FINAL SCRIPT: APPROVED FOR FILMING

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Title: Measurement of Pulmonary Artery Pressure in Rats Using Right Heart Catheterization

Authors and Affiliations:

Hantong Ding[1,2], Mengting Zeng[1,2], Binda Sun[1,3], Fang Deng[1,4], Jian Chen[1,3], Gang Xu[1,3]

[1]Key Laboratory of Extreme Environmental Medicine of the Ministry of Education`
[2]College of Basic Medicine, Army Medical University
[3]Institute of Medicine and Equipment for High Altitude Region, College of High Altitude Military Medicine, Army Medical University
[4]Department of Pathophysiology, College of High Altitude Military Medicine, Army Medical University

Corresponding Authors:

Jian Chen	jchenone@tmmu.edu.cn
Gang Xu	xg270251@tmmu.edu.cn

Email Addresses for All Authors:

Hantong Ding	m19122969134@163.com
Mengting Zeng	2160894203@qq.com
Binda Sun	sunbinda1209@tmmu.edu.cn
Fang Deng	celldf@126.com
Jian Chen	jchenone@tmmu.edu.cn
Gang Xu	xg270251@tmmu.edu.cn

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? **Yes, all done**

Videographer: Please record the computer screen for the shots labeled as SCREEN as back-up

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: 14
Number of Shots: 50 (8 SC)

Introduction

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

INTRODUCTION:

~~What are the most recent developments in your field of research~~?

1.1. **Jian Chen:** Our laboratory has optimized the traditional rat right heart catheterization procedure in many ways to improve efficiency and success rate.

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

~~What are the current experimental challenges~~?

1.2. **Jian Chen:** The challenge is that the technique of measuring pulmonary artery pressure in small animals using right heart catheterization is difficult for beginners to master.

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

CONCLUSION:

~~What advantage does your protocol offer compared to other techniques~~?

1.3. **Mengting Zeng:** Our protocol does not require a microscope, improves the process of vasotomy, and reduces the risk of puncturing the heart wall.

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

~~How will your findings advance research in your field~~?

1.4. **Hantong Ding:** This technology can help us measure pulmonary artery pressure in small animals more efficiently and accurately.

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

~~What new scientific questions have your results paved the way for~~?

1.5. **Mengting Zeng:** Our protocol provides support and a framework for the reproducibility of studies related to pulmonary hypertension models.

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

NOTE: The author being interviewed has changed.

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

Ethics Title Card

This research has been approved by the Institutional Animal Care Committee at the Army Medical University

Protocol

2. Preparing the Catheter

Demonstrator: Gang Xu

2.1. To begin, use scissors to cut off the needle tip of the infusion set **[1]** and select a venous infusion needle **[2-TXT]**. Thread a copper wire, with a diameter smaller than 1 millimeter, into the polyvinyl chloride tube **[3]**.

2.1.1, Talent cutting the needle tip from the tube using scissors.

2.1.2, WIDE: Talent picking up a venous infusion needle and reading the measurements labeled on the tube. **TXT: Outer diameter: 1.6 mm; Inner diameter: 1 mm: Length: 25 cm**

Author's NOTE: Move shot 2.1.2 above shot 2.1.1. VO inverted

2.1.3, Talent inserting a copper wire into the tube.

2.2. Wrap the tail end of the tube around a cylindrical object, such as a pencil, to form a circle with a diameter of 7 millimeters **[1]** and immerse the circular end in water heated to 100 degrees Celsius for 10 minutes **[2]**. After heating, remove the tube from the water **[3]**, then pull out the copper wire from the tube **[4]**. Ensure the tail end maintains a pigtail-like shape, and verify that the opposite end can be connected to a three-way stopcock **[5]**.

