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Title: Microelectrode Array Recording of Sinoatrial Node Firing Rate to Identify Intrinsic Cardiac Pacemaking Defects in Mice

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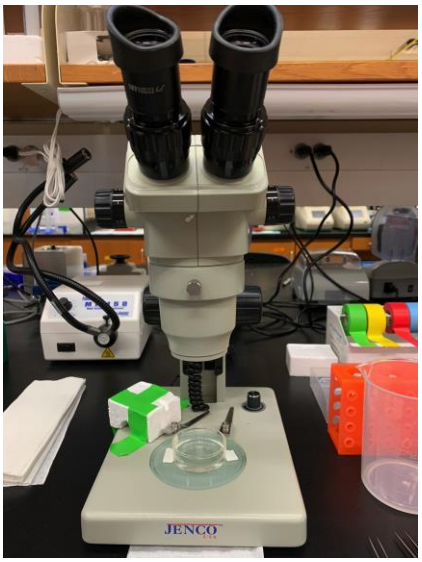
Author Questionnaire

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**.
   Please list the make and model of your microscope. **Inverted Microscope: Motic AE2000**

   Dissection Scope: Jenco



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

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 the 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: 21
Number of Shots: 53

Introduction

1. Introductory Interview Statements

Authors: When you decide who will deliver statements 1.1 and 1.2 during the shoot, remove the other author's name.

REQUIRED:

1.1. **Kelsey Paulhus/Edward Glasscock:** Measuring intrinsic heart rate is an important indicator of cardiac health. This technique accurately measures this rate while excluding the majority of confounding influences on the sinoatrial node.

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

1.2. **Kelsey Paulhus/Edward Glasscock:** MEA recording of intrinsic heart rate captures accurate firing rate data similar to single-cell recordings without extensive electrophysiology training.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 5.2.1*

OPTIONAL:

1.3. **Praveen Kumar:** Individuals using this technique may struggle to obtain healthy tissue preparations in early experiments. Practice the dissection and optimize the buffers and data collection settings before beginning real data collection.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.4.3*

Introduction of Demonstrator on Camera

1.4. **Edward Glasscock:** Demonstrating the procedure will be Drs. Man Si and Praveen Kumar, post-doctoral researchers in my laboratory.

1.4.1. INTERVIEW: Author saying the above.

1.4.2. The named demonstrator(s) looks up from the workbench or desk, or microscope and acknowledges the camera.

Ethics Title Card

1.5. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at the Southern Methodist University.

Protocol

NOTE: Videographer note: My only notes for the editor are that the scope segments were filmed in groups and not as single shots due to the nature of the procedure. As an example, 2.5.2 & 2.5.3 were filmed together etc. Otherwise, everything went rather smoothly.

2. Dissecting the sinoatrial node

2.1. Begin by holding the skin of the euthanized mouse with a hemostat **[1-TXT]** and use surgical scissors to make a transverse incision in the skin just beneath the bottom of the rib cage from the left costal arch to the right costal arch **[2]**.

2.1.1. WIDE: Talent holding the skin with a hemostat. **TEXT: See text for euthanasia details.**

2.1.2. Talent making an incision.

2.2. Use surgical scissors to cut open the peritoneum **[1]** and carefully separate the liver from the diaphragm without nicking the liver to prevent excessive bleeding **[2]**. Incise the diaphragm along the thorax to expose the thoracic cavity **[3]**.

2.2.1. Talent cutting the peritoneum.

2.2.2. Talent separating the liver.

2.2.3. Talent incising the diaphragm.

2.3. Use the surgical scissors to cut the lateral walls of the rib cage from the edges of the costal arches up to the clavicles to expose the heart **[1]**. Then, use a 23-gauge syringe needle to pin the rib cage over the shoulder **[2]**. Use a transfer pipette to drop warm heparinized complete Tyrode's solution on the heart to keep it moist **[3]**.

2.3.1. Talent cutting the rib cage.

2.3.2. Talent pinning the rib cage over the shoulder.

2.3.3. Talent dropping the heparinized complete Tyrode's solution onto the heart.

2.4. Hold the lungs with extra fine Graefe forceps and sever the trachea with surgical scissors to remove the lungs **[1]**. For removing the heart, hold the apex of the heart with extra fine Graefe forceps **[2]** and cut the aorta and inferior vena cava with surgical scissors **[3]**.

2.4.1. Talent cutting the trachea while holding the lung.

2.4.2. Talent holding the heart with extra fine Graefe forceps.

2.4.3. Talent cutting the aorta and inferior vena cava.

2.5. Transfer the heart to a Petri dish containing cured silicone elastomer **[1]** and use a transfer pipette for bathing the heart with 2 to 3 milliliters of warm heparinized complete Tyrode's solution **[2]**. Attach the apex of the heart to the dish with a dissection pin **[3]**.

