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Project Page Link: https://review.jove.com/account/file-uploader?src=20871503

Title: Seizure Activity Induced by Electroshock in *Drosophila* Larvae

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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?

   YES

   SCOPE shots: 3.5.2, 3.5.3, 3.7.2, 3.8.1.

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? **Yes**

   If **Yes**, how far apart are the locations? Within same building (50 metres)

Current Protocol Length
Number of Steps: 22
Number of Shots: 41 (6 SC, 4 SCOPE)

Introduction

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

REQUIRED:

1.1. **Richard Baines:** We use the larvae of the fruit fly *Drosophila* to identify novel treatments for epilepsy. Just like humans, flies can carry genetic mutations that make them prone to seizures and we can reduce seizure severity in flies with clinically-used antiseizure medications.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.1.2*

What are the current experimental challenges?

1.2. **Richard Baines:** The main challenges are to fully understand the workings of the fly nervous system such that we can map areas to their equivalent areas in the human brain. Author's NOTE: Richard Baines delivered this statement.

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*

What research gap are you addressing with your protocol?

1.3. **Jack Corke:** About 1% of the population have epilepsy and of that number, about one third do not respond well to the antiseizure drugs currently used. We are exploring new ways to treat epilepsy. Author's NOTE: Jack Corke delivered this statement.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.8.1* Author's NOTE: Incorrectly labelled as 1.4.1 in the shoot. Videographer noted.

What advantage does your protocol offer compared to other techniques?

1.4. **Jack Corke:** The use of fly larvae rather than adult flies is advantageous because larvae live in the food that they eat. This makes adding experimental drugs very simple. The technique we use to induce seizures is also simple but effective.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.10.1* Author's NOTE: Also labelled correctly as 1.4.1 in shoot.

What research questions will your laboratory focus on in the future?

1.5. **Richard Baines:** We have identified some candidate drugs that have potent anti-seizure activity. We will now focus on understanding how these drugs work and testing that they are safe to use in mammals.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 5.1.1*

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

Protocol

NOTE: The timestamps for the SCOPE shots were added at the postshoot stage. Please contact Debopriya Sadhukhan for queries related to **SCOPE shots**.

2. Larval Preparation, Probe Calibration, and Electroshock Procedure

Demonstrator: Jack Corke

2.1. To begin, construct the elctroschock probe for the procedure **[1]**. Ensure that stocks of approximately 100 wandering third instar larvae from both a wild-type control strain and a seizure mutant strain are available **[2]**.

2.1.1. Talent checking and labeling vials containing wild-type and seizure mutant larvae.

2.2. Remove a single wandering third instar larva from the vial wall **[1]** and place it into a small plastic Petri dish **[2-TXT]**. Using a small paintbrush, gently wash the larva with double-distilled water to remove any food residue **[3]**.

2.2.1. Talent removing a wandering larva from the side of a vial. Author's NOTE: 2.2.1 combined with 2.2.2.

2.2.2. Talent transferring the larva to a Petri dish using a paintbrush. **TXT: Use actively moving larvae that have left the food to crawl up**

2.2.3. Talent gently washing the larva in the Petri dish using the paintbrush.

2.3. Now, transfer the washed larva to an empty plastic dish **[1]**. With forceps, hold a small fragment of paper towel and gently dry the larva **[2]**. Remove excess water without fully drying the larva to prevent it from sticking to the dish **[3]**.

2.3.1. Talent transferring the washed larva to a dry dish using a paintbrush.

2.3.2. Talent using forceps to dab the larva with a paper towel fragment.

2.3.3. Close-up shot showing minimally dried larva.

2.4. Let the larva recover for 30 seconds to ensure easy handling during probe placement **[1]**.

2.4.1. Talent placing the dish with larva aside.

2.5. Next, view the larva under a dissection microscope at 15 to 20 times magnification **[1]**. Once the larva resumes normal crawling, gently place the electroshock probe on the

anterior dorsal surface above the central nervous system **[2]**. Apply enough pressure to compress the larva to about one-third to one-half of its depth to ensure proper conductivity without causing damage **[3]**.

