FINAL SCRIPT: APPROVED FOR FILMING

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Project Page Link: https://review.jove.com/account/file-uploader?src=21262818

Title: In Vivo Telemetry to Record Long-Term Cardiovascular Parameters, Temperature, and Activity in Spinal Cord Injury Rat Models

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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**

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

   No

   If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit.

   If your microscope does not have a camera port, the scope kit will be attached to one of the eyepieces and **you will have to perform the procedure using one eye**.

   Meijitechno 204780

   SCOPE: 2.5, 2.6, 2.7, 2.9 and 2.10
   Videographer: Please capture the shots labeled SCOPE with a SCOPE KIT

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. **Yes**

Current Protocol Length
Number of Steps: 22
Number of Shots: 45

Introduction

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

INTRODUCTION:

~~What is the scope of your research? What questions are you trying to answer?~~

1.1. **Sajeev Kaur:** The scope of my research is optimizing telemetric implant surgery to enable long-term recording of physiological parameters in spinal contused rats.

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

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

1.2. **Sajeev Kaur:** The most recent developments in our field include characterizing acute, chronic, and progressive cardiovascular, temperature, and activity changes following spinal cord injury.

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. **Samir Patel:** The advantage of our protocol is minimizing procedural challenges, improving survival, and enabling continuous recordings in awake, freely moving animals.

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. **Anna Baur:** Our findings will advance research by providing an in-depth understanding of autonomic changes during the acute and chronic phases of spinal cord injury.

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. **Dorottya Gal:** Our results pave the way for new questions by addressing key gaps and providing a foundation for deeper insight into autonomic dysfunction after spinal cord injury.

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.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

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

1.6. **Dr. Sajeev Kaur, Post-Doctoral Fellow, Spinal Cord & Brain Injury Research Center, University of Kentucky**: (authors will present their testimonial statements live)

1.6.1. 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.)

1.7. **Dr. Samir Patel, Assistant Professor, Spinal Cord & Brain Injury Research Center, Department of Physiology, University of Kentucky**: (authors will present their testimonial statements live)

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

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Kentucky

Protocol

2. Telemetric Implant Placement in the Descending Aorta of a Rat

Demonstrator: Sajeev Kaur, Anna Baur and Dorottya Gal

2.1. To begin, using a syringe, fill the pressure catheter tip of a telemetric implant with gel **[1]**. After sequentially sterilizing the implants in different agents, keep them submerged in 0.9% sodium chloride **[2-TXT]**.

2.1.1. WIDE: Talent using a syringe to fill the pressure catheter tip of the telemetric implant with gel.

2.1.2. Talent sequentially transferring the telemetric implant into containers with detergent, cold sterilant, and 0.9 percent sodium chloride. **TXT: Agents: Detergent, cold sterilant and 0.9% NaCl (Submerge 24 h each)**

2.2. Cover a heating pad with surgical underpads followed by sterile drape towels to maintain a sterile field **[1]**. Set the heating pad to 37 to 38 degrees Celsius **[2]**.

2.2.1. Talent covering the heating pad with surgical underpads and sterile drape towels.

2.2.2. Talent positioning a heating pad on the surgical table and adjusting the temperature settings.

2.3. Administer buprenorphine, meloxicam and 5 mL of0.9% normal saline subcutaneously **[1]**. Shave the abdominal area of an anesthetized rat from 1 centimeter above the urethral orifice to the mid abdomen using an electric shaver **[2-TXT]**. Clean the exposed skin three times with alcohol prep pads followed by povidone iodine application **[3]**. Then place a press and seal barrier and cut an opening to expose the surgical site **[4]**.