2.5.1. Talent placing the dissected heart in a petri dish containing cured silicone elastomer.

2.5.2. Talent dropping warm heparinized Complete Tyrode's solution over the heart.

2.5.3. Talent pinning the apex of the heart with the dish.

2.6. Hold the inferior vena cava with Dumont-2 laminectomy forceps **[1]**, insert a 22-gauge syringe needle through the inferior and superior vena cava to locate their position in the right atrium, which also identifies the approximate position of the sinoatrial node **[2]**.

2.6.1. SCOPE: The inferior vena cava being held with Dumont-2 laminectomy forceps.

2.6.2. SCOPE: A 22-gauge syringe needle being inserted.

2.7. Hold the left atrial appendage with Dumont-2 laminectomy forceps **[1]**, put a dissection pin through the left atrial appendage to hold it in place, and repeat the same procedure for the right atrial appendage **[2]**. Then, remove the syringe needle that spans the venae cavae **[3]**.

2.7.1. SCOPE: The left atrial appendage being held with Dumont-2 laminectomy forceps.

2.7.2. SCOPE: The left atrial appendage being pinned to the plate.

2.7.3. SCOPE: The syringe needle being removed.

2.8. Use Castroviejo scissors to remove the apex of the heart by making a transverse incision across the ventricles for releasing the blood from the heart **[1]**. Then, wash the heart by adding warm heparinized complete Tyrode's solution **[2]**.

2.8.1. SCOPE: A transverse incision across the ventricles being made.

2.8.2. Talent adding warm heparinized complete Tyrode's solution over the heart.

2.9. Use Castroviejo scissors to cut along the atrioventricular septum until the atria are separated from the ventricles and keep the incision closer to the ventricle **[1]**. Cut along the interatrial septum to remove the left atrium **[2]**.

2.9.1. SCOPE: The atrioventricular septum being incised.

2.9.2. SCOPE: The interatrial septum being cut.

2.10. Place the dissection pins in the periphery of the right atrium to make it lay flat **[1]**. Remove any remaining fat, vessels, or tissue from the atrium using the Castroviejo scissors **[2]** and Locate the sinoatrial node in the right atrium **[3]**. *Videographer: This step is important!*

2.10.1. SCOPE: The dissection pins being placed in the periphery of the right atrium.

2.10.2. SCOPE: Remaining tissue being removed.

2.10.3. SCOPE: The sinoatrial node being located.

3. Microelectrode array System Preparation for Recording

3.1. Add Tyrode's solution to the input solution bottle **[1]** and turn on the flow of carbogen gas to oxygenate the Tyrode's solution **[2]**. Set the peristaltic pump to 25 rpm, which gives a flow rate of 2 milliliters per minute **[3]**.

3.1.1. Talent adding Tyrode's solution in the input solution bottle.

3.1.2. Talent turning the knob of the gas cylinder.

3.1.3. Talent adjusting the pump rpm.

3.2. After starting the pump, ensure that the buffer is not leaking from the system **[1],** and set the temperature controller to 37 degrees Celsius **[2]**.

3.2.1. Talent starting the pump.

3.2.2. Talent setting the temperature at 37 ˚C.

4. Heart Tissue Placement on Microelectrode array Grid

4.1. Use a paintbrush to transfer the dissected tissue from the dissecting Petri dish onto the microelectrode array grid **[1]**. Gently position the tissue with a soft paintbrush to overlay the sinoatrial node region to the electrode grid **[2]**. Then, place the mesh over the tissue using the bone forceps or any curved forceps **[3]**.

4.1.1. Talent transferring the tissue from the plate to the grid.

4.1.2. SCOPE: The position of tissue being adjusted over the grid. *Videographer: This shot is important!*

4.1.3. SCOPE: The mesh being placed over the tissue.

4.2. Using the bone forceps, position the harp anchor on the mesh to hold everything in place **[1]**. Arrange the microelectrode array dish on the connector plate **[2]**. Carefully place the perfusion cap on the microelectrode array dish without disturbing the harp slice anchor **[3]** and secure the perfusion cap with tape **[4]**.

4.2.1. SCOPE: The harp anchor being place on the mesh.

4.2.2. Talent placing the MEA dish on the connector plate.

4.2.3. Talent placing the perfusion cap on the MEA dish.

4.2.4. Talent securing the perfusion cap with tape.

5. Data Acquisition

5.1. Turn on the amplifier **[1]** and set up a workflow for the recording in the software **[2]**. Select **Beat_recording.moflo** *(Beat recording dot mow-flow)* template, open it, and set the number of traces, trace duration, trace interval, input voltage, sampling rate, and other recording parameters according to the desired recording conditions **[3]**.

5.1.1. Talent turning on the amplifier.

5.1.2. LAB MEDIA: 62735_Screenshot_1: 00:00 to 00:21 *Video editor: Please speed up the video.*

5.1.3. LAB MEDIA: 62735_Screenshot_1: 00:24 to 00:54, 01:21 to 01:36, and 01:42 to 01:46 *Video editor: Please speed up the video.*

5.2. Click the **Record and Play** button to start the recording and acquire data for 10 traces of 1-minute duration with 2 minutes of intervals between the traces **[1]**.