2.2.1. Talent bending the tail end of the tube around a pencil to form a circular shape.

2.2.2. Talent placing the bent circular end of the tube into a beaker of boiling water.

2.2.3. Talent using forceps to remove the tube from the beaker after heating.

2.2.4. Talent pulling out the copper wire from the tube.

2.2.5. Talent exposes the catheter and connects the three-way stopcock. **Author's NOTE**: Action was modified

3. Venotomy and Catheter Insertion

3.1. Shave the right cervical region of the anesthetized rat to expose the skin surface **[1-TXT]** and disinfect the exposed area using a cotton swab soaked in rubbing alcohol **[2]**. Then, using tissue scissors, make a 2-centimeter incision in the skin on the right side of the neck **[3]**. With curved ophthalmic forceps, bluntly dissect the external jugular vein on the right side **[4]**.

3.1.1. Talent shaving the right cervical area with electric clippers. **TXT: Anesthesia: 0.3 mL of 2% Sodium Pentobarbital/100 g of body weight (IP)**

3.1.2. Talent wiping the shaved area with a cotton swab soaked in rubbing alcohol.

3.1.3. Talent making a 2 centimeter incision using tissue scissors.

3.1.4. Talent using curved ophthalmic forceps to bluntly dissect and expose the external jugular vein.

3.2. Carefully free approximately 1 centimeter of the exposed external jugular vein **[1]** and ligate the distal end of the vein using a 5-0 *(5-oh)* suture thread **[2]**. Tie a slipknot at the proximal end of the vein **[3]**. Use a hemostat to clamp the suture thread at the distal end and gently pull it toward the head to tighten the blood vessel moderately **[4]**.

3.2.1. Talent isolating and freeing 1 centimeter of the external jugular vein.

3.2.2. Talent ligating the distal end of the vein with a 5-0 suture thread.

3.2.3. Talent tying a slipknot at the proximal end of the vein.

3.2.4. Talent clamping the distal suture with a hemostat and pulling it gently toward the head.

3.3. Now, bend the prepared number 7 needle at a 45-degree angle in the opposite direction of the needle tip **[1]** ~~and puncture the external jugular vein in the direction toward the proximal end using the bent needle **[2]**~~.

3.3.1. Talent bending a number 7 needle at a 45 degree angle using Tissue scissors.

3.3.2. ~~Talent puncturing the external jugular vein with the bent needle toward the proximal end~~.

Author's NOTE: Shot 3.3.2 overlaps with that of Shot 3.5.1, so it is recommended to delete 3.3.2.

3.4. Then, fill the cardiac catheter, which is connected to the three-way stopcock, with heparin sodium solution **[1]**. After filling, close the three-way stopcock to prevent backflow **[2]**.

3.4.1. Talent filling the catheter and connected three-way stopcock with heparin sodium solution using a syringe.

3.4.2. Talent rotating the valve on the three-way stopcock to close it. **Author's**

NOTE: Combined Shot 3.4.1 and Shot 3.4.2 into one shot.

3.5. Insert the bent tip of the prepared number 7 needle into the external jugular vein **[1]**. Gently lift the needle upward and insert the curved ophthalmic forceps along the needle track into the vein to open it up **[2]**. Withdraw the needle carefully from the vein **[3]** and insert the catheter into the vein through the gap created by the forceps **[4]**.

3.5.1. Talent inserting the bent needle tip into the external jugular vein.

3.5.2. Talent lifting the needle and advancing curved ophthalmic forceps into the vein.

3.5.3. Talent withdrawing the needle while forceps remain in place.

3.5.4. Talent guiding the catheter into the vein through the channel opened by the forceps.

3.6. Next, carefully withdraw the curved ophthalmic forceps from the vein **[1]** and untie the previously placed slipknot at the proximal end of the vein **[2]**. Push the catheter further into the vein **[3]**, then tie a new slipknot around the vein to secure the catheter **[4]**. Ensure that the knot is not too tight and that there is no blood leakage while allowing smooth catheter movement **[5]**.

3.6.1. Talent removing the ophthalmic forceps from the vein.

3.6.2. Talent untying the slipknot at the proximal end of the vein.

3.6.3. Talent advancing the catheter further into the vein.

3.6.4. Talent tying a new slipknot around the vein to secure the catheter.

3.6.5. Talent pointing to the area to show that there is no bleeding.

4. Catheter Advancement and Waveform Identification

4.1. Turn on the polygraph device **[1]** and connect the pressure transducer to the polygraph **[2]**. Flush the pressure transducer with normal saline to remove any air bubbles **[3]**.

