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Title: Isolating and Imaging Live, Intact Pacemaker Regions of Mouse Renal Pelvis by Vibratome Sectioning

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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 (through a camera port or one of the oculars). Please list the make and model of your microscope.

   Nikon SMZ18

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

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 videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, 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? **No**

Current Protocol Length

Number of Steps: 17
Number of Shots: 38

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Nathan Grainger:** This protocol yields intact pacemaker regions of the renal pelvis, the smooth muscle organ within the kidney that pumps urine into the ureter and onto the bladder.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.4.*

1.2. **Nathan Grainger:** Unlike isolating cells from the renal pelvis, this approach provides intact, in situ environments of renal pelvis pacemaker regions and a more physiological preparation for studying renal pelvis pacemaking.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 5.1. and 5.2.*

OPTIONAL:

1.3. **Nathan Grainger:** An individual that has never performed this technique may struggle to cut uniform sections. A first time user must ensure the kidney is level on the vibratome stage and doesn't speed up the cutting process.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.5.2.*

Ethics Title Card

1.4. The protocols described in this study were approved by the Institutional Animal Care and Use Committee at the University of Nevada, Reno.

Protocol

2. Preparation and dissection of kidney and calibration of vibratome instrument

2.1. Using internal tissue forceps and internal dissection scissors, pinch the intestines and lift them away from the abdominal wall **[1]**. Simultaneously, cut the underside of the intestines free from the body at the proximal duodenum and distal colon to gain access to the retroperitoneal space containing the kidneys **[2]**.

2.1.1. Talent lifting the intestines using forceps and dissection scissors.

2.1.2. Talent cutting the underside of the intestine.

2.2. Gently pinch and lift the distal end of the ureter with tissue forceps **[2]**. Using the dissection scissors, cut underneath the pinched ureter towards the kidney until it has become liberated from the surrounding connective tissue **[3-TXT].** Once the kidneys are exposed, extract them individually [3].

2.2.1. Talent extracting the kidneys. **NOTE: Use as 2.2.3.**

2.2.2. Talent lifting the ureter with tissue forceps. **TEXT: ~4 mm away from the kidney**. **NOTE: Use as 2.2.1.**

2.2.3. Talent cutting under the ureter to liberate it from surrounding tissues. **NOTE: Use as 2.2.2.**

2.3. Fill a silicon elastomer-coated dish with ice-cold KRB solution **[2-TXT]**. Transfer the kidney to the dissection dish and ensure that is completely submerged **[1]**.

2.3.1. Talent transferring the kidney to dissection dish coated with silicon elastomer.- **NOTE: Use as 2.3.2.**

2.3.2. Talent filling the dish with KRB solution. **TEXT: KRB- Krebs-Ringer bicarbonate NOTE: Use as 2.3.1.**

2.4. Use fine spring scissors and internal forceps to remove adipose tissue from the base of the kidney to expose the distal renal pelvis, or RP, and proximal ureter **[1]**. Remove the proximal ureter and a portion of the distal RP from the base using fine spring scissors and forceps **[2]**.

2.4.1. SCOPE: 62040_Scope shots 2.4.1 and 2.4.2.avi 00:10-00:50

2.4.2. SCOPE: 62040_Scope shots 2.4.1 and 2.4.2.avi 00:55-01:25

2.5. Pierce the outer renal capsule with fine-tip forceps, angling the tips away from the kidney body **[1]**. Using forceps with each hand, pinch the loose ends of the capsule and peel them apart until the remaining renal capsule membrane is removed entirely **[2]**.

2.5.1. SCOPE: 62040_Scope shots 2.5.1 and 2.5.2.avi. 00:05-00:10

2.5.2. SCOPE: 62040_Scope shots 2.5.1 and 2.5.2.avi. 00:11-01:13. *Video Editor: Speedup the video and play.*

2.6. Insert a razor blade into the blade holder of the vibratome instrument **[1]** and adjust the blade clearance angle to approximately 18 degrees **[2]**.

2.6.1. Talent inserting the razor blade into the blade holder of vibratome instrument.

2.6.2. Talent adjusting the blade clearance angle.

2.7. Adjust the blade parameters as mentioned in the text manuscript, ensuring that kidney section thickness does not exceed 150 micrometers, which would negatively impact Calcium ion-imaging experiments **[1]**

2.7.1. Talent adjusting the parameters of the blade in the vibratome instrument.

3. Vibratome sectioning and kidney slice Ca^{2+} image acquisition

3.1. Use blunt-ended forceps to gently grasp and remove the prepared kidney from ice-cold KRB solution **[1]**. Immediately place the kidney on absorbent paper for approximately 2 to 4 seconds to remove excess external moisture **[2]**.

