FINAL SCRIPT: APPROVED FOR FILMING

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Title: Studying the Coding Profiles of Somatic Stimulation on Cardiac-Locked Neuronal Responses in the Rat Spinal Dorsal Horn

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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? **Yes, all done**

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

Current Protocol Length
Number of Steps: 14
Number of Shots: 30

Introduction

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

1.1. **Ziyi Zhang:** My research decodes acupuncture's spinal neuromodulatory effects on cardiovascular regulation via a thoracic spine-stabilized MEA-ECG protocol enabling millisecond-precise analysis of cardiac-locked SDHN dynamics **[1]**.

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. **Yun Liu:** Current experimental challenges involve achieving reliable thoracic spinal stabilization to prevent pneumothorax, optimizing anesthesia protocols for neuronal stability as well as ensuring millisecond-level MEA-ECG synchronization **[1]**.

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

What advantage does your protocol offer compared to other techniques?

1.3. **Yun Liu:** Our protocol provides a novel dual-monitoring approach by integrating real-time electrocardiograms with high-density microelectrode arrays. This design overcomes the temporal resolution limitations of traditional calcium imaging in neuro-cardiac interaction studies **[1]**. NOTE: Statement modified by the author. Suits our style.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.10*

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

1.4. **Hanqing Xi:** Our results prompt investigation into whether cardiac-locked spinal neurons drive MI progression and if acupoint-specific acupuncture modulation offers therapeutic benefits for myocardial infarction **[1]**.

1.4.1. 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.

Testimonial Questions (OPTIONAL):

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.5. **Ziyi Zhang:** Publishing with JoVE enhances visibility via visual protocols, boosting transparency and reaching broader, interdisciplinary audiences **[1]**.

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

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)

1.6. **Hanqing Xi:** Lab training time dropped 50% as new members used the video protocol, minimizing errors and accelerating project timelines **[1]**.

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

AUTHORS: Please deliver the testimonials in both Chinese and English

Videographer: Please capture the testimonials in both Chinese and English

问：您认为通过 JoVE 发表研究成果将如何提升其学术可见性与影响力？

1.5. 张子怡：通过 JoVE 的可视化实验方案，研究者不仅能提升方法透明度，还能以直观形式突破学科壁垒，使复杂技术更容易被跨领域同行理解、验证和借鉴。

问：能否分享您使用或在 JoVE 发表成果后的具体受益案例？（如促进合作、增加引用、获得资助、优化实验流程、缩短培训时间、降低实验成本或提升产出效率等）

1.6.奚晗清：采用视频实验方案后，实验室新人培训时间减少 50%，显著降低操作错误并加快项目进度。

Ethics Title Card

This research has been approved by the Ethics Committee at the Institute of Acupuncture and Moxibustion, China Academy of Chinese Medical Sciences

Protocol

2. Neural Recording and Classification in a Rat Model of Cardiac Stimulation

Demonstrator: Ziyi Zhang

2.1. To begin, examine a Y-shaped cannula to confirm that it is completely dry **[1]**. Using spring scissors, make a transverse incision in the trachea of an anesthetized rat **[2-TXT]**.

2.1.1. WIDE: Talent inspecting the Y-shaped cannula for dryness.

2.1.2. Talent using spring scissors to make a transverse incision in the trachea. **TXT: Anesthesia: 3 - 5% isoflurane inhalation; 50 mg/kg pentobarbital sodium (i.p)**

2.2. Insert the cannula into the tracheal opening **[1]**. Secure the tracheal cannula with 3-0 *(three-zero)* non-absorbable sutures to prevent air leakage and accidental extubation **[2]**.

2.2.1. Talent inserting the cannula into the tracheal opening.

2.2.2. Talent tying the sutures around the cannula to secure it in place.

2.3. Next, insert three electrodes into the rat's skin **[1]**. Place the positive electrode into the left lower limb, the negative electrode into the right upper limb, and the ground electrode into the right lower limb **[2]**.