NOTE: VO and shot numbers edited to accommodate the added shot

Added shot: Administration of buprenorphine, meloxicam and 5 mL of 0.9% saline subcutaneously

2.3.1. Talent shaving the abdominal region of the rat. **TXT: Anesthesia: 3 - 4 % isoflurane inhalation; Apply lubricating gel to eyes**

2.3.2. Talent cleaning the skin with alcohol prep pads and applying povidone iodine.

2.3.3. Talent placing the press and seal barrier and exposing the surgical field.

2.4. Now make a 4.5 to 5-centimeter midline skin incision from above the urethral orifice and perform an abdominal incision **[1]**. Secure the visceral organs with saline soaked 16

ply gauze sponges and metal retractors **[2]**.

2.4.1. Talent making a midline abdominal incision.

2.4.2. Talent positioning gauze sponges and retractors to secure visceral organs.
AUTHOR'S NOTE: Please use 2nd video for this step as saline dripped all over during filming this step and there was minor cut on the side of actual incision. So Lorenzo re- recorded this step in another animal.

2.5. Using two fine forceps, gently separate fat bodies to expose the descending aorta and vena cava **[1-TXT]**.
Videographer: Please capture the shots labeled SCOPE with a SCOPE KIT

2.5.1. SCOPE: Talent using forceps to separate fat bodies. **TXT: Hold fat bodies with one forceps and dissect with the other**

2.6. Perform blunt dissection approximately 0.5 centimeters below the renal artery to separate the descending aorta from the vena cava **[1]**. Further separate the aorta from underlying fat to create space for angled forceps insertion **[2]**.

2.6.1. SCOPE: Talent performing blunt dissection near the renal artery landmark.

2.6.2. SCOPE: Close up of space created beneath the descending aorta.

2.7. Insert angled forceps under the descending aorta through the dissected space **[1]**. Tear the connective tissue between the aorta and vena cava using forceps **[2].** Then pass a sterile 4-0 *(four-Oh)* silk thread underneath the descending aorta and secure both ends with a hemostat **[3-TXT]**. Temporarily mono occlude the descending aorta proximal to the catheter insertion point **[4]**.
NOTE: VO and shot numbers edited to accommodate the added shot

2.7.1. SCOPE: Talent carefully sliding angled forceps beneath the descending aorta.

Added shot: Tearing of the connective tissue between aorta and vena cava

2.7.2. SCOPE: Talent threading silk suture beneath the aorta and clamping with a hemostat. **TXT: Have another experimenter hold the hemostat at 45° angle and adjust to prevent backflow**

2.7.3. Shot of descending aorta being mono occluded with silk thread.

2.8. Remove the implant from the sodium chloride and inspect the pressure catheter tip for air bubbles or gaps **[1]**. After turning on the implant using a magnet and radio, add gel if needed before insertion **[2]**.

2.8.1. Talent inspecting the catheter tip.

2.8.2. Talent adding gel to the catheter tip.

2.9. Pierce the upper wall of the descending aorta using a twisted 21-gauge needle **[1]** and

insert the pressure catheter tip 1.5 to 2 centimeters into the descending aorta **[2-TXT]**.

2.9.1. SCOPE: Shot of needle puncturing the aortic wall.

2.9.2. SCOPE: Shot of pressure catheter tip being inserted. **TXT: Use vein pick if needed; loosen occlusion thread to advance catheter**

2.10. Clean surrounding blood and apply tissue adhesive to secure the implant **[1]**, and check for blood leakage **[2]**. Cut and remove occlusion thread using fine scissors and forceps **[3-TXT].**

2.10.1. SCOPE: Talent cleaning the insertion site and applying tissue adhesive.

2.10.2. SCOPE: Talent checking site for blood leakage.

Added shot: 2.10.3:SCOPE: Cut and remove occlusion thread using fine scissors and forceps. **TXT: Remove retractor and gauze before suturing; confirm implant status via radio**

2.11. Anchor the implant body to the abdominal wall subcutaneously using non-absorbable sutures **[1]**. Then close the abdominal wall using absorbable sutures in an interrupted pattern and close the skin using an intradermal continuous technique **[2]**.

