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Title: Noninvasive Electrocardiography in the Perinatal Mouse

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Author Questionnaire

1. Microscopy: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **N**

3. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Lindsey A. Fitzsimons**: This unique protocol allows the assessment of neonatal mouse cardiovascular function using electrocardiography technology immediately after birth in a non-invasive manner without the use of anesthetics **[1]**.

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

REQUIRED:

1.2. **Lindsey A. Fitzsimons**: Assessing murine cardiovascular function in the early postnatal period will expand our understanding of the neonatal heart in a manner that could directly correlate with human clinical data **[1]**.

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

Introduction of Demonstrator on Camera

~~1.3. **Lindsey A. Fitzsimons**: Demonstrating the procedure will be Victoria Brewer, a student researcher in the laboratory of Kerry L. Tucker **[1][2]**.~~

~~1.3.1. INTERVIEW: Author saying the above~~
~~1.3.2. Named demonstrator(s) looks up from workbench or desk or microscope and acknowledges camera~~

Ethics Title Card

1.4. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at the University of New England.

Protocol

2. Device and Animal Preparation

2.1. To prepare the measuring device for the analysis, plug the device into the USB port of a computer **[1]**. The measuring device will automatically begin heating up to 37 degrees Celsius **[2]**.

2.1.1. WIDE: Talent plugging in device

2.1.2. Shot of temperature readout on device or **other appropriate shot** *Videographer/Video Editor: can skip if no visual* NOTE: The device does not have a temperature readout function. Shot 2.1.2 alternative was a video of the demonstrator (LF) using fingers to touch the surface of the device to ensure that it is "warm to the touch."

2.2. After about 15 minutes, remove a mouse pup from its home cage **[1]** and wipe the thorax with 70% ethanol **[2]**.

2.2.1. Talent selecting pup

2.2.2. Thorax being wiped

2.3. Place the pup on the heated surface of the plastic **[1]** and allow the animal to acclimate to the surface for approximately 2-5 minutes in the dark **[2]**.

2.3.1. Talent placing pup onto device
2.3.2. Talent setting timer, with mouse on device visible in frame

3. Mouse and Electrode Platform Setup

3.1. While the mouse is acclimating, collect a small droplet of adhesive electrical conducting gel **[1]** and gently press the droplet down onto the top of each of the four, flattened electrode surfaces **[2]**, carefully pulling the conducting gel away at an oblique angle from the center of the electrode construct after contact **[3]**.

3.1.1. WIDE: Talent collecting droplet *Videographer: Important step*
3.1.2. Droplet being pressed onto electrode NOTE: This and next shot combined *Videographer: Important step*
3.1.3. Gel being pulled form center of construct *Videographer: Important/difficult step*

3.2. When each electrode is completely covered with gel **[1]**, place the neonatal mouse pup onto the platform in the prone position with the head of the pup facing the outgoing USB edge of the platform **[2]** with each electrode covering a portion of the pup's upper thorax **[3-TXT]**.

3.2.1. Shot of electrode completely covered with gel *Videographer: Important step*
3.2.2. Pup being placed onto platform *Videographer: Important step*
3.2.3. Shot of chest covered with electrode(s) *Videographer: Important/difficult step*
TEXT: Active pups may require multiple placement attempts NOTE: 3.2.1-3.5.1 were combined into a single shot for convenience. the mouse actually lies on top of the electrodes which are anchored to the platform; thus, the shot depicts the mouse on top of the platform from the top as well as from the front to describe this visually from different angles

3.3. Gently restrain the forearms while simultaneously applying gentle pressure to the electrodes for approximately 1 minute **[1]**.

3.3.1. Forearms being restrained

3.4. When the conducting gel is set, secure the pup on the right and left sides with rubber silicone bumpers **[1]**.

3.4.1. Talent placing bumper(s) *Videographer: Important step*

3.5. Monitor the mouse for a moment, adjusting the bumper placement as needed **[1-TXT]**.

3.5.1. Shot of mouse between bumpers, then bumper being adjusted **TEXT: Caution: Do not compress pup too tightly**

3.6. When the pup is secure, apply any remaining conducting gel to the grounding tail electrode **[1]** and use gentle pressure to place the electrode on the rump of the pup to allow the gel to set before releasing the pup **[2]**.