2.5.1. Talent adjusting the microscope and aligning the dish under it.

2.5.2. SCOPE: 68431_scope_1.mp4 00:00-00:02.

2.5.3. SCOPE: 68431_scope_1.mp4 00:03-00:08.

2.6. Apply a 2-second pulse of constant voltage using an isolated voltage stimulator, the intensity of which was previously calibrated through a titration curve **[1]**.

2.6.1. Talent pressing the activation button on the voltage stimulator.

2.7. Immediately start a timer as soon as the shock is delivered **[1]**. Observe the larva for signs of transitory paralysis, spasms, or rolling behavior **[2]**.

2.7.1. Talent starting a timer.

2.7.2. SCOPE: 68431_scope_2.mp4 00:20-00:26.

2.8. Stop the timer once the larva clearly moves away from its original position, indicating recovery through forward peristaltic movement **[1]**.

2.8.1. SCOPE: 68431_scope_3.mp4 00:07-end.

2.9. Then, rinse the probe wires first with 100 percent ethanol, followed by double-distilled water **[1]**. After inspecting the wires under magnification, gently scrape off any residue using forceps without changing the wire spacing **[2]**.

2.9.1. Talent rinsing probe wires with ethanol.

2.9.2. Talent using forceps to scrape residue cautiously.

2.10. Use the probe to deliver a 2-second pulse of varying voltages to around 15 larvae per voltage for each genotype **[1]**. Shock each larva only once **[2]**.

2.10.1. Talent adjusting voltage on stimulator and applying shock to a larva. **TXT: Voltages: 0, 2, 4, 6, 8, 10, and 12 V**

2.10.2. Talent placing the larva back onto the vial or dish.

2.11. Measure the recovery time for each larva and compute the average recovery time for each voltage across genotypes **[1]**.

2.11.1. SCREEN: Screen-Recording---probe-calibration 00:00-00:06.

2.12. Plot the average recovery times on a graph and fit a straight line to the data **[1]**.

2.12.1. SCREEN: Screen-Recording---probe-calibration 00:07-00:20.

2.13. Choose a voltage that shows a distinct and significant difference in recovery time between wild-type and seizure mutant larvae **[1]**. Ensure the selected voltage does not cause prolonged recovery that would hinder throughput **[2]**.

2.13.1. SCREEN: Screen-Recording---probe-calibration 00:29-00:34. Author's NOTE: 2.13.1 and 2.13.2 are combined in videographer's shot. We were unsure as to what was required here, so we recorded them (again). Thus, if you already have what you need, then you can ignore the videographer shots that you will receive.

2.13.2. SCREEN: Screen-Recording---probe-calibration 00:34-00:40.

3. Drug Screening to Check the Effects on Larval Seizure

Demonstrator: Jack Corke

3.1. For drug preparation, add drugs dissolved in an appropriate solvent directly onto the surface of solid fly food **[1]** and allow time for it to soak in and evaporate **[2]**.

3.1.1. Talent pipetting drug solution onto food in open vials.

3.1.2. Talent keeping the vial aside.

3.2. Alternatively, scoop out the food from vials and re-melt it **[1]**. When the temperature cools to 40 degrees Celsius, add the drug solution **[2]** and mix thoroughly using a vortex mixer **[3]**. Pour approximately 5 milliliters of the treated food back into vials and let it solidify before use **[4]**.

3.2.1. Talent scooping out the food from the vial.

3.2.2. Talent adding the drug to the molten food. Author's NOTE: 3.2.2. combined with 3.2.4.

3.2.3. Talent vortexing the mixture.

3.2.4. Talent pouring treated food into fresh vials and keeping them aside.

3.3. Run a concentration gradient by testing a range of drug concentrations to determine

the optimal dose **[1]**.

3.3.1. Shot of several vials placed in series.

3.4. Apply electroshock to larvae of the test genotype or those exposed to a drug and measure the seizure recovery time **[1-TXT]**.

3.4.1. Talent applying electroshock to larvae and starting the timer. **TXT: Use a sample size of 20 larvae; Have a negative and positive control** Author's NOTE: Use 2.6.1 instead.

3.5. Apply a cutoff time of 300 seconds to eliminate excessively long recovery durations. Only record seizures with recovery times greater than 30 seconds as quantifiable **[1]**.