3.1.1. Talent removing the kidney from KRB solution using blunt-end forceps.

3.1.2. Talent placing the kidney on absorbent paper.

3.2. Gently roll the kidney across the absorbent paper to ensure that all sides of the parenchyma have dried so that there is optimal adhesion of the kidney to the vibratome stage **[1]**.

3.2.1. Talent rolling the placed kidney on the absorbent paper.

3.3. Immediately apply a thin layer of cyanoacrylate glue to the base of the vibratome specimen plate **[1]** and use blunt-ended forceps to place the kidney, ureter side down, on the area covered in glue **[2]**. *Videographer: This step is important!*

3.3.1. Talent applying cyanoacrylate glue to the base of the vibratome specimen plate.

3.3.2. Talent placing the kidney on the specimen plate using forceps.

3.4. Gently apply downward pressure to the top of the kidney with the flat edge of the forceps for approximately 10 to 20 seconds to dry the glue **[1]**. Firmly secure the specimen plate to the bottom of the buffer tray **[2]** and adjust the level of KRB solution so that the top of the kidney is fully immersed **[3]**. *Videographer: This step is important!*

3.4.1. Talent applying pressure on top of the kidney.

3.4.2. Talent placing the specimen plate to the bottom of the buffer tray.

3.4.3. Talent adding KRB solution to the specimen plate.

3.5. For automatic vibratome sectioning, select the start and end positions of the vibratome blade-cutting cycle 0.5 to 1 centimeter clear of the kidney to ensure that the entire kidney plane is getting sectioned **[1]**. Start the automatic cutting process, making sure that the blade makes contact with the kidney **[2]**.

3.5.1. Talent selecting the start and end positions of the vibratome blade cutting.

3.5.2. Talent starting the machine to initiate cutting process.

3.6. Using forceps, collect sections that are liberated from the kidney **[1]** and immediately transfer them to individual wells **[2]**. Continue the sectioning protocol and use a light microscope to identify PKJ regions in kidney slices until the PKJ regions become more apparent **[3-TXT]**. *Videographer: This step is important!*

3.6.1. Talent collecting the sections of the kidney using forceps.

3.6.2. Talent transferring the sections in individual wells.

3.6.3. SCOPE: 62040_Scope shot 3.6.3.avi. 00:10-00:39. **TEXT: PKJ-Pelvis-kidney junction**

4. Kidney slice Ca^{2+} image acquisition

4.1. Fill a silicon elastomer-coated imaging dish with ice-cold KRB solution **[2]**. Transfer an individual kidney slice to the dish **[1]**. Insert minutien pins around the periphery of a kidney slice to secure the section to the base of the imaging dish **[3]**.

4.1.1. Talent transferring the slices to silicon elastomer-coated imaging dish.-**NOTE: Use as 4.1.2.**

4.1.2. Talent filling the dish with KRB solution. **NOTE: Use as 4.1.1.**

4.1.3. SCOPE: 62040_Scope shot 4.1.3.avi. 00:05-00:27

4.2. Place the imaging dish on the stage of an upright spinning-disk confocal microscope **[1]** and immediately start perfusing with KRB solution **[2]**. Use a low magnification, water-immersion objective lens to locate the kidney slice **[3-TXT]**.

4.2.1. Talent placing the dish on the stage of confocal microscope.

4.2.2. Talent perfusing the dish with KRB solution.

4.2.3. SCREEN: 62040_screenshot_1.m4v. 00:05-00:14

4.3. Center the imaging field on areas of the slice where the PKJ is present using landmarks **[1]**. Once the PKJ is located, use a higher magnification water-immersion objective lens to magnify the area of interest **[2]**. *Videographer: This step is difficult!*

4.3.1. SCREEN: 62040_screenshot_2.m4v. 00:01-00:05

4.3.2. SCREEN: 62040_screenshot_3.m4v. 00:01-00:10

4.4. Distinguish different cells of interest and different types of Calcium ion transient durations in the PKJ wall **[1]**. Once cells of interest have been identified, adjust the laser intensity to yield a good signal-to-noise ratio **[2]** and record images at a temporal sampling frequency between 16 and 32 Hertz **[3]**. *Videographer: This step is difficult!*

4.4.1. SCREEN: 62040_screenshot_4.m4v. 00:01-00:22

4.4.2. SCREEN: 62040_screenshot_5.m4v. 00:01-00:12

4.4.3. SCREEN: Talent recording the images at temporal frequency. **NOTE: Not filmed**

Results

5. Imaging of spontaneous calcium ion transient in vibratome sections of whole kidneys

5.1. Light microscopic images of whole kidney sections show annotated landmarks of a single PKJ region **[1]**, multiple PKJ regions present close to the inner medulla **[2]**, and the distal kidney **[3]**.

