Submission ID #: 61330
Scriptwriter Name: Bridget Colvin
Project Page Link: https://www.jove.com/account/file-uploader?src=18705698

Title: A Model of Epileptogenesis in Rhinal Cortex-Hippocampus Organotypic Slice Cultures

Authors and Affiliations: Cláudia A. Valente[1,2], Francisco J. Meda[1,2,*], Mafalda Carvalho[1,2,*], Ana M. Sebastião[1,2]
*These authors contributed equally

[1]Instituto de Farmacologia e Neurociências, Faculdade de Medicina da Universidade de Lisboa
[2]Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina da Universidade de Lisboa

Corresponding Author:
Cláudia A. Valente
cvalentecastro@medicina.ulisboa.pt

Co-authors:
fmeda@medicina.ulisboa.pt
mafaldacarvalho@medicina.ulisboa.pt
anaseb@medicina.ulisboa.pt

Author Questionnaire

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

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

Videographer: screen capture file provided, do not film

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

Script length:

Number of shots: 50

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Cláudia A. Valente**: Epilepsy is a prevalent neurological disease. Rhinal cortex-hippocampus organotypic slices depict evolving epileptic-like events that resemble in vivo epilepsy and can therefore be considered an ex vivo model of epileptogenesis **[1]**.

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

REQUIRED:

1.2. **Cláudia A. Valente**: This system is an excellent tool for monitoring the dynamics and progression of epileptogenesis, as well as for screening potential therapeutic targets for this brain pathology **[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. **Cláudia A. Valente**: Demonstrating the procedures with me will be my students, Francisco J. Meda and Mafalda Carvalho **[1][2][3]**.

1.3.1. INTERVIEW: Author saying the above
1.3.2. ADDED SHOT: Francisco acknowledges the camera in the library (without the mask)
1.3.3. ADDED SHOT: Mafalda acknowledges the camera in the library (without the mask)

Ethics Title Card

1.4. The Portuguese law and European Union guidelines (2010/63/EU) were respected in all procedures regarding the protection of animals for scientific purposes. Procedures involving animal subjects have been approved by the the iMM's Institutional Animal Welfare Body (ORBEA-iMM) and the National competent authority (DGAV – Direção

Geral de Alimentação e Veterinária).

Protocol

2. Rhinal Cortex-Hippocampus Slice Preparation

2.1. To harvest the brain from a postnatal day 6-7 Sprague Dawley pup **[1-TXT]**, first wash the head three times in 5 milliliters of GBSS (G-B-S-S) **[2-TXT]**.

2.1.1. WIDE: Talent placing head in dish *Videographer: More Talent than head in shot* **TEXT: Euthanasia: Decapitation**. Talent washing head, with GBSS container visible in frame *Videographer: More Talent than head in shot* **TEXT: GBSS: Gey's Balanced Salt Solution** NOTE: 2.1.1 and 2.1.2 in one shot

2.2. Working in a biosafety cabinet, firmly insert sharp forceps into the eye sockets to hold the head in place and use thin-tipped scissors to cut the scalp along the midline from the vertebral foramen to the frontal lobes **[1-TXT] [2]**.

2.2.1. Head being held in place and incision being made. **TEXT: UV-sterilize all tools before use** NOTE: 2.2.1 and 2.2.2 in one shot

2.3. Cut the skull in the same manner and along the cerebral transverse fissure and use curved long forceps to move the skull pieces apart **[1]**.

2.3.1. Skull being cut and pieces being opened

2.4. Use a spatula to discard the olfactory bulbs and to transfer the brain dorsal-side up into a 60-millimeter plate containing 5 milliliters of cold GBSS **[1]**.

2.4.1. Bulbs being discarded and brain being transferred

2.5. Insert fine forceps into the cerebellum and insert the spatula along the midline to carefully open the hemisphere **[1] [2]**.

2.5.1. Forceps being inserted and hemisphere being opened/separated from the rest of the brain NOTE: 2.5.1 and 2.5.2 in one shot

2.6. Use short curved forceps to carefully remove the excess tissue that covers the hippocampus without touching the hippocampal structure **[1].** Use the spatula to cut below the hippocampus **[2] [3-TXT]**.

2.6.1. Excess tissue being removed *Videographer: Important/difficult step*

2.6.2. Tissue below hippocampus being cut (TAKE 1) and exposed hippocampus (TAKE 2). *Videographer:* NOTE: Use both takes

2.6.3. ADDED SHOT: Two hemispheres with exposed hippocampus. **TEXT: Repeat for second hemisphere**

2.7. Transfer one hemisphere at a time, hippocampus-side up and parallel to each other, onto a piece of filter paper **[1]**.