5.2.1. LAB MEDIA: 62735_Screenshot_2: 00:20 to 00:22, 00:53 to 00:57,and 01:09 to 01:15 *Video editor: Please speed up the video.*

5.3. Pause the pump **[1]** and switch the pump inflow tubing from the normal recording solution to Tyrode's solution containing the desired drug of choice **[2]**. Restart the pump **[3]** and resume the recording **[4]**.

5.3.1. Talent pressing pause button on pump controller.

5.3.2. Talent changing the tubing.

5.3.3. Talent pressing start button on pump controller.

5.3.4. LAB MEDIA: 62735_Screenshot_2: 05:46 to 05:49.

5.4. Once the drug-infused Tyrode's solution has reached the tissue **[1]**, record 10 traces in the same manner as done previously for the baseline recordings **[2]**. Take a final picture of the positioning of the tissue on the microelectrode array **[3]**.

5.4.1. The solution is reaching the tissue.

5.4.2. LAB MEDIA: 62735_Screenshot_2: 05:49 to 05:55.

5.4.3. Talent at the microscope, capturing the image.

6. Analysis of Microelectrode Array Recordings for Sinoatrial Node Beat Frequency Measurement

6.1. Open the saved recorded data file in the beat frequency analysis template of the analysis software. Click on the **Play** and allow the entire recording to run for data visualization and then assign appropriate analysis parameters **[1]**.

6.1.1. LAB MEDIA: 62735_Screenshot_3: 00:26 to 00:37 and 00:42 to 00:53. *Video editor: Please speed up the video.*

6.2. Select the channels to be included in the analysis and set the desired amplitude maxima or amplitude minima threshold values for automated waveform peak identification. Click on the **Play** icon again to rerun the data set and confirm that the analysis parameters are appropriate for spike extraction **[1]**.

6.2.1. LAB MEDIA: 62735_Screenshot_3: 00:56 to 01:01, 01:07 to 01:17, and 01:33 to 01:39.

6.3. For analysis, identify the three most stable consecutive traces that exhibit a stable beating rate for each trace across most channels during the baseline period of the experiment and another three consecutive stable traces during the drug exposure period **[1]**. Click the **Play and Record** icon to start the analysis **[2]**.

6.3.1. LAB MEDIA: 62735_Screenshot_3: 01:57 to 02:03 and 02:38 to 02:51.

6.3.2. LAB MEDIA: 62735_Screenshot_3: 03:34 to 03:59. *Video editor: Please speed up the video.*

Results

7. Results: The Sinoatrial Node Beat Frequency Measurement

7.1. The data was collected from a 45-day old male wildtype Black Swiss mouse for the sinoatrial node beat frequency measurement. The waveforms with different shapes and amplitudes were observed in different channels, and all the channels showed identical interspike intervals and firing frequencies **[1]**.

7.1.1. LAB MEDIA: Figure 8

7.2. However, the degree of tissue contact with the electrode may also influence the waveform characteristics, such as amplitude **[1]**.

7.2.1. LAB MEDIA: Figure 8 *Video editor: Please emphasize the red box.*

7.3. From the 10 recorded traces **[1]**, the three consecutive channels with stable beat frequency **[2]** and interspike interval were chosen for further analysis **[3]**.

7.3.1. LAB MEDIA: Figure 10 A

7.3.2. LAB MEDIA: Figure 10 A *Video editor: Please emphasize the top panel in the screenshot image.*

7.3.3. LAB MEDIA: Figure 10 A *Video editor: Please emphasize the middle panel in the screenshot image.*

7.4. The bad extracted spike patterns **[1]** should be absent **[2]** but, if present, are either influenced by noise **[3]** or unstable **[4]**. The waveforms that correspond to individual heartbeats reflect intrinsic cardiac pacemaking activity **[5]**.

7.4.1. LAB MEDIA: Figure 11

7.4.2. LAB MEDIA: Figure 11 A

7.4.3. LAB MEDIA: Figure 11 B

7.4.4. LAB MEDIA: Figure 11 C

7.4.5. LAB MEDIA: Figure 11 D

7.5. The microelectrode array system allows easy application of drug agents to analyze the pharmacological effects **[1]**. The intrinsic firing rate of selected three traces across all 64 channels was found to be approximately 320 beats per minute in the sample data **[2]**.

7.5.1. LAB MEDIA: Figure 12

7.5.2. LAB MEDIA: Figure 12 A

7.6. The introduction of 4-aminopyridine increased the interspike intervals as expected, which decreased the beat frequency from 320 to 210 beats per minute **[1]**.

7.6.1. LAB MEDIA: Figure 12 B

Conclusion

8. Conclusion Interview Statements

8.1. **Praveen Kumar:** It is important to confirm that the tissue is healthy during the recording by verifying that the traces are stable and meet the standard criteria to collect reliable data for analysis.

8.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 6.2.1 and 6.3.1*

8.2. **Kelsey Paulhus:** MEAs can be used to record cardiac activity in other regions of the heart, allowing detailed, region-specific characterization that is amenable to study the effects of genetic and pharmacological manipulation.

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