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## Title: Acupuncture in a Rat Model of Asthma

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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 similar? **N**

**2. Software:** Does the part of your protocol being filmed demonstrate software usage? **Y**

*Videographer: Screen capture files provided; do not film*

**3. Interview statements:** Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one.**



Interviewees wear masks until the videographer steps away ( $\geq 6$  ft/2 m) and begins filming. The interviewee then removes the mask for line delivery only. When the shot is acquired, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

**4. Filming location:** Will the filming need to take place in multiple locations (greater than walking distance)? **N**

### Protocol Length

Number of Shots: **45**

# Introduction

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## 1. Introductory Interview Statements

### REQUIRED:

- 1.1. **Yong-Qing Yang**: Acupuncture is an effective method for treating asthma, although the underlying mechanism is not clear. This protocol mimics acupuncture in the clinic to study these mechanisms in an animal model [1].

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

### REQUIRED:

- 1.2. **Yu Wang**: The main advantage of this technique is that the high platform can be used to fix the rats without restriction, allowing full exposure of the dorsal acupoints [1].

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

### OPTIONAL:

- 1.3. **Guang-Quan Zhang**: Beginners should have basic rat handling skills and should practice inserting the acupuncture needles into a piece of cardboard before attempting to treat animals [1].

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

### OPTIONAL:

- 1.4. **Dong-Dong Zhou**: Visualization of the technique will help beginners to become familiar with the skills necessary to successfully perform the experiment [1].

- 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Videographer: Can cut for time*

## Ethics Title Card

- 1.5. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at Shanghai University of Traditional Chinese Medicine.

# Protocol

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## 2. High Platform Fabrication

- 2.1. The length and width of the supporting device should 86 x 17 centimeters [1].
  - 2.1.1. WIDE: Talent measuring device
- 2.2. Vertically affix a 30-centimeter long by 14-centimeter wide connecting device to the supporting device [1] and fix a 5- x 5-centimeter horizontal pedal to the connecting device [2].
  - 2.2.1. Shot of connecting device connected to supporting device *Videographer: Important step; Video Editor: please emphasize connecting and supporting devices when mentioned*
  - 2.2.2. Shot pedal connected to connecting device *Videographer: Important step; Video Editor: please emphasize pedal and connecting device when mentioned*
- 2.3. The suspended height at the bottom of the horizontal pedal should be greater than or equal to 50 centimeters [1].
  - 2.3.1. Shot of height at bottom of pedal *Video Editor: please add bracket emphasize height at bottom of pedal when mentioned*

## 3. Rat Asthma Model Establishment

- 3.1. For OVA (OH-vah) sensitization, grab and lift the tail of an adult rat [1-TXT] and place the animal on a rough surface while gently pulling back on the tail [2].
  - 3.1.1. WIDE: Talent grabbing/lifting tail/rat **TEXT: OVA: ovalbumin**
  - 3.1.2. Talent placing rat onto rough surface while pulling tail
- 3.2. Firmly but gently grasp the skin of both ears and neck with the left thumb and index finger [1] while tightly holding the back skin with the remaining three fingers and palm [2] and use the right hand to intraperitoneally inject 1 milliliter of freshly prepared sensitization solution into the rat abdomen [3-TXT].
  - 3.2.1. Ears and neck being grasped

- 3.2.2. Back skin being held
- 3.2.3. Rat being injected **TEXT: See text for all solution preparation details**
- 3.3. On the 14<sup>th</sup> day after sensitization, drill a small hole in the side of a 39- x 28- x 23-centimeter box **[1]** and use a rubber tube to connect the box to a nebulizing cup and compression nebulizer **[2]**.
  - ~~3.3.1.~~
  - 3.3.2. Talent connecting materials with tube
- 3.4. Place the rat into the box **[1]** and place the box in a biosafety cabinet **[2]**.
  - 3.4.1. Talent placing rat into box
  - 3.4.2. Talent placing box into cabinet
- 3.5. Turn on the ultrasonic nebulizer **[1]** and load 30 milliliters of a freshly prepared 300-milligram OVA in saline challenge solution into the nebulizing cup **[2-TXT]**.
  - 3.5.1. Talent turning on nebulizer
  - 3.5.2. Talent loading solution into cup **TEXT: Adjust dosage and rat number according to sealed box volume**
- 3.6. The nebulization can be repeated several times in one day or according to the severity of the asthma symptoms to be induced **[1]**.
  - 3.6.1. Talent taking rat out of box

