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Title: Activity of Posterior Lateral Line Afferent Neurons During Swimming in Zebrafish

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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, but not while doing the experiment

   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.

   Olympus BX51WI

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: 20
Number of Shots: 42

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Elias Lunsford:** This protocol enables the researcher to monitor the activity and response of single sensory neurons to controlled stimulation in an intact, behaving vertebrate.

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

1.2. **James Liao:** Loose-patch electrophysiology is a quick and direct method for recording the spiking activity of individual neurons in real-time, which provides better time resolution and sensitivity at a lower cost than most optical imaging techniques.

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

OPTIONAL:

1.3. **James Liao:** Lateral line hair cells are homologous to those found in the human inner ear. This technique is poised to reveal properties of hair cell circuits that apply to human deafness and hearing disorders.

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

1.4. **Elias Lunsford:** Electrophysiology is a craft using fine-motor skills, which needs to be seen and physically imitated. Text-only instructions can leave too much room for misinterpretation.

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

Ethics Title Card

1.5. All animal care and experiments were performed in accordance with protocols approved by the University of Florida's Institutional Animal Care and Use Committee.

Protocol

2. Preparation of materials for electrophysiological recordings

2.1. To begin, dispense a thin layer of self-mixing silicone elastomer such as Sylgard into a cover glass bottomed tissue culture dish **[1]** to make a silicone elastomer-bottomed recording dish **[2].**

2.1.1. Talent dispensing sylgard into the dish

2.1.2. Silicone elastomer-bottomed recording dish

2.2. To make dissection pins, provide a negative charge of 5 Volts to a 100-milliliter beaker of etchant using a DC power supply **[1-TXT]** and attach a tungsten wire to the positively charged output **[2]**.

2.2.1. Talent providing negative charge to the beaker **TEXT: Etchant: 3M KOH, Tungsten wire: 0.002 inch; 50.8 µm diameter** NOTE: 2.2.1 – 2.3.1 are combined

2.2.2. Talent attaching tungsten to the positive terminal

2.3. Repeatedly dip the tip of the wire into the etchant bath until the tip narrows to a sharp point **[1]**. Under a stereomicroscope, cut the wire approximately 1 millimeter from the tip with a straight edge razor blade **[2]**. Repeat this three times, then insert pins into the cured recording dish using fine forceps **[3]**.

2.3.1. Talent dipping the wire in etchant

2.3.2. SCOPE: Talent cutting the wire using blade

2.3.3. Talent inserting the pins into the dish

2.4. To prepare recording electrodes, pull borosilicate glass capillary tubes using a micropipette puller with box filament **[1-TXT]**. The electrode should have a 30-micrometer diameter tip with a taper that will be used to record afferent neurons from the posterior lateral line **[2]**.

2.4.1. Talent pulling the capillary tube into electrode **TEXT: Capillary tube- ID: 0.86 mm, OD: 1.50 mm**

2.4.2. LAB MEDIA: Figure 1 Ai

2.5. Pull an additional borosilicate glass capillary tube into a pair of electrodes with smaller 1 to 5 micrometer tip diameters **[1]**. Holding one electrode in each hand, gently run the tips across one another to break them at a 30-degree angle **[2]**.

2.5.1. Talent pulling capillary tube into the electrodes

2.5.2. Talent breaking the tips using electrode

2.6. Using a microforge, polish the beveled tip until smooth **[1]**. The final tip diameter should be between 30 to 50 micrometers **[2].**

2.6.1. Talent polishing the tip

2.6.2. LAB MEDIA: Figure 1 Aii

3. Preparation of larvae for electrophysiological recordings

NOTE: Authors went through this on the day of filming regardless, so videographer filmed b-roll of the scope / hand action.

3.1. Immobilize zebrafish larvae and transfer them into a 35-millimeter Petri dish using a large-tipped transfer pipette **[1]**. Remove as much of the surrounding solution as possible **[2].** Immerse larvae in 10 microliters of 0.1% alpha-bungarotoxin for approximately 5 minutes **[3]**.

3.1.1. SCREEN: 62233_Liao_screencapture_1. 0:00 – 0:21. NOTE: Vid footage 3.1.1 – 3.1.3 are combined

3.1.2. SCREEN: 62233_Liao_screencapture_1. 0:23 – 2:05. *Video editor: Speed up the alignment running*

3.1.3. SCREEN: 62233_Liao_screencapture_1. 2:09 – 2:25.

3.2. Wash the paralyzed larva with extracellular solution for 10 minutes **[1]**. Then, use a transfer pipette to move the larva from the extracellular solution bath to the silicone-bottomed recording dish **[2]**.