2.7.1. Tissue being placed onto filter paper *Videographer: Important step*

2.8. Place the filter paper onto a tissue chopper with the hemispheres perpendicular to the blade and cut the hemispheres into 350-micron slices **[1] [2-TXT]**.

2.8.1. + 2.8.2 Paper being placed onto chopper and tissue being sliced. **TEXT: Adjust slice thickness to 350 microns** NOTE: 2.8.1 and 2.8.2 in one shot

2.9. Transfer the sliced tissue into a new Petri dish containing 5 milliliters of cold GBSS **[1-TXT]** and use round tip electrodes to carefully separate the slices **[2-TXT]**, keeping only the samples with a structurally intact rhinal cortex and hippocampus **[3-TXT]**.

2.9.1. Talent placing sliced tissue into dish

2.9.2. Slices being separated/excess cortex being cut *Videographer: Important/difficult step* **TEXT: Carefully separate the slices**

2.9.3. NOTE: Use TAKE 2 and TAKE 3.

TAKE 2: Three intact slices

TAKE 3: Close-up of one intact slice. *Video Editor: please emphasize rhinal cortex and hippocampus when mentioned* **TEXT: Structural deficits will negatively impact viability**

2.10. The DG (D-G) and CA (C-A) areas should be perfectly defined, as well as the entorhinal and perirhinal cortex regions **[1-TXT]**.

2.10.1. Use 2.9.3. *Video Editor: please emphasize DG, CA, entorhinal, and perirhinal cortex areas when mentioned* **TEXT: DG: Dentate Gyrus, CA: Cornu Ammonis** NOTE: Use Take 3

2.11. Use a spatula and a round-tip electrode to place up to four intact slices onto individual inserts in the appropriate number of wells of a 6-well plate containing 1.1 milliliter of culture medium per well **[1-TXT].** Use a P20 pipette to remove any excess dissection medium from around each slice **[2].** Place the plate in the cell culture incubator **[3].**

2.11.1. Slice being placed in the spatula and onto insert *Videographer: Important step* **TEXT: See text for all medium and solution preparation details**

2.11.2. Dissection medium being aspirated from around the slice. *Videographer: Important step*

2.11.3. ADDED SHOT: Use 4.3.1. Close-up of temperature and CO_2 and talent placing plate into incubator.

2.12. Change the medium every 2-3 days. Use forceps to lift the insert. Aspirate the medium from the corresponding well and place the insert back in place **[1]**.

2.12.1. Insert being lift and medium being aspirated from well, with insert at side of plate visible in frame as possible, and insert being returned to the well.

2.13. Use a P1000 pipette to add 1 milliliter of fresh, 37-degree Celsius medium to each well **[1-TXT]**.

2.13.1. Medium being added, with medium container visible in frame. **TEXT: See text for all medium and solution preparation details**

3. Electrophysiological Recordings

3.1. Prepare the electrophysiology setup in a closed circuit. Verify that the flow rate of the interface-type chamber is set to 2 milliliters/minute **[1]** and open the carbox valve **[2]**.

3.1.1. WIDE: Talent checking flow rate with a glass beaker

3.1.2. Talent opening valve

3.2. Check the water level in the system **[1]** and place a piece of filter paper into the interface recording chamber to drain any excess medium **[2]**.

3.2.1. Talent checking water level. Close-up of interface chamber.

3.2.2. Talent placing filter paper into chamber

3.3. Place a piece of lens cleaning paper beneath the frame to supply medium to the slice **[1]** and turn on the temperature controller, amplifiers and micromanipulators **[2]**. Use a syringe to load a glass electrode with freshly prepared artificial cerebrospinal fluid **[3]**.

3.3.1. Talent placing lens cleaning paper beneath frame

3.3.2. ADDED SHOT: Talent turning on temperature controller, amplifiers and micromanipulators

3.3.3. Talent loading fluid into electrode, with fluid container visible in frame. **TEXT: See text for all medium and solution preparation details**

3.4. Place the glass electrode into the receiving electrode **[1]** and wait for the temperature in the interface chamber to stabilize at 37 degrees Celsius **[2]**. Working in a biosafety cabinet, place the insert in a 60-millimeter plate with a drop of medium **[3].**

3.4.1. Glass electrode being placed into receiving electrode

3.4.2. ADDED SHOT: Close-up of 37ºC in the temperature controller

3.4.3. Talent placing a drop of medium into a 60-millimeter plate and placing insert over the drop

3.5. Use a sharp blade to cut a slice from the insert **[1]** and place the slice in the interface chamber with the hippocampus to the bottom right **[2]**.