2.3.1. Shot of 3 electrodes.

2.3.2. Talent placing electrodes into the specified limbs of the rat.

2.4. Place the rat in a supine position **[1]**. After disinfecting the skin, perform a thoracotomy to expose the thymus **[2]**. Then use the tip of a glass dissecting needle to make a small opening in the pericardium **[3]**.

2.4.1. Talent placing the rat in a supine position.

2.4.2. Shot of the exposed thymus.

2.4.3. Talent carefully creating a small incision in the pericardium using a glass dissecting needle.

2.5. Now insert a silicone catheter, 10 to 15 centimeters in length with multiple small holes at its distal end, through the incision in the pericardium **[1]**. Secure the catheter to the chest wall tissue with bioglue **[2]**.

2.5.1. Talent inserting the silicone catheter into the pericardial opening.

2.5.2. Talent applying bioglue to affix the catheter to the chest wall.

NOTE: Place step 2.11 here.

2.6. Remove the muscles attaching to the head clamp and the straight portion of the long neck muscles to expose the spinous processes of the second thoracic vertebra **[1]**. Then displace the semispinalis and spinalis muscles to expose the vertebral arch from T2 to T6 **[2]**. Using rongeurs, remove the spinous process of the T3 vertebra to expose the T3 spinal cord **[3]**.

2.6.1. Talent dissecting the neck muscles to expose the T2 spinous processes.

2.6.2. Talent retracting the semispinalis and spinalis muscles to reveal vertebral arches

2.6.3. Talent using rongeurs to remove the spinous process of T3.

2.7. For thoracic vertebrae fixation, use a custom spinal clamp to secure the articular processes of T2 and T6 **[1]**. Moisten the surrounding muscles with saline to maintain hydration **[2]**.

2.7.1. Talent positioning and securing the spinal clamp to T2 and T6.

2.7.2. Talent applying saline to keep the muscle tissues hydrated.

2.8. Now attach the electrode array to the micromanipulator of a stereotactic instrument **[1]**. Insert it vertically into the T3 dorsal horn of the spinal cord through the dorsal median sulcus **[2-TXT]**. Then insert the reference electrode into the back muscle **[3]**.

2.8.1. Talent mounting the electrode array onto the stereotactic micromanipulator.

2.8.2. Talent inserting the array into the spinal cord at the specified location. **TXT: Insertion: 500 µm lateral to midline; Depth: 1500 µm**

2.8.3. Talent positioning and inserting the reference electrode into the back muscle.

2.9. For stimulation, load a micro-syringe connected to a silicone catheter having multiple holes, with bradykinin solution **[1-TXT]**. Inject 4 microliters of the solution and induce cardiac nociceptive stimulation **[2]**.

2.9.1. Talent loading microsyringe connected to silicone catheter with bradykinin solution. **TXT: Bradykinin solution: 1 µg/mL**

2.9.2. Talent injecting the solution.

2.10. Observe heart rate and neuronal discharge changes in the T3 spinal cord dorsal horn within 30 minutes of injection **[1]**.

2.10.1. SCREEN: 2.10.heart-rate.mp4 00:00-00:20
AND
2.10.neuronal-discharge-changes.mp4 00:20
Video Editor: Please play both shots side by side

NOTE: Move this step 2.11 after 2.5

2.11. Then, perform manual acupuncture at the PC6 acupoint using a stimulation parameter of 1 hertz **[1]**. Insert acupuncture needles into the PC6 acupoints at a depth of approximately 3 millimeters **[2]**.

2.11.1. Talent performing acupuncture on the rat's PC6 points.

2.11.2. Shot of needle insertion into the PC6 acupoint to the specified depth.

2.12. Launch the software to import the recorded neural data in ns6 *(N-S-Six)* format **[1]**. Drag and drop the ns6 file into the program **[2]**.

2.12.1. SCREEN: 2.12-2.14.mp4 00:01-00:07

2.12.2. SCREEN: 2.12-2.14.mp4 00:08-00:12

2.13. Select **File** and click on **Save As** then choose the **.nex5** *(dot-nex-five)* format to generate standardized spike train data **[1]**.