3.6.1. Gel being applied to electrode NOTE: 3.6.1-3.7.1 were combined to accommodate the animal movement
3.6.2. Electrode being pressed onto rump

3.7. Carefully place the final silicon bumper on top of the rump to hold the grounding electrode in place **[1]** and gently place the entire platform into the Faraday cage **[2]**.

3.7.1. Bumper being placed *Videographer: Important step*

3.7.2. Platform being placed into cage. NOTE: Combined with 3.8.1 and 3.8.2 *Videographer: Important step*

3.8. Then check that the pup is not moving excessively **[1]** and that the body and head of the mouse appear secure **[2]**.

3.8.1. Shot of pup between bumpers *Videographer: Important step*
3.8.2. Shot of head *Videographer: Important step*

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
3.1., 3.2., 3.4., 3.7., 3.8.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
3.1., 3.2. Try to keep a small but sufficient amount of electrode gel on each electrode. Always take the time to ensure each electrode is sufficiently covered and there are no interfering strands. Try to place the mouse so that the upper part of the thorax is on the electrodes. This can be difficult depending on the activity level of the mouse and may often take several attempts.

Results

4. Results: Representative Electrocardiogram (ECG) Reads and Analyses

4.1. An ideal ECG (E-C-G) will have a clear, prominent signal that allows all of the waves to be analyzed in several different time frames **[1]**.

4.1.1. LAB MEDIA: 61074_screenrecording1. Good quality ECG recording with cardiac cycle waveforms occurring at regular intervals with minimal interference; recorded using LabScribe software (iWorx Systems Inc., Dover, NH USA). 00:00 – 00:16

4.2. A poor-quality reading has no discernable beats, shows clear interference **[1]**, and exhibits waves or inconsistencies across the reading **[2]**.

4.2.1. LAB MEDIA: 61074_screenrecording2. Poor quality ECG recording; discernable cardiac cycle waveforms are absent and there is pronounced electrical interference; recorded using LabScribe software (iWorx Systems Inc., Dover, NH USA). 00:00 –00:17

4.2.2. LAB MEDIA: Figure 2 *Video Editor: please emphasize green data line*

4.3. The program provides analysis of the key aspects of the ECG reading **[1]**, including the heart rate **[2]**, R-R intervals **[3]**, QRS complex interval **[4]**, QT interval **[5]**, and PR interval **[6]**.

4.3.1. LAB MEDIA: Table 1

4.3.2. LAB MEDIA: Table 1 *Video Editor: please emphasize Heart Rate column*

4.3.3. LAB MEDIA: Table 1 *Video Editor: please emphasize R-R Interval column*

4.3.4. LAB MEDIA: Table 1 *Video Editor: please emphasize QRS Duration column*

4.3.5. LAB MEDIA: Table 1 *Video Editor: please emphasize QT Duration column*

4.3.6. LAB MEDIA: Table 1 *Video Editor: please emphasize PR Duration column*

4.4. Using these data to establish a set of normative values, in this representative analysis **[1]**, it was determined that pups analyzed in the first postnatal day had an average heart rate of 357.2 beats per minute **[2]**.

4.4.1. LAB MEDIA: Table 1

4.4.2. LAB MEDIA: Table 1 *Video Editor: please emphasize P1 row from Pup Age to Heart rate*

4.5. For a pup in the first postnatal day, the average R-R, PR, QRS, and QT intervals were 169.1, 16.9, 45.4, and 36.3 milliseconds, respectively **[1]**.

4.5.1. LAB MEDIA: Table 1 *Video Editor: please emphasize P1 data cell and R-R, PR, QRS, and QT P1 data*

4.6. Importantly, the setup can be used to analyze ECG patterns from neonatal mice suffering from congenital heart defects **[1]**.

4.6.1. LAB MEDIA: Supplemental Figure 2 *Video Editor: please emphasize CHD MUT ECGs*

Conclusion

5. Conclusion Interview Statements

5.1. **Lindsey Fitzsimons**: The device is effective and reliable but also highly sensitive to surrounding electrical interference. Be sure to always remove any unnecessary equipment, including laptop chargers, and smart devices prior to recording **[1]**.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (2.1.)

5.2. **Lindsey Fitzsimons**: Additional possible measurements include respiration and Lead III, which is calculated from Leads I-II. Additional variables could allow for further overlap with other physiological symptoms and/or specific cardiac arrhythmias **[1]**.

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