3.5.1. Slice being cut from insert *Videographer: Important step*

3.5.2. Talent placing slice into interface chamber

3.6. Then place the receiving electrode into the CA3 (C-A-three) pyramidal cell layer **[1]**, initiate the continuous acquisition protocol **[2]**, and record the slice for 30 minutes **[3]**.

3.6.1. Electrode being placed into pyramidal cell layer *Videographer: Important step*

3.6.2. Talent starting acquisition, with monitor visible in frame

3.6.3. SCREEN: SCREEN_Slice being recorded: 00:00-00:10

4. Propidium Iodide (PI) Uptake Assay and Immunohistochemistry

4.1. To perform the propidium iodide uptake assay work in a biosafety cabinet. Lift the insert from the well **[1]** and add propidium iodide in medium to a final concentration of 2-micromolar per well **[2]**.

4.1.1. WIDE: Talent lifting insert

4.1.2. Talent adding PI to well(s), with PI container visible in frame

4.2. Slowly agitate the plate before placing the insert back into the well, taking care that there are no bubbles beneath the slices **[1]**.

4.2.1. Plate being agitated and insert being placed back. **TEXT: Repeat for other inserts**

4.3. When all slices have been treated, return the plate to the cell culture incubator **[1]**.

4.3.1. Close-up of temperature and CO_2 and talent placing plate into incubator. *Videographer/Video Editor: this shot will be used again in 2.11.3*

4.4. For immunostaining of the slices, at the end of the propidium iodide incubation, aspirate the medium from the bottom of each well **[1]** and add 1 milliliter of 4% paraformaldehyde to the top and bottom of each insert **[2]**.

4.4.1. Talent aspirating medium

4.4.2. Talent adding PFA to well(s), with PFA container visible in frame

4.5. After 1 hour at room temperature, wash the slices two times for 10 minutes and 1 milliliter of PBS per wash **[1]** and use a hydrophobic pen to draw two rectangles on microscope slides **[2]**.

4.5.1. Talent adding PBS to well(s), with PBS container visible in frame. NOTE: Two takes were shot. Please choose TAKE 2.

4.5.2. Rectangle being drawn onto slide

4.6. Use a sharp blade to cut the slices from the inserts **[1]** and place one slice into each rectangle **[2]**.

4.6.1. Slice being cut

4.6.2. Second slice being placed into rectangle, with first slice on slide visible in frame

4.7. Add permeabilization-blocking solution onto each slice for a 3-hour incubation at room temperature **[1]**.

4.7.1. Talent adding blocking solution to slide(s), with solution container visible in frame

4.8. At the end of the incubation, add the primary antibodies of interest to each slice for a 4-degree Celsius incubation overnight **[1]**.

4.8.1. Talent adding antibod(ies) to slice, with antibody container(s) visible in frame

4.9. The next morning, wash the slices with PBS-Tween three times for 10 minutes per wash **[1]** and incubate the slices with the appropriate secondary antibodies for 4 hours at room temperature **[2]**.

4.9.1. Talent adding PBS-T to slide, with PBS-T container visible in frame *Videographer/Video Editor: this shot will be used again*

4.9.2. Talent adding antibod(ies) to slice, with antibody container(s) visible in frame

4.10. At the end of the incubation, wash the slices three times as demonstrated **[1]** and add 50 microliters of Hoechst (pronounce 'hookst') solution onto each slice for a 20-minute incubation at room temperature **[2]**.

4.10.1. Use 4.9.1. Talent adding PBS-T to slide, with PBS-T container visible in frame

4.10.2. Talent adding Hoechst solution to slice(s), with solution container visible in frame

4.11. At the end of Hoechst incubation, wash the slices three times **[1]** and add 50 microliters of mounting medium to each slice **[2]**.

4.11.1. Use 4.9.1. Talent adding PBS-T to slide, with PBS-T container visible in frame

4.11.2. Mounting medium being added to slice, with medium container visible in frame.

4.12. Then place a coverslip onto each slice **[1]** and seal the slices with nail polish **[2]**.

4.12.1. Coverslip being placed

4.12.2. Coverslip being sealed with nail polish

4.13. After allowing the slides to dry for 24 hours at room temperature, visualize the immunostaining and propidium iodide uptake by confocal microscopy **[1-TXT]**.

