FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 67005
Scriptwriter Name: Pallavi Sharma
Project Page Link: https://review.jove.com/account/file-uploader?src=20436573

Title: Developing a Behavioral Box for Assessing Prepulse Inhibition and Neural Activity in Psychiatric Animal Models

Authors and Affiliations:

Lorena Andreoli[1], Fernando da Silva Fiorin[1,2], Hougelle Simplicio[1], Mirian Hayashi[2], Edgard Morya[1]*

[1]Edmond and Lily Safra International Institute of Neuroscience, Santos Dumont Institute
[2]Laboratory of Molecular Pharmacology, Departamento de Farmacologia, Escola Paulista de Medicina (EPM), Universidade Federal de São Paulo (UNIFESP)

Corresponding Authors:
Edgard Morya (edgard.morya@isd.org.br)

Email Addresses for All Authors:

(lori.andreoli@gmail.com)
(fernandofiorin@hotmail.com)
(hougelle.simplicio@isd.org.br)
(edgard.morya@isd.org.br)
(mhayashi@unifesp.br)

Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

√ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

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

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **16/10/2024**

When you are ready to submit your video files, please contact our Content Manager, Utkarsh Khare.

Current Protocol Length

Number of Steps: 17
Number of Shots: 35

Introduction

1.1. **Lorena Andreoli:** This protocol outlines a straightforward method for evaluating animal models of schizophrenia, designed for behavioral researchers without programming or electronics expertise. We created a cost-effective behavioral box to assess prepulse inhibition in rats affected by neurodevelopmental issues stemming from early-life distress.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 2.8.1.*

What are the most recent developments in your field of research?

1.2. **Lorena Andreoli:** Recent advancements in psychiatric animal models using the prepulse inhibition (PPI) test focus on understanding sensory gating deficits seen in disorders like schizophrenia. This includes genetic modifications, neuroimaging, and pharmacological interventions to investigate PPI disruptions, ultimately improving diagnostics and therapies.

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

What new scientific questions have your results paved the way for?

1.3. **Lorena Andreoli:** We hope that our protocol will popularize the study of prepulse inhibition and animal models of schizophrenia and labs that study molecular, behavioral, and physiological aspects of the disease can use it to contribute to the understanding of this complex psychiatric disorder.

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

Ethics Title Card

This research has been approved by the Ethics Committee of CEUA Santos Dumont Institute

Protocol

2. Developing a Behavioral Box to Assess Prepulse Inhibition to Investigate the Post-Weaning Isolation Rat Model

Demonstrator: Fernando Fiorin

2.1. To begin, make an acrylic transparent box to accommodate a freely behaving rat equipped with a wired headstage **[1]**. Use self-adhesive rubber pads to isolate the acrylic box from vibrations and secure the accelerometer below without contact with other parts **[2]**.

2.1.1. WIDE: Talent at the bench with the completed transparent acrylic box placed in front.

2.1.2. Close-up of talent attaching rubber pads to the base of the acrylic box.

2.2. Using a soldering iron, solder a ribbon cable directly to the four pinholes on the accelerometer board: VCC *(V-C-C)*, GND *(G-N-D)*, SCL *(S-C--L)*, and SDA *(S-D-A)* **[1]**. Clean the center of the acrylic and the flat faceboard of the accelerometer **[2-TXT]**.

2.2.1. Close-up of the talent soldering the ribbon cable to the accelerometer board pinhole.

2.2.2. Shot of the talent cleaning the center of the acrylic box and the flat faceboard of the accelerometer. **TXT: Wait until the cleaning and curing process is fully complete**

2.3. Then, connect the opposite end of the ribbon cable to the microcontroller by connecting VCC to 3.3 volts, GND to GND, SCL to SCL, and SDA to SDA **[2]**.

2.3.1. Close-up of talent connecting the ribbon cable from the accelerometer to the microcontroller board.

2.4. Next, construct a soundproof chamber with a frontal door using 80-centimeter edges of 15 millimeters thick medium-density fiberboard **[1-TXT].** ~~Line the interior of the chamber with acoustic foam **[2].**~~ Position a speaker 20 centimeters above the acrylic box, ensuring that the head stage and cable have a full range of motion **[3]**.

