Talent connecting the WBP chamber to the flow sensor and bias flow tubing. **TXT: Calibrate the entire setup using the software interface**

3.3. Attach a skull-fixed silicone tube to a thirty-centimeter extension tube **[1]** and place the mouse into the whole-body plethysmography chamber **[2]**. Extend the tube through the top port of the chamber **[3]** and seal the port using modeling clay around the tubing **[4]**. Verify airtightness by confirming that the respiratory waveform peaks fall within four plus or minus two **[5]**.

3.3.1. Talent connecting the skull-fixed silicone tube to a 30 cm extension tube.

3.3.2. Talent gently placing the prepared mouse into the WBP chamber.

3.3.3. Talent guiding the extension tube through the chamber's top port.

3.3.4. Talent applying modeling clay to seal the port tightly around the tube.

3.3.5. SCREEN: 3.3.5.mp4 00:10-00:20 ***Videographer: Please record the computer screen for the shots labeled as SCREEN***

3.4. Connect a one-milliliter syringe loaded with five milliliters of capsaicin solution to the extension tube **[1]** and mount it onto the microinjection pump **[2-TXT]**.

3.4.1. Talent connecting the loaded syringe to the extension tubing.

3.4.2. Talent mounting the syringe onto the pump. **TXT: Flow rate: 10 µL/min**

3.5. Position a high-speed camera facing the chamber **[1]**. Adjust the camera distance and focus for a clear view **[2]** and set the sampling rate to one hundred sixty frames per second **[3]**.

3.5.1. Talent positioning the high-speed camera directly in front of the chamber.

3.5.2. Talent adjusting the camera's focal length and distance.

3.5.3. Talent adjusting the camera settings.

3.6. Now, simultaneously start the whole-body plethysmography, ultrasound, and camera software **[1]**. Trigger the pump to begin capsaicin infusion **[2]** and record cough counts for ten minutes **[3]**.

3.6.1. Talent clicking Start on the WBP, ultrasound, and camera software simultaneously.

3.6.2. Talent triggering capsaicin infusion from the microinjection pump.

3.6.3. SCREEN: 3.6.3-Added-shot-.mp4 00:03-00:15.

4. Establishment of Sneeze-Like Behavior in Mice

4.1. Prepare the animals and all the materials required for the procedure **[1]**.

4.1.1. TEXT ON PLAIN BACKGROUND:

- Sterile PE tube (ID 0.5 mm, OD 1 mm) for stimulation cannula
- Sterilized nylon filament (0.25 mm, 5 cm) calibrated to 0.6 g at ~45°
- Set up the WBP (Whole-Body Plethysmography) system, ultrasound recorder, and high-speed camera
- Randomly assign mice to 3 groups (n=6/group):
    - Control: Untreated baseline
    - Sham: Cannula fixation, no stimulation
    - Stimulation: Cannula fixation + filament-induced sneeze

4.2. After anesthetizing and preparing the mouse, secure it in a stereotaxic frame **[1-TXT]**. Disinfect the surgical area with iodophor **[2]**. Incise the skin over the skull and nasal bone using a scalpel to expose the nasal bone **[3]**.

4.2.1. WIDE: Talent placing and fixing the anesthetized mouse into a stereotaxic frame. **TXT: Anesthesia: Tribromoethanol (125 - 250 mg/kg, i.p.)**

4.2.2. Talent applying iodophor over the skull and nasal area.

4.2.3. Talent making incisions over the skull and nasal bridge and retracting the skin to expose the nasal bone.

4.3. Using a micro-drill, create a 0.5-millimeter hole at the junction between the nasal bone

and the lateral process of the nasal septal cartilage **[1]**. Now, expose the nasal mucosa through the burr hole **[2]**.

4.3.1. SCOPE: 4.3.1.mp4 00:10-00:25.

4.3.2. SCOPE: 4.3.2.mp4 .

4.4. Gently lift the nasal mucosa using a thirty-two-gauge needle to expose the nasal cavity **[1]**.

4.4.1. SCOPE: 4.4.1.mp4 00:05-00:20.

4.5. Then, mount the polyethylene tube into the stereotaxic arm **[1]**. Align the tip of the tube flush with the burr hole opening **[2]** and secure the tube onto the nasal bone using dental cement **[3]**.

