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Title: A Behavioral Screen for Heat-Induced Seizures in Mouse Models of Epilepsy

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Author Questionnaire

1. **Microscopy**: Does your protocol demonstrate the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **NO**

2. **Software:** Does the part of your protocol being filmed demonstrate software usage? **NO**

Protocol Length
Number of Steps: **18**
Number of Shots: **38**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Martin Smith**: GEFS+ mutations cause febrile seizures in patients. The ability to study hyperthermia-induced seizures in mice with GEFS+ mutations can help define the relationship between specific mutations and seizure properties **[1]**.

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

1.2. **Antara Das**: The temperature gradient in the heating box is set via the digital temperature controller in a reproducible manner. Thus, the mouse body temperature can be increased at a slow, steady rate **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Suggested B-roll: 3.3.1 for 'the digital temperature controller'*

OPTIONAL:

1.3. **Martin Smith**: This protocol can be used to identify potential therapies, such as drugs or dietary restrictions, that reduce or eliminate febrile seizures. **[1]**.

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

1.4. **Antara Das**: To insert the rectal temperature probe into the mouse without injury, we recommend briefly anesthetizing the mouse and lubricating the tip of the rectal probe **[1]**.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.4.2 for 'lubricate the tip'*

Ethics Title Card

1.5. All animal procedures were performed in accordance with the guidelines of Institutional Animal Care and Use Committee (IACUC) at University of California, Irvine.

Protocol

2. Preparation for the Heat-Induced Seizure Assay

2.1. Start by switching on the mouse heat chamber **[1]** with the **Power On** button and then press the **Heat On** button **[2]**. Using the keypad on the digital temperature controller, set the temperature of the heat chamber to 50 degrees Celsius **[3]**.

2.1.1. WIDE: Talent at the lab bench; the heat chamber in view.

2.1.2. Talent pressing the **Power On** and the **Heat On** button.

2.1.3. Talent setting the temperature of the heat chamber.

2.2. Line the floor of the heat chamber with cob bedding **[1]**. Mount a video recording camera in front of the heat chamber **[2]**. Line a 140-millimeter diameter Petri dish with thick layers of tissue paper **[3]** and place it on ice to serve as a cooling pad **[4]**.

2.2.1. Talent adding cob bedding in the heat chamber.

2.2.2. Talent mounting a camera in front of the heat chamber.

2.2.3. Talent lining a Petri dish with tissue paper.

2.2.4. Talent placing Petri dish on the ice.

2.3. For the screening assay, ensure the body weight of the mouse is 15 grams or more **[1]**.

2.3.1. Talent recording the body weight of the mouse; the mouse on the weighing balance.

2.4. Before starting the procedure, confirm that the mouse is completely anesthetized with a toe pinch **[1-TXT]**. Coat the metal tip of the rectal temperature probe with a lubricant **[2]** and gently insert it into the mouse **[3]**. Secure the rectal probe to the tail of the mouse with tape **[4]**.

2.4.1. Talent pinching the mouse. **TEXT: Anesthesia: a few drops of isoflurane in a bell jar for 10–15 s**

2.4.2. Talent applying lubricant to the metal tip of the rectal temperature probe.

2.4.3. Talent inserting the probe into the mouse.

2.4.4. Talent securing the probe with a tape.

2.5. Place the mouse in a new recovery cage lined with cob bedding **[1]**.

2.5.1. Talent placing the mouse in the recovery cage.

2.6. Start a timer **[1]** and observe the mouse for 5 minutes while monitoring the core body temperature **[2]** until the mouse completely recovers from anesthesia **[3]** and the temperature stabilizes at 35 to 36 degrees Celsius **[4]**.

2.6.1. Talent starting the timer.

2.6.2. Talent monitoring the core body temperature of the mouse.

2.6.3. The mouse moving in the cage.

2.6.4. Body temperature of the mouse at 35–36 ˚C

2.7. At the end of 5 minutes, note the body temperature of the mouse as the initial body temperature at zero minute **[1]**.

2.7.1. Talent recording the body temperature of the mouse on the sheet.

2.8. Transfer the mouse quickly to the floor of the preheated mouse heat chamber to START the experiment trial **[1]**.

2.8.1. Talent transferring the mouse into the preheated mouse heat chamber.

3. Heat-induced Seizure Assay

3.1. After the mouse is placed into the preheated mouse chamber, start the camera **[1]** and the stopwatch **[2]**.

3.1.1. WIDE: Talent turning on the camera. Videographer NOTE: Do not use tk1

3.1.2. Talent starting the stopwatch.

3.2. Increase the temperature of the heat chamber at regular intervals such that the body temperature of the mouse increases at a rate of 0.25 to 0.5 degrees Celsius per minute **[1]**. Begin recording the body temperature of the mouse at 1-minute intervals for the duration of the experiment **[2]**.

3.2.1. Talent increasing the temperature of the mouse heat chamber.

3.2.2. Talent recording the body temperature of the mouse on a worksheet.

3.3. At 9.5 minutes, set the temperature of the heat chamber to 55 degrees Celsius **[1]**, to stabilize the temperature of the heat chamber to 55 degrees Celsius by the tenth minute **[2]**.

3.3.1. Talent setting the temperature of the heat chamber to 55 ˚C.

3.3.2. The temperature of the heat chamber stabilized at 55 ˚C. Videographer's NOTE: shot begins at 90 seconds

3.4. Similarly, achieve a stable temperature of the heat chamber to 60 degrees Celsius by the twentieth minute. Each seizure screening trial lasts for 30 minutes **[1]**.