2.4.1. Shot of talent assembling the MDF panels to create a soundproof chamber. **TXT: Line the interior of the chamber with acoustic foam**

2.4.2. ~~Close-up of the interior lining process, showing acoustic foam placement.~~ **NOTE: Since shot was not provided,it has been added as on-screen text**

2.4.3. View of the speaker positioned above the acrylic box with the headstage cable positioned freely.

2.5. Connect the audio-out from the computer to the microcontroller board **[1-TXT].** ~~and the speaker system through a commercial audio detector **[2]**.~~

2.5.1. Talent connects the audio-out to analog input and ground on the microcontroller. **TXT: Connect speaker through a commercial audio detector**

2.5.2. ~~Close-up of speaker connection through the audio detector.~~
NOTE: Since shot was not provided,it has been added as on-screen text

2.6. Using a universal serial bus cable, connect the microcontroller board to the computer **[1]**. Then, open the integrated development environment software for the microcontroller board on the computer **[2]**.

2.6.1. Shot of talent connecting the USB cable from the microcontroller board to the computer.

2.6.2. SCREEN: 2.6.1-2.7.3.mp4 00:02-00:16

2.7. After selecting the corresponding **microcontroller board**, **COM port** *(Com-Port)*, and **baud rate [1]**, open the file sketch, compile, and upload the code to the microcontroller board **[2]**. Click on the **monitor serial port** to check for incoming data, then close the development environment software **[3]**.

2.7.1. SCREEN: 2.6.1-2.7.3.mp4. 00:27-00:36

2.7.2. SCREEN: 2.6.1-2.7.3.mp4 00:17-00:26

2.7.3. SCREEN: 2.6.1-2.7.3.mp4. 01:00-01:20

2.8. Now, position a digital sound level meter within the acrylic box where the animal will be placed **[1-TXT]**. ~~With the chamber door closed, check the background noise level **[2]**~~. Play the acoustic stimuli and adjust the detection range on the sound level meter to calibrate the stimulus levels being sent by the computer **[3]**.

2.8.1. Shot of the talent placing the sound level meter in the animal's position inside the box. **TXT: Check the background noise level with the chamber door closed**

2.8.2. ~~Close-up of the sound level meter reading the background noise in the closed chamber.~~
NOTE: Since shot was not provided,it has been added as on-screen text

2.8.3. Talent adjusting the detection range on the sound level meter during acoustic stimulus playback.

2.9. Then, open the preferred open-source software for acquiring and saving data from the serial port. Configure the software settings, including the COM port, microcontroller board, and baud rate **[1]**. Click **Connect** to read raw data or the chart view **[2]**, then click **Save** to start recording the experiment's data **[3]**.

2.9.1. SCREEN: 2.9.1-2.9.3.mp4 00:05-00:25

2.9.2. SCREEN: 2.9.1-2.9.3.mp4 00:26-00:36

2.9.3. SCREEN: 2.9.1-2.9.3.mp4. 00:50-01:01

3. Synchronization of the Prepulse Inhibition (PPI) Box with Neuronal Recordings from the Model

3.1. After implanting the electrode array in the rat's brain **[1],** carefully connect the head stage to the connector implant on the rat's skull **[2]**.

3.1.1. Establishing shot of the talent with the rat implanted with an electrode array placed in front of him.

3.1.2. Talent attaching the headstage to the connector implant on the rat's skull.

3.2. Then, power on the neuronal data acquisition system **[1]** and confirm that it receives data from the acoustic stimuli through either the microcontroller or a TTL *(T-T-L)* generator connected to the audio detector or microphone **[2-TXT]**. Turn on the open-source serial port recorder set up earlier **[3]**.

3.2.1. Talent turning on the neuronal data acquisition system.

3.2.2. Shot of talent confirming data reception from the acoustic stimuli through the system. **TXT: TTL: Transistor-Transistor Logic**

3.2.3. SCREEN: 3.2.3-3.5.1.mp4. 00:06-00:10

3.3. Now, use electrophysiology acquisition software to collect electrophysiological and accelerometer data **[1]**.

3.3.1. SCREEN: 3.2.3-3.5.1.mp4 00:30-00:50

3.4. Expose the animal to white background noise for 7 minutes with random presentation of all types of pulses, repeating each pulse type 10 times **[1]**.

3.4.1. SCREEN: 3.2.3-3.5.1.mp4. 01:07-01:31

3.5. At the end of the recording session, turn off the acquisition software **[1]**. Then, carefully disconnect the headstage connector from the implant on the animal's skull **[2]**.

3.5.1. SCREEN: 3.5.1.endmp4 00:38-00:51

3.5.2. Close-up of talent disconnecting the headstage from the rat's implant.

3.6. To perform electrophysiological and behavioral analysis, import the raw data containing brain activity, acceleration, and acoustic stimuli into a signal processing platform **[1]**. Extract epochs centered on each acoustic stimulus **[2]**.
NOTE: 3.6-3.6 were not provided. Please add missing media title cards

3.6.1. SCREEN: Signal processing platform showing data import and organization of raw brain, acceleration, and acoustic stimulus data.