4.1.1. Talent powering on the polygraph device.

4.1.2. Talent attaching the pressure transducer to the polygraph input port.

4.1.3. Talent flushing the transducer with normal saline using a syringe to ensure no air bubbles remain.

4.2. Next, open the recording software on the connected computer **[1]**. Zero the pressure transducer by exposing it to atmospheric pressure **[2]** and perform two-point calibration using a mercury sphygmomanometer **[3]**. In the software, set the filter type to **low pass**, the cutoff frequency to **40 hertz**, and the sampling rate to **1000 samples per second [4]**.

4.2.1. SCREEN: 69559_screenshot_1.mp4. 00:00-00:19

4.2.2. SCREEN: 69559_screenshot_2.mp4.

4.2.3. SCREEN: 69559_screenshot_3.mp4. 00:00-00:10

4.2.4. SCREEN: 69559_screenshot_4.mp4.

4.3. Connect the three-way stopcock, which is attached to the catheter, to the pressure transducer **[1]**. Ensure there are no air bubbles in the three-way stopcock **[2]**. Open and rotate the switch on the three-way stopcock to allow communication between the catheter and the pressure transducer, and observe the venous waveform **[3]**.

4.3.1. Talent connecting the three-way stopcock to the transducer.

4.3.2. Close-up shot showing no air bubbles.

4.3.3. Talent opening the three-way stopcock and rotating.

4.4. Rotate the catheter while slowly pushing it forward **[1]**. If resistance is encountered, stop pushing and raise the pad made with a surgical blade **[2]**. Slightly withdraw the catheter **[3]**, then rotate and push it forward again until a sense of emptiness is felt and a ventricular waveform appears **[4]**.

4.4.1. Talent gently rotating and advancing the catheter.

4.4.2. Talent raising the surgical pad under the catheter entry point.

4.4.3. Talent withdrawing the catheter slightly.

4.4.4. SCREEN: 69559_screenshot_5.mp4. 00:00-00:20

4.5. Push the catheter forward to reach the pulmonary artery **[1]**. Observe the sensation of the catheter tip sliding along the heart wall during advancement **[2]**. Monitor the screen to identify the appearance of the pulmonary artery waveform **[3]**.

4.5.1. Talent slowly advancing the catheter further.

4.5.2. Talent pausing the advancement and looking at the screen.

4.5.3. SCREEN: 69559_screenshot_6.mp4.

4.6. If the waveform on the screen shows a ventricular pattern, indicating difficulty entering the pulmonary artery **[1]**, withdraw the catheter slightly **[2]**. Rotate the catheter and gently push it forward again **[3]** until the ventricular waveform appears clearly, indicating the correct path **[4]**. Finally, advance the catheter to enter the pulmonary artery **[5]**.

Added shot: SCREEN: 69559_screenshot_NEW

4.6.1. Talent pulling the catheter back by a small distance.

4.6.2. Talent rotating and pushing the catheter gently.

4.6.3. SCREEN: 69559_screenshot_7.mp4.

4.6.4. SCREEN: 69559_screenshot_8.mp4.

Results

5. Results

5.1. Two models of hypoxic pulmonary hypertension were established through the plateau environment simulation chamber, simulating an altitude of 5800 meters and chemically injecting 1 milliliter of 60% monocrotaline into the neck for 28 days **[1]**.

5.1.1. LAB MEDIA: Table 1.

5.2. The right ventricular systolic pressure and mean pulmonary arterial pressure measured in both the chronic hypobaric hypoxia model and the monocrotaline model were significantly higher **[1]** than those in the control group **[2]**.