2.11.1. Talent suturing the implant body to the abdominal wall.

2.11.2. Talent suturing the abdominal wall and the skin with intradermal sutures.

2.12. Clean the incision with hydrogen peroxide followed by povidone iodine **[1]**. Apply triple antibiotic cream to the incision site and follow the post-surgical care **[2]**.

2.12.1. Talent cleaning the incision area.

2.12.2. Talent applying antibiotic cream.

AND

TEXT ON PLAIN BACKGROUND:

Post-surgical Care

1. Inject enrofloxacin (5 mg/kg) immediately post-surgery
2. Place rat on heating pad for recovery: 37 - 38 °C for at least 24 - 72 h
3. Fit e-collar around neck for 3 days
4. Subcutaneous injections: Buprenorphine and meloxicam: 2x daily for 3 days
   Enrofloxacin: 1x daily for 5 days

Provide food and water ad libitum

Video Editor: Please play both shots side by side in a split screen

2.13. Perform spinal cord injury 2 weeks later using IH *(I-H)* impactor device **[1].** Then transfer the injured rats onto telemetric plates in a cage for data acquisition **[2].**

2.13.1. Shot of the IH impactor device.

2.13.2. Shot of the SCI rat on the telemetric plates in a cage.

3. Telemetric Data Acquisition and Export Using Ponemah

Demonstrator: Sajeev Kaur

3.1. To begin data recording, launch the Ponemah software **[1].** Click **Create** under the **Experiment** tab and label the experiment **[2]**.

3.1.1. SCREEN: 69714_screenshot_1 00:00-00:11

3.1.2. SCREEN: 69714_screenshot_1. 00:12-00:30

3.2. Under the **Hardware** tab, click on **Edit APR** *(A-P-R)* **configuration,** then choose the **APR** and click on **Add** to move to the available region **[1]**. Now, press **Edit PhysioTel/HD MX2** *(M-X-Two)* **Configuration** in the hardware tab to select the **MX-2**, then click on **Add**, and confirm that it appears in the left panel **[2].**

3.2.1. SCREEN: 69714_screenshot_2. 00:04-00:13

3.2.2. SCREEN: 69714_screenshot_2. 00:17-00:25

3.3. Add implants assigned to different animals from the implant inventory on the right side to the middle and left panels **[1].** Click on each implant and connect it to a different receiver plate then save and exit once all connections are made **[2]**.

3.3.1. SCREEN: 69714_screenshot_3. 00:00-00:13

3.3.2. SCREEN: 69714_screenshot_3. 00:20-00:35

3.4. Now, navigate to the **Set-Up** tab then press **Experimental Setup** and click on **Enable Page [1]**. Choose the black background then select the **subject** before adding the **pressure** in the label and changing the unit to **mmHg** *(millimeters-of-mercury)* **[2].**

3.4.1. SCREEN: 69714_screenshot_4. 00:02-00:14

3.4.2. SCREEN: 69714_screenshot_4. 00:20-00:35

3.5. Go to the **Set-up** tab again and choose **Subject Setup**. Click on the **animal number**, select the **gender** and **species**, and click on **Pressure** to enable parameters **[1]**. If heart rate needs to be displayed, click on **Heart Rate [2]**. Click **Apply Channel Settings** to **Similar Channels** if connecting more than one implant. Then, click **OK [3]**.

3.5.1. SCREEN: 69714_screenshot_5 00:02-00:15

3.5.2. SCREEN: 69714_screenshot_5. 00:16-00:23

3.5.3. SCREEN: 69714_screenshot_5 00:33-00:43

3.6. Next, click on **All Continuous** at the top of the screen to visualize blood pressure recording traces **[1]**.

3.6.1. SCREEN: 69714_screenshot_6 00:05-00:30

3.7. For data exporting, go to the **Experiment** tab, click on **Open**, and select the data from the intended folder **[1]**.

3.7.1. SCREEN: 69714_screenshot_7 00:02-00:18

3.8. Navigate to the **Action** tab, click on **Start Review**, and select the subject number, desired signal types, and time range **[1]**. Set the logging rate to determine how the data are segmented in seconds, minutes, or hours **[2]**.