#### 4. Acupuncture Treatment

- 4.1. For acupuncture treatment of the asthmatic animal, select acupoints, including GV14 (**G-V-fourteen**), bilateral BL12, and bilateral BL13, according to the theory of traditional Chinese medicine for treating asthma **[1]** and administer manual acupuncture on day 2 and every other day for a total of 7 session after asthma establishment **[2]**.
  - 4.1.1. LAB MEDIA: Supplementary Figure 2 *Video Editor: please emphasize pressure point texts and lines when mentioned*
  - 4.1.2. LAB MEDIA: Figure 2 *Video Editor: please add/emphasize needles*
- 4.2. For needle placement, transfer the rat to the high platform **[1]** and, grasping the handle of disposable stainless-steel needle with the thumb and index finger of the dominant hand **[2]**, firmly press the back of the rat with the palm of the hand **[3]**.

- 4.2.1. Talent placing rat into platform *Videographer: Important/difficult step*
- 4.2.2. Needle being grasped *Videographer: Important/difficult step*
- 4.2.3. Rat back being pressed *Videographer: Important/difficult step*
- 4.3. For GV14 needle placement, touch the depression in the middle of the upper edge of the scapula between the seventh cervical vertebra and the first thoracic vertebra on the midline of the back [1] and gently prick the skin with the needle point to locate the acupoint [2].
  - 4.3.1. Depression being touched
  - 4.3.2. Skin being pricked
- 4.4. Then quickly insert the needle [1] and rapidly remove the right hand [2].
  - 4.4.1. Needle being inserted
  - 4.4.2. Hand being removed
- 4.5. Place the BL13 needles below the third thoracic vertebra on both sides of the intercostal spaces [1] and place the BL12 needles below the second thoracic vertebra on both sides of the intercostal spaces as demonstrated [2].
  - 4.5.1. Second needle being placed, with first placed needle visible in frame *Videographer: Important/difficult step*
  - 4.5.2. Second needle being placed, with first placed needle visible in frame *Videographer: Important/difficult step*
- 4.6. When all of the needles have been placed, twist each needle 360 degrees 60 times over a period of 10-20 seconds every 5 minutes for 20-30 minutes [1] before removing the needles [2].
  - 4.6.1. Needle being twisted *Videographer: Important step*
  - 4.6.2. Talent removing needle(s) *Videographer: Important step*

## 5. Penh Measurement

- 5.1. To measure Penh in the acupuncture-treated animals, first turn on the signal amplifier [1] and click **Calibrate** to return the coarse adjustment baseline to 0 [2].
  - 5.1.1. WIDE: Talent turning on amplifier
  - 5.1.2. SCREEN: 5.2-5.4: 00:00-00:03 **Note: 5.1.2 was filmed during the shoot, use it instead of the screen capture**
- 5.2. Switch to the direct current position [1] and fine tune the baseline to 0 [2].