5.2.1. LAB MEDIA: Table 1. *Video editor: Highlight the row for model group*

5.2.2. LAB MEDIA: Table 1. *Video editor: Highlight the row for control group*

1. Infusion
   Pronunciation link: https://www.merriam-webster.com/dictionary/infusion
   IPA: /ɪnˈfjuːʒən/
   Phonetic Spelling: in·fyoo·zhuhn
2. Venous
   Pronunciation link: https://www.merriam-webster.com/dictionary/venous
   IPA: /ˈviːnəs/
   Phonetic Spelling: vee·nuhs
3. Polyvinyl chloride
   Pronunciation link: https://www.merriam-webster.com/dictionary/polyvinyl%20chloride
   IPA: /ˌpɑːliˈvaɪnəl ˈklɔːraɪd/
   Phonetic Spelling: paa·lee·vye·nuhl klor·ide
4. Catheter
   Pronunciation link: https://www.merriam-webster.com/dictionary/catheter
   IPA: /ˈkæθɪtər/
   Phonetic Spelling: kath·uh·ter
5. Stopcock
   Pronunciation link: https://www.merriam-webster.com/dictionary/stopcock
   IPA: /ˈstɑːpˌkɑːk/
   Phonetic Spelling: staap·kaak
6. Venotomy
   Pronunciation link: https://www.merriam-webster.com/dictionary/venotomy
   IPA: /vɪˈnɑːtəmi/
   Phonetic Spelling: vih·naa·tuh·mee

7. Ophthalmic
   Pronunciation link: https://www.merriam-webster.com/dictionary/ophthalmic
   IPA: /ɑːfˈθæl.mɪk/
   Phonetic Spelling: aaf·thal·mik
8. Jugular
   Pronunciation link: https://www.merriam-webster.com/dictionary/jugular
   IPA: /ˈdʒʌgjələr/
   Phonetic Spelling: juhg·yuh·ler
9. Hemostat
   Pronunciation link: https://www.merriam-webster.com/dictionary/hemostat
   IPA: /ˈhiːməˌstæt/
   Phonetic Spelling: hee·muh·stat
10. Heparin
    Pronunciation link: https://www.merriam-webster.com/dictionary/heparin
    IPA: /ˈhɛpərɪn/
    Phonetic Spelling: hep·uh·rin
11. Polygraph
    Pronunciation link: https://www.merriam-webster.com/dictionary/polygraph
    IPA: /ˈpɑːliˌgræf/
    Phonetic Spelling: paa·lee·graf
12. Transducer
    Pronunciation link: https://www.merriam-webster.com/dictionary/transducer
    IPA: /trænzˈduːsər/
    Phonetic Spelling: tranz·doo·ser
13. Sphygmomanometer
    Pronunciation link: https://www.merriam-webster.com/dictionary/sphygmomanometer
    IPA: /ˌsfɪgmoʊməˈnɑːmətər/
    Phonetic Spelling: sfig·moh·muh·naa·muh·ter
14. Pulmonary
    Pronunciation link: https://www.merriam-webster.com/dictionary/pulmonary
    IPA: /ˈpʊlməˌnɛri/
    Phonetic Spelling: pool·muh·ner·ee
15. Hypoxic
    Pronunciation link: https://www.merriam-webster.com/dictionary/hypoxic
    IPA: /haɪˈpɑːksɪk/
    Phonetic Spelling: hye·paak·sik
16. Hypertension
    Pronunciation link: https://www.merriam-webster.com/dictionary/hypertension
    IPA: /ˌhaɪpərˈtɛnʃən/
    Phonetic Spelling: hye·per·ten·shuhn
17. Monocrotaline
    Pronunciation link: https://www.merriam-webster.com/dictionary/monocrotaline
    IPA: /ˌmɑːnoʊˈkroʊtəˌliːn/
    Phonetic Spelling: maa·noh·kroh·tuh·leen

18. Hypobaric
    Pronunciation link: https://www.merriam-webster.com/dictionary/hypobaric
    IPA: /ˌhaɪpoʊˈbærɪk/
    Phonetic Spelling: hye·poh·bar·ik
19. Hypoxia
    Pronunciation link: https://www.merriam-webster.com/dictionary/hypoxia
    IPA: /haɪˈpɑːksiə/
    Phonetic Spelling: hye·paak·see·uh