3.2.1. SCREEN: 62233_Liao_screencapture_1. 4:25 – 4:35. NOTE: Vid footage 3.2.1 – 3.2.2 are combined

3.2.2. SCREEN: 62233_Liao_screencapture_1. 4:46 – 5:09. NOTE: this also has solo shot as take 2

3.3. Under a stereomicroscope, gently position the larvae with fine-tipped forceps above the center of the silicone mat, lateral side up, with the body's anterior and posterior ends running left to right **[1]**.

3.3.1. SCREEN: 62233_Liao_screencapture_1. 5:59 – 6:06. NOTE: Vid footage 3.3.1 – 3.4.2 are combined

3.4. Using fine-tipped forceps, insert the etched pin orthogonally to the silicone through the dorsal notochord of the larvae directly dorsal to the anus **[1]**. Insert the second pin through the notochord near the end of the tail and insert the third pin through the notochord dorsal of the gas bladder **[2]**.

3.4.1. SCREEN: 62233_Liao_screencapture_1. 6:38 – 6:58.

3.4.2. SCREEN: 62233_Liao_screencapture_1. 7:29 – 7:38.

3.5. Insert the fourth pin through the otic vesicle while providing slight rotation as the pin inserts into the encapsulant. As a slight rotation is applied, watch for the tissue between the cleithrum and otic vesicle to reveal the cluster of afferent somata **[1]**.

3.5.1. SCREEN: 62233_Liao_screencapture_1. 8:29 – 9:03. *Video editor: Speed up the alignment running*

4. Ventral root recording

NOTE: Videographer filmed the computer screen for some scope shots

4.1. Place pinned larva under the 10x objective on a fixed stage of the DIC microscope and orient the myseptal clefts of the muscle blocks parallel to the left headstage vector **[1]**. Place the ground wire into the bath solution and ensure that it is connected to the left headstage **[2]**.

4.1.1. Talent placing the larvae on the stage of microscope

4.1.2. Talent placing the wire in solution

4.2. Fill the VR recording electrode with 30 microliters of extracellular solution using a flexible gel-loading pipette tip **[1]** and insert it into the left headstage pipette holder **[2]**. Place the VR electrode onto the myoseptum and lower it until it is above the larva **[2].**

4.2.1. Talent filling the electrode with the solution

4.2.2. Talent inserting the electrode in holder

4.3. Increase the magnification to 40x and lower the recording pipette into the dish solution while applying positive pressure produced by a pneumatic transducer **[1-TXT].** Bring the electrode tip over a myoseptum between the two myomeres ventral to the lateral line until the cleft is centered in the VR electrode tip aperture **[2]**.

4.3.1. Talent lowering the pipette into the dish solution **TEXT: Pressure: 1–2 mmHg**

4.3.2. SCOPE: 62233_Liao_scopecapture_4.3.2. 0:00 – 0:23. Talent bringing the electrode tip over the position

4.4. Lower the pipette until the lagging edge of the tip aperture gently contacts the epithelium **[1]**. After initial contact, maneuver the pipette diagonally to ensure the leading edge makes contact and can generate a seal **[2]**.

4.4.1. SCOPE: 62233_Liao_scopecapture_4.4.1-4.4.2. 0:00 – 0:10. Talent lowering the pipette

4.4.2. SCOPE: 62233_Liao_scopecapture_4.4.1-4.4.2. 0:10 – 0:16. Talent moving the pipette diagonally

4.5. Apply negative pressure with the pneumatic transducer and hold it **[1-TXT]**.

4.5.1. Talent applying negative pressure **TEXT: Pressure: 100 mm Hg**

4.6. In the patch clamp software, click on the Play button on the tool bar to monitor the VR signal **[1]**. Ensure that the VR recording is being achieved once motor neuron activity with well-stereotyped burst signal dynamics are observed **[2].**

4.6.1 SCREEN: 62233_Liao_screencapture_2. 00:07-00:15.

4.6.2 SCREEN: 62233_Liao_screencapture_2. 00:16-00:22.

5. Afferent neuron recording

5.1. Fill the afferent recording electrode with 30 microliters of extracellular solution and insert it into the right headstage pipette holder **[1]**, then lower it into the dish solution while applying positive pressure produced by a pneumatic transducer **[2]**.