- 5.2.1. Talent selecting DC position
- 5.2.2. SCREEN: 5.2-5.4: 00:04-00:15
- 5.3. Use a syringe to inject 5 milliliters of air [1] before switching to the alternating current position [2].
  - 5.3.1. SCREEN: 5.2-5.4: 00:26-00:58 *Video Editor: please speed up*
  - 5.3.2. SCREEN: 5.2-5.4: 00:59-01:06
- 5.4. After checking the channels, ensure that the error value is less than 0.5% [1].
  - 5.4.1. SCREEN: 01:17-01:22 *Video Editor: please emphasize Error value*
- 5.5. Next, turn on the aerosol distribution and rodent bias flow supply systems [1] and adjust the flow rate to 4.5 milliliters according to the liquid level on the mercury ball [2].
  - 5.5.1. Talent turning on system(s)
  - 5.5.2. Talent adjusting flow rate
- 5.6. Under **Create Study Options**, enter “Test” for the **Study** and select “Rat” for the **Species** [1].
  - 5.6.1. SCREEN: 5.6-5.7: 00:01-00:22 *Video Editor: please speed up*
- 5.7. Confirm that **Simple Dose List** is selected and set the **Number of Doses** to 6 [1].
  - 5.7.1. SCREEN: 5.6-5.7: 00:26-00:34
- 5.8. To select the **Penh** and **Area** parameters [1], click **Next** and set the animal adaptation time to 5 minutes, the nebulization time to 2 minutes, the response time to 3 minutes, and the recovery time to 2 minutes [1].
  - 5.8.1. SCREEN: 5.8: 00:00-00:13
  - 5.8.2. SCREEN: 5.8.: 00:14-00:29 *Video Editor: please speed up*
- 5.9. Then add 200 microliters of each freshly prepared concentration of methacholine in PBS to the nebulizer in sequence as indicated [1-TXT].
  - 5.9.1. Talent adding methacholine to nebulizer **TEXT: 0 -> 3.125 -> 6.25 -> 12.5 -> 25 -> 50 mg/mL**
- 5.10. Real-time changes in Penh should be observed [1].



5.10.1. SCREEN: 5.10: 00:00-00:19 *Video Editor: please speed up*

5.11. When all 6 solutions with different concentrations have been delivered and assessed, click **File** and **End Session [1]**.

5.11.1. SCREEN: 5.11: 00:00-00:18 *Video Editor: please speed up*

## Protocol Script Questions

**A.** Which steps from the protocol are the most important for viewers to see?

2.2., 4.2., 4.5., 4.6., 5.8.

**B.** What is the single most difficult aspect of this procedure and what do you do to ensure success?

4.2. For acupuncture manipulation, transfer the rat to the high platform and, grasping the handle of disposable stainless-steel needle with the thumb and index finger of the dominant hand, press the back of the rat with the palm of the hand. The harder the palm of the right hand presses the rat back, the easier it is to insert the needle.

4.5. Place the BL13 needles below the third thoracic vertebra on both sides of the intercostal spaces and place the BL12 needles below the second thoracic vertebra on both sides of the intercostal spaces.

# Results

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## 6. Results: Representative Acupuncture Needle Placement and Penh Measurement

- 6.1. With acupuncture at the GV14, bilateral BL12, and bilateral BL13 points, the rat maintains a prone position [1] and can twist its head to the outside only, as one direction is blocked with a wooden board [2].
  - 6.1.1. LAB MEDIA: Figure 5 *Video Editor: please emphasize pin location texts and/or needles in left image when mentioned*
  - 6.1.2. LAB MEDIA: Figure 5 *Video Editor: please emphasize board in right and/or left image*
- 6.2. As illustrated in the graph, after asthma induction, as the methacholine concentration increases [1], the Penh concentration increases [2], promoting a gradual increase in the growth rate [3].
  - 6.2.1. LAB MEDIA: Figure 6 *Video Editor: please stretch arrow along x-axis to emphasize methacholine concentration increase*
  - 6.2.2. LAB MEDIA: Figure 6 *Video Editor: please stretch arrow along y-axis to emphasize Penh % change increase*
  - 6.2.3. LAB MEDIA: Figure 6 *Video Editor: please emphasize data line*

# Conclusion

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## 7. Conclusion Interview Statements

7.1. **Yu-Dong Xu**: Western blot and real-time PCR can also be used to study specific protein and gene changes after acupuncture treatment [1].

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

7.2. **Yan-Jiao Chen**: This technique can help researchers to explore specific changes in key factors and signal pathways in asthma after acupuncture [1].

7.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Videographer: Can cut for time*