3.4.1. The temperature of the heat chamber stabilized at 60 ˚C.

3.5. If the mouse experiences a seizure, record the body temperature of the mouse during the seizure as a seizure threshold temperature **[1]**. Take note of the seizure behavior characteristics displayed by the mouse **[2-TXT]**. Then, quickly pick up the mouse from the chamber and place it on the cooling pad **[3]**.

3.5.1. Talent recording the seizure threshold temperature.

3.5.2. The mouse experiencing a seizure. **TEXT: Refer to TEXT** Videographer NOTE: Do not use tk1 or tk2

3.5.3. Talent picking up the mouse and placing it on the cooling pad.

3.6. If a mouse does not experience heat-induced seizures within the 30-minute observation period **[1]** or the body temperature of the mouse reaches 44 degrees Celsius, place the mouse on the cooling pad **[2]**.

3.6.1. Mouse in the heating chamber.

3.6.2. Talent placing the mouse on the cooling pad.

3.7. When body temperature of the mouse comes down to 36 to 37 degrees Celsius, transfer the mouse to a recovery cage **[1-TXT]**.

3.7.1. Talent placing the mouse in a recovery cage. **TEXT: Only one mouse in a recovery cage at a time**

3.8. To remove the rectal probe from the mouse, carefully cut the tape between the mouse tail and rectal probe wire **[1]**. Wipe and clean the metal tip of the rectal probe with 70% alcohol **[2]**.

3.8.1. Talent cutting the tape and removing the probe.

3.8.2. Talent cleaning the metal tip of the rectal probe with 70% alcohol.

3.9. Observe the mouse until it recovers before returning it to the home cage. Keep monitoring the status of the mouse **[1]**. Mark the END of the experiment trial **[2]**.

3.9.1. Talent returning the mouse to its home cage.

3.9.2. The mouse in the home cage/ Talent viewing data on the spreadsheet. Videographer NOTE: Can also use 3.9.1 tk3

3.10. Reset the temperature of the mouse heat chamber to 50 degrees Celsius and allow it to equilibrate before the next assay **[1]**.

3.10.1. The temperature at 50 ˚C on the digital temperature controller.

Results

4. Results: Screening for Occurrence of Heat-induced Seizures in Mice with the Different Genetic Backgrounds

4.1. In the heat seizure assay, the mean body temperature of the mice was recorded every minute using the heating protocol **[1],** and the rate of the body temperature change over time was evaluated **[2]**. There was no difference in the rate of change of body temperature between 129X1 *(1-2-9-X-1)* heterozygous mutant mice and B6NJ *(B-6-N-J)* wild-type littermates **[3]**.

4.1.1. LAB MEDIA: Figure 2 A.

4.1.2. LAB MEDIA: Figure 2 B, C.

4.1.3. LAB MEDIA: Figure 2 B, C. *Video Editor: Emphasize Scn1a$^{KT/+}$ and data line with orange circles in both graphs*

4.2. Different species of mice displayed different behavior when exposed to the periodic increase in body temperature **[1]**. All heterozygous mutant mice from 129X1 or B6NJ genetic backgrounds exhibited heat-induced seizures **[2]**.

4.2.1. LAB MEDIA: Figure 2 D.

4.2.2. LAB MEDIA: Figure 2 D. *Video Editor: Emphasize orange bars*

4.3. None of the wild-type mice in the 129X1 enriched background experienced seizures **[1],** while only a third of the mice from the seizure-sensitive B6NJ background showed seizures **[2]**.

4.3.1. LAB MEDIA: Figure 2 D. *Video Editor: Emphasize Scn1a$^{+/+}$ bar from 129X1 columns*

4.3.2. LAB MEDIA: Figure 2 D. *Video Editor: Emphasize Scn1a$^{+/+}$ black bar from B6NJ columns*

4.4. A mean seizure threshold temperature of 129X1 mutant mice **[1]** was not significantly different from the mean seizure threshold temperature seen in B6NJ mice **[2]**. In contrast, the average seizure threshold temperature differed among the species of similar genetic backgrounds **[3]**.

4.4.1. LAB MEDIA: Figure 2 E. *Video Editor: Emphasize Scn1a$^{KT/+}$ from 129X1 column*

4.4.2. LAB MEDIA: Figure 2 E. *Video Editor: Emphasize Scn1a$^{KT/+}$ from B6NJ column*

4.4.3. LAB MEDIA: Figure 2 E. *Video Editor: Sequentially emphasize both bars from 129X1 and B6NJ*

4.5. The severity of the seizures was measured using the modified Racine scale **[1]**. The maximum Racine score of heterozygous mutant mice in 129X1 enriched background **[2]** was not significantly different from heterozygous mutant mice in B6NJ genetic background **[3]**.

4.5.1. LAB MEDIA: Table 1, Figure 2 F.

4.5.2. LAB MEDIA: Figure 2 F. *Video Editor: Emphasize 129X1 column*

4.5.3. LAB MEDIA: Figure 2 F. *Video Editor: Emphasize B6NJ column*

Conclusion

5. Conclusion Interview Statements

5.1. **Antara Das**: While setting the heating protocol, please ensure that the body temperature of the mouse does not increase at a rate faster than 0.25 ^{o}C/min, or it can be harmful to the health of the animal **[1]**.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggetsed B-roll: 3.2.1 for 'increase at a rate'*

5.2. **Martin Smith**: It would be very cool to combine the heating protocol with simultaneous EEG recording. That allows us to identify patterns of brain activity with febrile seizure behavior **[1]**.

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