3.8.1. SCREEN: 69714_screenshot_8. 00:02-00:06, 00:12-00:20

3.8.2. SCREEN: 69714_screenshot_8 00:33-00:43

3.9. Once selections are complete, go to the **Experiment** tab and choose **Save Marked Sections**, followed by **Save Derived Data [1]**. Finally, go to the **Actions** tab and click on **Close Review Session** to end the data export **[2]**.

3.9.1. SCREEN: 69714_screenshot_9. 00:02-00:10, 00:25-00:35

3.9.2. SCREEN: 69714_screenshot_9. 00:46-01:01

Results

4. Results

4.1. Eight weeks after spinal cord injury, colorectal distension induced a minimum increase of 20 millimeters of mercury in systolic blood pressure during 1-minute stimulation compared with baseline, demonstrating development of autonomic dysreflexia **[1]**.

4.1.1. LAB MEDIA: USE THIS-LAB MEDIA FINAL.pptx *Video editor: Please use image in slide 1 (For reference see image 1D in Figure 1). Highlight the increase in the curve between 60 to-120 sec.*

4.2. Systolic blood pressure **[1],** Diastolic blood pressure **[2]** and mean arterial pressure were elevated 1 to 2 days post spinal cord injury compared with pre-injury recordings **[1]**.

4.2.1. LAB MEDIA: USE THIS-LAB MEDIA FINAL.pptx *Video editor: Please use image in slide 2 (For reference see image 1E (i-iii) in Figure 1). Show the corresponding graphs and Highlight the SBP 1-2 days, DBP 1- 2 days and MAP 1-2 days graphs*

4.3. Heart rate showed increased fluctuations following spinal cord injury compared with pre-injury recordings **[1]**. Core body temperature regulation was disrupted during the first two days after spinal cord injury **[2]**. and animal activity was reduced substantially following spinal cord injury compared with pre-injury activity **[3]**.

4.3.1. LAB MEDIA: USE THIS-LAB MEDIA FINAL.pptx *Video editor: Please use image in slide 2 (For reference see image 1E (iv) in Figure 1). Show the corresponding graphs and Highlight the HR 1-2-days graph*

4.3.2. LAB MEDIA: USE THIS-LAB MEDIA FINAL.pptx *Video editor: Please use image in slide 2 (For reference see image 1E (v) in Figure 1). Show the corresponding graphs and Highlight the Core body temp 1-2-days graph*.

4.3.3. LAB MEDIA: USE THIS-LAB MEDIA FINAL.pptx *Video editor: Please use image in slide 2 (For reference see image 1E (vi) in Figure 1). Show the corresponding graphs and Highlight the activity 1-2-days graph*

Pronunciation Guide:

 Telemetry
Pronunciation link: https://www.merriam-webster.com/dictionary/telemetry
IPA: /təˈlɛmɪtri/
Phonetic Spelling: tuh·lem·uh·tree
 Cardiovascular
Pronunciation link: https://www.merriam-webster.com/dictionary/cardiovascular
IPA: /ˌkɑrdi.oʊˈvæskjələr/
Phonetic Spelling: kahr·dee·oh·vas·kyuh·ler
 Spinal
Pronunciation link: https://www.merriam-webster.com/dictionary/spinal
IPA: /ˈspaɪnəl/
Phonetic Spelling: spy·nuhl
 Autonomic
Pronunciation link: https://www.merriam-webster.com/dictionary/autonomic
IPA: /ˌɔtəˈnɑmɪk/
Phonetic Spelling: aw·tuh·nom·ik
 Telemetric
Pronunciation link: https://www.merriam-webster.com/dictionary/telemetric
IPA: /ˌtɛləˈmɛtrɪk/
Phonetic Spelling: teh·luh·met·rik
 Contused
Pronunciation link: https://www.merriam-webster.com/dictionary/contused
IPA: /kənˈtjuːzd/
Phonetic Spelling: kuhn·tyoozd
 Buprenorphine
Pronunciation link: https://www.merriam-webster.com/dictionary/buprenorphine
IPA: /ˌbjuːprəˈnɔrfiːn/
Phonetic Spelling: byoo·pruh·nor·feen
 Meloxicam
Pronunciation link: https://www.merriam-webster.com/dictionary/meloxicam
IPA: /məˈlɑksɪˌkæm/
Phonetic Spelling: muh·lok·sih·kam
 Isoflurane
Pronunciation link: https://www.merriam-webster.com/dictionary/isoflurane
IPA: /ˌaɪsoʊˈflʊreɪn/
Phonetic Spelling: eye·soh·floor·ayn
 Povidone
Pronunciation link: https://www.merriam-webster.com/dictionary/povidone
IPA: /ˈpɑvɪˌdoʊn/
Phonetic Spelling: pah·vih·dohn