3.6.2. SCREEN: Process of selecting and extracting epochs centered on acoustic stimuli.

3.7. Then, normalize the means to enable comparison between animals **[1]**. Set the response to a single pulse as the maximum response and normalize the reflex responses to other pulses relative to this value **[2]**.

3.7.1. SCREEN: View of normalization settings, with pulse 5 selected as the maximum response.

3.7.2. SCREEN: Reflex responses being normalized relative to the pulse 5 value.

3.8. Finally, define the startle amplitude for pulse 5 as 100 percent and represent responses to other pulses as a function of the pulse 5 amplitude **[1]**.

3.8.1. SCREEN: Platform showing pulse 5 amplitude set to 100 percent. Comparison view showing other pulse amplitudes calculated relative to pulse 5.

Results

4. Results

4.1. Local field potential analysis indicated no significant power modulation in the prelimbic and infralimbic cortices post-stimulation **[1],** while areas like the ventral tegmental area, amygdala, nucleus accumbens, and hippocampus showed decreased power spectrum density in delta, theta, and alpha bands, with a notable beta modulation **[2].**

4.1.1. LAB MEDIA: Figure 8. *Video editor: Highlight the panels for prelimbic and infralimbic*

4.1.2. LAB MEDIA: Figure 8 *Video editor: Highlight the panels for ventral tegmental area, amygdala, nucleus accumbens, and hippocampus*

4.2. Both commercial and DIY *(D-I-Y)* accelerometers demonstrated consistent amplitude and time-based data, with baseline readings and startle response amplitudes showing close alignment across devices **[1].**

4.2.1. LAB MEDIA: Figure 9

Pronunciation Guide:

1. Prepulse

- **Pronunciation link**: https://www.oed.com/dictionary/prepulse_n
- **IPA**: /ˈpriːˌpʌls/
- **Phonetic Spelling**: pree-puhlsoed.com+5oed.com+5oed.com+5howtopronounce.com+2howtopronounce.com+2howtopronounce.com+2

---

2. Inhibition

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/inhibition
- **IPA**: /ˌɪn.hɪˈbɪʃ.ən/
- **Phonetic Spelling**: in-huh-bish-uhnhowtopronounce.com+8merriam-webster.com+8merriam-webster.com+8howtopronounce.com

---

3. Accelerometer

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/accelerometer
- **IPA**: /əkˌsɛl.əˈrɒm.ɪ.tər/
- **Phonetic Spelling**: ak-sel-uh-rah-muh-termerriam-webster.com+3merriam-webster.com+3merriam-webster.com+3

---

4. Microcontroller

- **Pronunciation link**: https://www.howtopronounce.com/microcontroller
- **IPA**: /ˈmaɪ.kroʊ.kənˌtroʊ.lər/
- **Phonetic Spelling**: my-kroh-kuhn-troh-lerhowtopronounce.com

---

5. Electrophysiology

- **Pronunciation link**: https://www.howtopronounce.com/electrophysiology
- **IPA**: /ɪˌlɛk.troʊˌfɪz.iˈɒl.ə.dʒi/
- **Phonetic Spelling**: ih-lek-troh-fiz-ee-ol-uh-jeehowtopronounce.com+5howtopronounce.com+5howtopronounce.com+5

---

6. Hippocampus

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/hippocampus
- **IPA**: /ˌhɪp.əˈkæm.pəs/
- **Phonetic Spelling**: hip-uh-kam-puhsoed.com+2merriam-webster.com+2howtopronounce.com+2

---

7. Amygdala

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/amygdala
- **IPA**: /əˈmɪg.də.lə/
- **Phonetic Spelling**: uh-mig-duh-luh

---

8. Prefrontal

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/prefrontal
- **IPA**: /ˌpriːˈfrʌn.təl/
- **Phonetic Spelling**: pree-fruhn-tuhl

9. Neurodevelopmental

- **Pronunciation link**: https://www.howtopronounce.com/neurodevelopmental
- **IPA**: /ˌnʊə.roʊ.dɪˌvɛl.əpˈmɛn.təl/
- **Phonetic Spelling**: noor-oh-di-vel-up-men-tuhlhowtopronounce.com+9howtopronounce.com+9howtopronounce.com+9

10. Schizophrenia

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/schizophrenia
- **IPA**: /ˌskɪt.səˈfriː.ni.ə/
- **Phonetic Spelling**: skit-suh-free-nee-uh