5.1.1. LAB MEDIA: Figure 2C. *Video editor focus on the white dashed rectangle.*

5.1.2. LAB MEDIA: Figure 2B and 2D. *Video editor focus on the dashed rectangles labelled G and H in Figure 2D along with the labelled Figure 2B.*

5.1.3. LAB MEDIA: Figure 2E.

5.2. The locations of the PKJ **[1]**, renal arterioles **[2]**, and PKJ boundaries are shown here **[3]**.

5.2.1. LAB MEDIA: Figure 2F, 2G, and 2H. *Video editor focus on the black arrow heads.*

5.2.2. LAB MEDIA: Figure 2F and 2G. *Video editor focus on the white arrow heads.*

5.2.3. LAB MEDIA: Figure 2F, 2G, and 2H. *Video editor focus on the white dashed lines.*

5.3. A PKJ section from a mouse expressing GCaMP *(pronounce 'G-camp')* in PDGFRα^{+} *(pronounce 'P-D-G-F-R-alpha-positive')* cells is shown here **[1]**. The thin PKJ wall suspended between parenchymal tissue can be distinguished **[2]** using landmarks such as the renal arteriole **[3]**.

5.3.1. LAB MEDIA: Figure 3A and B. **TEXT: PDGFRα^{+}-platelet-derived growth factor receptor-alpha-positive**

5.3.2. LAB MEDIA: Figure 3B. *Video editor focus on the white asterisk.*

5.3.3. LAB MEDIA: Figure 3A. *Video editor focus on the white asterisk.*

5.4. The expression of GCaMP6f in this specific transgenic tissue is spread across the entire width of the PKJ, across both the muscle and adventitial layers **[1]**.

5.4.1. LAB MEDIA: Figure 3 C.

5.5. In PDGFRα GCaMP6f$^+$ (*pronounce 'G-camp six f-positive'*) kidney slices, a network of cells that typically extends over the width of the PKJ wall is fluorescent and displays oscillating Calcium ion transients of various durations and frequencies **[1]**. In SMC GCaMP3$^+$ (*pronounce 'G-camp three-positive')* kidney slices, a layer of GCaMP3$^+$ cells are present in the muscle layer **[2-TXT]**.

5.5.1. LAB MEDIA: Figure 3C.

5.5.2. LAB MEDIA: Figure 3D. **TEXT: SMC-Smooth muscle cells.** *Video editor focus on white/light grey cells close to white dashed rectangle.*

5.6. A spatiotemporal map shows that PDGFRα$^+$ cells located in the PKJ adventitia elicit long-duration, low-frequency Calcium ion transients **[1]**, whereas SMCs fired shorter duration Calcium ion transients more frequently **[2]**.

5.6.1. LAB MEDIA: Figure 3E. *Video editor play Video 1.avi in the inset to show the long duration of fluorescence.*

5.6.2. LAB MEDIA: Figure 3F. *Video editor play Video 2.avi in the inset to show the short duration of fluorescence.*

5.7. Similarly, an array of fluorescent calcium ion signals within and surrounding collecting ducts **[1]** and oscillating calcium ion transients in the SMCs were also observed **[2]**.

5.7.1. LAB MEDIA: Video 3.avi. *Video editor play the full video.*

5.7.2. LAB MEDIA: Video 4.avi. *Video editor play the full video.*

Conclusion

6. Conclusion Interview Statements

6.1. **Nathan Grainger:** When attempting this procedure, it is important to keep the kidney as level as possible during vibratome sectioning to ensure uniform kidney slices are liberated from the block.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.4*

6.2. **Nathan Grainger:** Following this procedure, other methods such as single cell isolation, immunohistochemistry and molecular studies can be performed to increase our understanding of pacemaker mechanisms in the renal pelvis.

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