4.13.1. LAB MEDIA: Figure 4A **TEXT: Optional: Store slices at -20 °C until imaging**

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see? Please list 4 to 6 individual steps.
2.6.-2.6., 2.11., 3.4., 3.6.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success? Please list 1 or 2 individual steps from the script above.
2.6., 2.9.

Results

5. Results: Representative Rhinal Cortex-Hippocampus Organotypic Slice Functional and Phenotypic Characterization

5.1. Rhinal cortex-hippocampus organotypic slices **[1]** depict mixed interictal and ictal-like activity at 7 days in vitro **[2]**.

5.1.1. LAB MEDIA Figure 3A
5.1.2. LAB MEDIA: Figure 3A *Video Editor: please emphasize bottom trace*

5.2. At 14 days in vitro, spontaneous activity is characterized by ictal discharges **[1]**, which evolve to an overwhelming ictal activity at 21 days in vitro, with ictal events lasting greater than 1 minute **[2]**.

5.2.1. LAB MEDIA: Figure 3B *Video Editor: please emphasize bottom trace*
5.2.2. LAB MEDIA: Figure 3C *Video Editor: please emphasize bottom trace*

5.3. Propidium iodide uptake and immunohistochemistry against the neuronal marker NeuN (pronounce 'Ne-u-N') **[1]** revealed some neuronal death in 7 days in vitro slices **[2]** that is considerably increased at 14 days in vitro in all areas of the hippocampus **[3]**.

5.3.1. LAB MEDIA: Figure 4
5.3.2. LAB MEDIA: Figure 4 *Video Editor: please emphasize Figure 4A*
5.3.3. LAB MEDIA: Figure 4 *Video Editor: please emphasize arrows in bottom panels (Figures 4-B1a, 4-B2a and 4-B3a)*

5.4. At 7 days in vitro, ramified microglia with a low CD68 (C-D-sixty-eight) expression **[1]** are more abundant than Iba1 (E-ba-one)-positive, CD68-positive reactive microglia **[2]**, whereas at 14 days in vitro, in all areas of the hippocampus, Iba1-positive, CD68-positive bushy or amoeboid M1 microglia **[3]** exceed microglia with a low CD68 expression **[4]**.

5.4.1. LAB MEDIA: Figure 5 *Video Editor: please emphasize arrows in Figure 5-A4a*
5.4.2. LAB MEDIA: Figure 5 *Video Editor: please emphasize arrowheads in Figure 5-A4a*
5.4.3. LAB MEDIA: Figure 5 *Video Editor: please emphasize arrowheads in Figure 5-B5a*
5.4.4. LAB MEDIA: Figure 5 *Video Editor: please emphasize arrows in Figure 5-B5a*

5.5. At 14 days in vitro some Iba1-positive, CD68-positive cells with a hyper-ramification appearance can be pinpointed **[1]**, suggesting the possibility of an M2 anti-inflammatory microglia phenotype **[2]**.

5.5.1. LAB MEDIA: Figure 5 *Video Editor: please emphasize open arrowheads in Figure 5-B6A*

5.5.2. LAB MEDIA: Figure 5

5.6. At 7 days in vitro, the expression of C3d (C-three-D) is barely detectable **[1]**, while in 14-day in vitro slices, hypertrophic glial fibrillary acidic protein-positive, C3d-positive astrocytes can be observed **[2]**, suggesting a progressive activation of A1 astrocytes **[3]**.

5.6.1. LAB MEDIA: Figure 6 *Video Editor: please emphasize Figure 6-A*

5.6.2. LAB MEDIA: Figure 6 *Video Editor: please emphasize arrowheads in Figure 6-B4a*

5.6.3. LAB MEDIA: Figure 6

Conclusion

6. Conclusion Interview Statements

6.1. **Cláudia A. Valente**: Prepare rhinal cortex-hippocampus slices with extreme care. Make sure to remove the excess tissue above the hippocampus, as it can compromise slice's integrity. Slices with structural deficits will not be viable **[1]**.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (2.2.-2.11.)

6.2. **Cláudia A. Valente**: In addition to the demonstrated approaches, a variety of assays, such as western blot analysis, RT-PCR, and ELISA, can be applied to this system to address specific questions about epileptogenesis **[1]**.

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