 Urethral
Pronunciation link: https://www.merriam-webster.com/dictionary/urethral
IPA: /jʊˈriːθrəl/
Phonetic Spelling: yuh·reeth·ruhl
 Visceral
Pronunciation link: https://www.merriam-webster.com/dictionary/visceral
IPA: /ˈvɪsərəl/
Phonetic Spelling: vih·suh·ruhl
 Descending
Pronunciation link: https://www.merriam-webster.com/dictionary/descending
IPA: /dɪˈsɛndɪŋ/
Phonetic Spelling: dih·sen·ding
 Aorta
Pronunciation link: https://www.merriam-webster.com/dictionary/aorta
IPA: /eɪˈɔrtə/
Phonetic Spelling: ay·or·tuh
 Vena cava
Pronunciation link: https://www.merriam-webster.com/dictionary/vena%20cava
IPA: /ˌviːnəˈkeɪvə/
Phonetic Spelling: vee·nuh·kay·vuh
 Renal
Pronunciation link: https://www.merriam-webster.com/dictionary/renal
IPA: /ˈriːnəl/
Phonetic Spelling: ree·nuhl
 Hemostat
Pronunciation link: https://www.merriam-webster.com/dictionary/hemostat
IPA: /ˈhiːməˌstæt/
Phonetic Spelling: hee·muh·stat
 Occlude
Pronunciation link: https://www.merriam-webster.com/dictionary/occlude
IPA: /əˈkluːd/
Phonetic Spelling: uh·klood
 Catheter
Pronunciation link: https://www.merriam-webster.com/dictionary/catheter
IPA: /ˈkæθɪtər/
Phonetic Spelling: kath·ih·ter
 Subcutaneously
Pronunciation link: https://www.merriam-webster.com/dictionary/subcutaneously
IPA: /ˌsʌbkjuˈteɪniəsli/
Phonetic Spelling: sub·kyoo·tay·nee·uhs·lee
 Enrofloxacin
Pronunciation link: https://www.merriam-webster.com/dictionary/enrofloxacin

IPA: /ˌɛnroʊˈflɑksəsɪn/
Phonetic Spelling: en·roh·flok·suh·sin
 Colorectal
Pronunciation link: https://www.merriam-webster.com/dictionary/colorectal
IPA: /ˌkoʊloʊˈrɛktəl/
Phonetic Spelling: koh·loh·rek·tuhl
 Distension
Pronunciation link: https://www.merriam-webster.com/dictionary/distension
IPA: /dɪˈstɛnʃən/
Phonetic Spelling: dih·sten·shuhn
 Systolic
Pronunciation link: https://www.merriam-webster.com/dictionary/systolic
IPA: /sɪˈstɑlɪk/
Phonetic Spelling: sis·tah·lik
 Diastolic
Pronunciation link: https://www.merriam-webster.com/dictionary/diastolic
IPA: /ˌdaɪəˈstɑlɪk/
Phonetic Spelling: dye·uh·stah·lik