2.13.1. SCREEN: 2.12-2.14.mp4 00:13-00:17

2.14. Import the converted **.nex5** files into the classification software **[1]**. Execute the relevant code for filtering and categorizing the signals **[2]**. Then sort spike waveforms based on waveform characteristics and principal component analysis, with threshold parameters set at plus or minus 3 standard deviations from baseline noise **[3]**.

2.14.1. SCREEN: 2.12-2.14.mp4 00:21-00:27

2.14.2. SCREEN: 2.12-2.14.mp4 00:55-01:11

2.14.3. SCREEN: 2.12-2.14.mp4 01:28-02:00

2.15. Analyze the cardiac-locked spinal dorsal horn neurons by launching MATLAB. Define the experimental parameters. Click **Run** to execute **[1].**

2.15.1. SCREEN: 2.15.Analyze-cardiac-locked-the-spinal-dorsal-horn-neurons.mp4
00:03-00:45
Video Editor: Please speed up the video

Results

3. Results

3.1. Neurons recorded from channel 19 showed dense, rhythmic spike trains **[1]**, with a symmetrical autocorrelation pattern **[2]**, and tightly clustered waveform groups on PCA *(P-C-A)* **[3]**.

3.1.1. LAB MEDIA: Fig 2B. *Video Editor: Highlight raster for chan19.*

3.1.2. LAB MEDIA: Fig 2C. *Video Editor: Please Zoom in on chan19a autocorrelation*

3.1.3. LAB MEDIA: Fig 2D. *Video Editor: Focus on PCA cluster for chan19.*

3.2. Neurons from channel 11 split into three distinct firing profiles **[1]**, each with unique autocorrelation features **[2]** and PCA waveform clusters **[3]**.

3.2.1. LAB MEDIA: Fig 2E. *Video Editor: Please highlight chan11a–c raster rows*.

3.2.2. LAB MEDIA: Fig 2F.

3.2.3. LAB MEDIA: Fig 2G. *Video Editor: Please emphasize three PCA waveform clusters.*

3.3. Cardiac-locked neurons on channels 17c *(Seventeen-C)* and 21a *(Twenty-one-A)* fired rhythmically with ECG R-waves *(E-C-G-R-Waves)* at baseline **[1]**, but bradykinin disrupted this pattern and introduced new spike clusters between the P and Q waves **[2]**.

3.3.1. LAB MEDIA: Fig 3A.

3.3.2. LAB MEDIA: Fig 3B.

3.4. MAPC6 *(Map-C-Six)* restored R-wave-locked firing **[1]**, although this rhythmicity declined again post-MAPC6 **[2]**. Bradykinin sharply increased the proportion of excited neurons **[3]** while MAPC6 reduced this response **[4]**.

3.4.1. LAB MEDIA: Fig 3C

3.4.2. LAB MEDIA: Fig 3D

3.4.3. LAB MEDIA: Fig 3E. *Video Editor: Please emphasize the BK column*

3.4.4. LAB MEDIA: Fig 3E. *Video Editor: Please emphasize the post-MAPC6 column*

3.5. Conversely, inhibitory neuron proportions dropped with bradykinin **[1]** but recovered after MAPC6 **[2]**.

3.5.1. LAB MEDIA: Fig 3F. *Video Editor: Please emphasize the BK column*

3.5.2. LAB MEDIA: Fig 3F. *Video Editor: Please emphasize the post-MAPC6 column*

3.6. Chan21a fired in sync with the ECG P wave at baseline **[1]**, clustered between P and Q waves after bradykinin **[2]**, and re-locked to the P wave following MAPC6 **[3]**.

3.6.1. LAB MEDIA: Fig 3A. *Video Editor: Please show the middle row corresponding to chan21a*

3.6.2. LAB MEDIA: Fig 3B. *Video Editor: Please show the middle row corresponding to chan21a*

3.6.3. LAB MEDIA: Fig 3D. *Video Editor: Please show the middle row corresponding to chan21a*