4.5.1. Talent mounting the stimulation cannula into the stereotaxic arm.

4.5.2. SCOPE: 4.5.2.mp4. 00:10-00:20

4.5.3. SCOPE: 4.5.3..mp4. 00:03-00:15

4.6. Place the mouse on a heating pad and allow it to recover until consciousness returns **[1]**.

4.6.1. Talent transferring the mouse onto a heating pad and monitoring recovery.

5. Sneeze Recording in Murine Models

5.1. Connect the whole-body plethysmography chamber to the flow sensor and bias flow system according to the manufacturer's manual **[1-TXT]**.

5.1.1. Talent attaching tubing between the plethysmography chamber, flow sensor, and bias flow module. **TXT: Calibrate the setup using the software interface**

~~5.1.2. SCREEN: Display the software calibration interface and execute the calibration process.~~ NOTE: Not provided, VO moved as on-screen text

5.2. Next, fit the ultrasonic probe with a port-adapted gasket **[1]**. Insert the probe into the plethysmography chamber and verify an airtight seal **[2]**.

5.2.1. Talent sliding a gasket onto the ultrasonic probe.

5.2.2. Talent inserting the probe into the chamber port and pressing gently to confirm an airtight seal.

5.3. Transfer the mouse into the plethysmography chamber **[1]** and extend the polyethylene tube through the top port **[2]**. Seal the port using modeling clay to secure the tubing **[3]**. Verify chamber airtightness and setup the camera as demonstrated earlier **[4]**.

5.3.1. Talent placing the prepared mouse into the WBP chamber.

5.3.2. Talent guiding the PE tube out through the chamber's top port.

5.3.3. Talent sealing the port with modeling clay molded around the tubing.

5.3.4. Talent fine-tuning the focus and adjusting the distance for optimal framing.

5.4. Finally, advance a 0.25-millimeter diameter nylon filament through a soft tube into the nasal cavity to a depth of zero to three millimeters **[1]**. Keep five centimeters of the filament exposed beyond the hand-held tube **[2]**.

5.4.1. Talent inserting the nylon filament through a soft guide tube into the mouse's nostril.

5.4.2. Close-up of five centimeter segment of filament protruding from the hand-held tube.

5.5. During each stimulation, press the free end of the filament until it bends to approximately forty-five degrees, delivering around 0.6 grams of force to the nasal mucosa for one second **[3]**. Repeat the stimulation once every thirty seconds while recording sneezing responses for ten minutes **[4]**.

5.5.1. Talent pressing the filament until it bends to 45 degrees inside the nasal cavity for one second.

5.5.2. SCREEN: 5.5.2,mp4 00:00-00:15.

Results

6. Results

6.1. Mice subjected to tracheal or nasal surgery with mechanical nasal stimulation and capsaicin tracheal challenge significantly increased sneeze and cough events, respectively **[1]**.

6.1.1. LAB MEDIA: Figure 2A,B. *Video editor: Highlight the taller bars labeled "Nasal" in the sneeze graph and "trachea" in the cough graph*.

6.2. Whole-body plethysmography recordings showed both single- and double-peak respiratory traces for capsaicin-induced coughing **[1]** and mechanically induced sneezing **[2]**.

6.2.1. LAB MEDIA: Figure 3A,C. *Video editor: Highlight the single and double peaks above the '0' mark on the Y-axis in both A and C*

6.2.2. LAB MEDIA: Figure 3E,G. *Video editor: Highlight the single and double peaks in above the '0' mark on the Y-axis in both E and G*

6.3. Sound oscillograms revealed that cough audio had an abrupt onset, peak intensity at the start, and was concentrated in the 0 to 30 kilohertz range **[1]**, whereas sneeze audio built progressively in intensity, spanned a 0 to 80 kilohertz range, and had a longer duration **[2]**.