3.5.1. SCREEN: 00:05-00:20.

Results

4. Results

4.1. The probe was calibrated, and it was evident from the line fits that the mutant showed an increased seizure recovery time at all voltages compared to the wild type **[1]**.

4.1.1. LAB MEDIA: Figure 3 *Video editor: Highlight blue line*

4.2. Seizure recovery times varied significantly across *Drosophila* genotypes, with para-bang-senseless exhibiting the longest recovery time **[1]** and julius seizure the shortest among mutants **[2]**.

4.2.1. LAB MEDIA: Figure 4 *Video editor: Highlight data points for "para-bss"*

4.2.2. LAB MEDIA: Figure 4 *Video editor: Highlight data points for "jus"*

4.3. Sodium valproate and Phenytoin treatment significantly reduced seizure recovery time in the para-bang-senseless mutant **[1]** compared to the vehicle controls **[2]**.

4.3.1. LAB MEDIA: Figure 5 *Video editor: Highlight data points for "para-bss+ PHY" in A and "para-bss+ VPA" in B*

4.3.2. LAB MEDIA: Figure 5 *Video editor: Highlight data points for "para-bss" in A and B*

Pronunciation Guides:

1. instar
Pronunciation link: https://www.merriam-webster.com/dictionary/instar
IPA: /ˈɪnˌstɑr/
Phonetic Spelling: IN-star
2. peristaltic
Pronunciation link: https://www.merriam-webster.com/dictionary/peristaltic
IPA: /ˌpɛr.ɪˈstɔl.tɪk/
Phonetic Spelling: per-ih-STAWL-tik
3. phenytoin

Pronunciation link: https://www.merriam-webster.com/dictionary/phenytoin
IPA: /fəˈnɪˌtoʊɪn/
Phonetic Spelling: fuh-NIT-oh-in

4. valproate
Pronunciation link: https://www.merriam-webster.com/medical/valproate
IPA: /ˈvæl.proʊ.eɪt/
Phonetic Spelling: VAL-proh-ate

5. electroshock
Pronunciation link: https://www.merriam-webster.com/dictionary/electroshock
IPA: /ɪˈlɛk.troʊ.ʃɑk/
Phonetic Spelling: ih-LEK-troh-shock

6. probe
Pronunciation link: https://www.merriam-webster.com/dictionary/probe
IPA: /proʊb/
Phonetic Spelling: prohb

7. larva / larvae
Pronunciation link (larva): https://www.merriam-webster.com/dictionary/larva
IPA: /ˈlɑr.və/ (larva), /ˈlɑr.vi/ (larvae)
Phonetic Spelling: LAR-vuh (singular), LAR-vee (plural)

8. vial
Pronunciation link: https://www.merriam-webster.com/dictionary/vial
IPA: /ˈvaɪ.əl/
Phonetic Spelling: VY-uhl

9. magnification
Pronunciation link: https://www.merriam-webster.com/dictionary/magnification
IPA: /ˌmæg.nə.fəˈkeɪ.ʃən/
Phonetic Spelling: mag-nuh-fuh-KAY-shuhn

10. calibration
Pronunciation link: https://www.merriam-webster.com/dictionary/calibration
IPA: /ˌkæl.əˈbreɪ.ʃən/
Phonetic Spelling: kal-uh-BRAY-shuhn

11. titration
Pronunciation link: https://www.merriam-webster.com/dictionary/titration
IPA: /taɪˈtreɪ.ʃən/
Phonetic Spelling: ty-TRAY-shuhn

12. spasm
Pronunciation link: https://www.merriam-webster.com/dictionary/spasm
IPA: /ˈspæz.əm/
Phonetic Spelling: SPAZ-um

13. genotype
Pronunciation link: https://www.merriam-webster.com/dictionary/genotype
IPA: /ˈdʒi.noʊˌtaɪp/
Phonetic Spelling: JEE-noh-type

14. ethanol
Pronunciation link: https://www.merriam-webster.com/dictionary/ethanol
IPA: /ˈɛθ.ə.nɔl/
Phonetic Spelling: ETH-uh-nawl