5.1.1. Talent putting the solution filled electrode into the holder

5.1.2. Talent lowering the electrode into the dish solution **TEXT: 1–2 mm Hg**

5.2. Locate the electrode and bring the electrode tip over the specimen. **[1]**. Using a micromanipulator, lower the afferent electrode tip until it is holding position above the cleithrum **[2]**.

5.2.1. SCOPE: 62233_Liao_scopecapture_5.2.1-5.2.2. 0:00 – 0:14. Talent locating the electrode on larvae

5.2.2. SCOPE: 62233_Liao_scopecapture_5.2.1-5.2.2. 0:14 – 0:22. Talent lowering the electrode

5.3. Increase the magnification to 40x immersion and locate the intersection of the posterior lateral line nerve and cleithrum **[1]**.

5.3.1. LAB MEDIA: Figure 1F

5.4. Bring the electrode tip over the afferent ganglion and lower the pipette until the tip contacts the epithelium. Gently, maneuver the electrode so that the entire tip circumference contacts the afferent ganglion **[1]**. Apply negative pressure with the pneumatic transducer and hold it **[2-TXT]**.

5.4.1. SCOPE: 62233_Liao_scopecapture_5.4.1

5.4.2. Talent applying negative pressure **TEXT: Pressure: 20–50 mm Hg**

5.5. After clicking on play in the patch clamp software **[1]**, Ensure that the whole cell, loose patch recording of afferent neurons is achieved once spikes occur spontaneously, roughly every 100 to 200 milliseconds **[2].**

5.5.1. SCREEN: 62233_Liao_screencapture_2. 00:25-00:30.

5.5.2. SCREEN: 62233_Liao_screencapture_2. 00:31-00:37.

5.6. Once afferent neuron and motor neuron activity are both detected, click on the Record button on the tool bar in pClamp10 to capture simultaneous gap free recordings in both the channels **[1]**.

5.6.1. SCREEN: 62233_Liao_screencapture_2. 00:38-00:58.

5.7. Perform data pre-processing as described in the text manuscript **[1]**.

5.7.1. Talent at the computer, processing the data.

Results

6. Results: Simultaneous electrophysiological recording of afferent neuron and ventral motor root activity

6.1. Using this gap-free recording protocol, the real-time activity of afferent and VR neurons can be measured simultaneously. Custom written pre-processing scripts generate plots to assist in visualization of spike detection, using parameters such as threshold, minimum duration, and minimum inter-spike interval **[1].**

6.1.1. LAB MEDIA: Figure 2

6.2. Isolated signals were obtained by adjusting the lower-bound and upper-bound detection variables in the pre-processing script **[1]**.

6.2.1. LAB MEDIA: Figure 2A

6.3. Ventral root spike detection follows identical parameters with additional inputs. Bursts within a motor command are defined by a minimum of two spikes within 0.1 millisecond lasting for 5 milliseconds at least and delineated by a minimum of three bursts with inter-burst intervals of less than 200 milliseconds **[1].**

6.3.1. LAB MEDIA: Figure 2B

6.4. Pre-processing scripts will overlay sections of afferent activity centered on a well-defined period of interest. In this case, mean spontaneous activity shows dramatic changes in response to the onset of motor activity such as the onset of a swim bout **[1].**

6.4.1. LAB MEDIA: Figure 2C

6.5. Significant differences were detected in afferent spike rates between swimming spike rates and spike rates of both pre- and post-swim periods **[1]**. Afferent spike rate was negatively correlated with swim duration **[2]**. There was no correlation detected between relative spike rate and swim frequency or duty cycle **[3].**

6.5.1. LAB MEDIA: Figure 3A

6.5.2. LAB MEDIA: Figure 3B

6.5.3. LAB MEDIA: Figure 3C

Conclusion

7. Conclusion Interview Statements

7.1. **Elias Lunsford:** Animal health is the most important thing when attempting this procedure. It is crucial to monitor fast blood flow to ensure animal welfare and increase likelihood of neural recording success.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.3.1*

7.2. **Elias Lunsford:** By leveraging genetic tools available in zebrafish, this electrophysiology protocol can be complemented by transgenic lines to powerfully investigate anatomical and functional circuit connectivity in hair cell systems and beyond.

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

7.3. **James Liao:** This application of Noble laurate Neher and Sakmann's patch-clamp electrophysiology to hair cell systems *in vivo* underscores the importance of understanding how sensory systems are influenced by internal and external states.

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