6.3.1. LAB MEDIA: Figure 3B,D. *Video editor: Highlight the black sharp waveforms and red regions in the 0–30 kHz range in the cough spectrograms*.

6.3.2. LAB MEDIA: Figure 3F,H. *Video editor: Highlight the black waveform and red regions spread from 0–80 kHz in the sneeze spectrograms*.

6.4. The compression phase of single-peak coughs averaged around 32.39 milliseconds **[1]**, significantly shorter than double-peak coughs at around 53.17 milliseconds **[2]**.

6.4.1. LAB MEDIA: Figure 4C. *Video editor: Highlight the orange bar labeled "Single" for coughs*.

6.4.2. LAB MEDIA: Figure 4C. *Video editor: Highlight the green bar labeled "Double" for coughs*.

6.5. The average compression phase of single-peak sneezes was around 44.03 milliseconds

[1], while double-peak sneezes averaged at around 74.24 milliseconds and were significantly longer **[2]**.

6.5.1. LAB MEDIA: Figure 4G. *Video editor: Highlight the orange bar labeled "Single" for sneezes.*

6.5.2. LAB MEDIA: Figure 4G. *Video editor: Highlight the green bar labeled "Double" for sneezes.*

6.6. Single-peak patterns occurred in 77.55% of coughs **[1]**, whereas only 32.42% of sneezes exhibited single peaks, with double-peak patterns dominating at 67.58% **[2]**.

6.6.1. LAB MEDIA: Figure 4D and H. *Video editor: Highlight the larger pie slice labeled "77.55%" for single-peak coughs in D*.

6.6.2. LAB MEDIA: Figure 4D and H. *Video editor: Highlight the smaller pie slice labeled "32.42%" for single-peak sneezes in H*.

6.6.3. LAB MEDIA: Figure 4D and H. *Video editor: Highlight the larger pie slice labeled "67.58%" for double-peak sneezes in H*.

6.7. Sneezing audio durations were significantly longer than coughing durations, though with overlapping ranges **[1]**.

6.7.1. LAB MEDIA: Figure 5A. *Video editor: Highlight the taller bar labeled "Sneeze"*

6.8. Transection of nasal sensory afferents significantly reduced capsaicin-induced sneezing **[1]**, while coughing remained unaffected **[2]**.

6.8.1. LAB MEDIA: Figure 5H. *Video editor: Highlight the much shorter bar labeled "Surgery" compared to "Sham" for sneezing*.

6.8.2. LAB MEDIA: Figure 5I.

- **Cannula**

Pronunciation link: https://www.merriam-webster.com/dictionary/cannula
IPA: /ˈkæn.jə.lə/
Phonetic spelling: ˈkan-yuh-luh

- **Abrasive**

Pronunciation link: https://www.merriam-webster.com/dictionary/abrasive

IPA: /əˈbreɪsɪv/
Phonetic spelling: uh-bray-siv

- **Plethysmography**

Pronunciation link: https://www.howtopronounce.com/plethysmography
IPA: /pləˌθɪzˈmɑːgrəfi/
Phonetic spelling: pluh-thiz-MOG-ruh-fee

- **Cricoid**

Pronunciation link: https://www.merriam-webster.com/dictionary/cricoid
IPA: /ˈkraɪkɔɪd/
Phonetic spelling: kry-koyd

- **Suture**

Pronunciation link: https://www.merriam-webster.com/dictionary/suture
IPA: /ˈsuːtʃər/
Phonetic spelling: soo-cher

- **Dental (as in "dental cement")**

Pronunciation link: https://www.merriam-webster.com/dictionary/dental
IPA: /ˈdɛntəl/
Phonetic spelling: den-tuhl

- **Calibration**

Pronunciation link: https://www.merriam-webster.com/dictionary/calibration
IPA: /ˌkælɪˈbreɪʃən/
Phonetic spelling: kal-i-BRAY-shun

- **Oscillogram / Oscillograms**

Pronunciation link: https://www.howtopronounce.com/oscillogram
IPA: /ˈɒsɪləˌgræm/
Phonetic spelling: OS-i-lo-gram