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Title: Culture of Neurospheres Derived from the Neurogenic Niches in Adult Prairie Voles

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Author Questionnaire

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

   If **Yes**, can you record movies/images using your own microscope camera?

   No

   If **No**, JoVE will need to record the microscope images using our scope kit (through a camera port or one of the oculars). Please list the make and model of your microscope.

   Stereomicroscope SZX16 (Olympus)

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Wendy Portillo:** This method can help to investigate sex-dependent differences in the neurogenic niches and to understand the neuroplasticity changes that underlie social interactions in the prairie vole.

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

1.2. **Daniela Ávila-González:** The assay of neurospheres is an excellent tool to determine the differentiation and proliferation potential of neural stem and progenitor cells.

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

Ethics Title Card

1.3. This study was approved by the Research Ethics Committee of the Instituto de Neurobiología, Universidad Nacional Autónoma de México, México and Instituto Nacional de Perinatología. The reproduction, care and humane endpoints of the animals were established following the Official Mexican Standard.

Protocol

2. Microdissection of the Neural Tissue

2.1. To begin, place a Petri dish on a surface surrounded by ice **[1]**, deposit the brain on the dish, and add 20 milliliters of cold wash solution **[2]**. In the coronal plane, divide the brain into two blocks of tissue using a scalpel, performing the cut at Bregma level in the anterior-posterior axis **[3]**.

2.1.1. WIDE: Establishing shot of talent placing a Petri dish on ice.

2.1.2. Talent putting the brain in the dish and adding wash solution.

2.1.3. Talent cutting the brain into 2 blocks.

2.2. Extract the subventricular zone, or VZ, tissue from the rostral block and the dentate gyrus, or DG, tissue from the caudal block **[1]**. To dissect the VZ, hold one of the hemispheres with a Dumont forceps and insert the fine tips of a second forceps under the tissue that lines the caudate-putamen **[2]**. *Videographer: This step is important!*

2.2.1. Talent positioning the dish under the microscope.

2.2.2. SCOPE: Talent inserting the tip of the forceps under the tissue.

2.3. Open the forceps along the dorsoventral axis to separate the tissue and collect the VZ **[1]** into a centrifuge tube with 2 milliliters of cold wash solution. Do not pool the tissue of more than two animals **[2]**. Repeat the microdissection in the other hemisphere and store the tube with the tissue on ice **[3]**. *Videographer: This step is difficult and important!*

2.3.1. SCOPE: Talent opening the forceps and isolating the tissue.

2.3.2. Talent putting the tissue into a tube with wash solution.

2.3.3. Talent placing the tube on ice.

2.4. To dissect the DG, use a scalpel to make a coronal cut into the caudal block to obtain two slices in which the hippocampal formation is observed **[1]**. Use Dumont forceps to hold one of the slices and make a horizontal cut between DG and CA1 with another forceps. Then, make a vertical incision between the DG and CA3 to separate the DG **[2]**. *Videographer: This step is difficult and important!*

2.4.1. SCOPE: Talent cutting the caudal block.

2.4.2. SCOPE: Talent cutting the tissue to isolate the DG.

2.5. Repeat the dissection in the other hemisphere of the first slice, then in both hemispheres in the second slice **[1]**. Collect the four DG pieces of each vole in a centrifuge tube **[2]**. *Videographer: This step is important!*

2.5.1. Talent working at the microscope.

2.5.2. Talent putting the DG pieces in the centrifuge tube.

3. Isolation of Neural Cells

3.1. Place the centrifuge tubes inside the biosafety cabinet and wait approximately 10 minutes for the tissue fragments to precipitate by gravity **[1]**. Then, remove the wash solution **[2]** and add 1 milliliter of warm enzymatic solution to each tube **[3]**. Incubate the tubes at 37 degrees for 10 minutes **[4]**.

3.1.1. Tubes in the cabinet.

3.1.2. Talent removing the wash solution.

3.1.3. Talent adding enzymatic solution to a tube.

3.1.4. Talent putting the tubes in the incubator and closing the door. *Videographer: Obtain multiple usable takes because this will be reused in 3.2.2.*

3.2. To disintegrate the tissue fragments, pipette them up and down with a 1-milliliter tip, but do not pipette more than 30 times **[1]**. Repeat the incubation at 37 degrees Celsius **[2]**, then pipette the tissue again **[3]**.

3.2.1. Talent pipetting the tissue up and down. *Videographer: Obtain multiple usable takes because this will be reused in 3.2.3.*

3.2.2. *Use 3.1.4.*

3.2.3. *Use 3.2.1.*

3.3. Add 9 milliliters of N2 medium to each tube to dilute the enzymatic treatment **[1]** and centrifuge the tubes at 200 *x g* for 4 minutes **[2]**. Discard the supernatant **[3]**, wash the cells with 10 milliliters of N2 medium, and repeat the centrifugation **[4]**.

3.3.1. Talent adding N2 medium to a tube, with the medium container in the shot.

3.3.2. Talent putting the tube in the centrifuge and closing the lid.

3.3.3. Talent discarding the supernatant.

3.3.4. Talent washing the cells with N2.

3.4. Remove the supernatant from each tube **[1]** and resuspend the cell pellets of the VZ and DG in 2 milliliters and 1 milliliter of the B27 medium, respectively **[2]**. Filter each cell suspension with a 40-micrometer cell strainer to remove any non-digested tissue **[3]**.

3.4.1. Talent removing supernatant from a tube.

3.4.2. Talent resuspending the cells in one tube.

3.4.3. Talent filtering the cell suspension.

4. Neurospheres Formation

4.1. Culture the filtered cells in an ultra-low attachment, 24-well plate, using two wells for the VZ and one well for the DG **[1]**.

4.1.1. Talent transferring the cells to the plate.

4.2. Add 20 nanograms per milliliter of Fibroblast Growth Factor 2 and 20 nanograms per milliliter of Epidermal Growth Factor to each well **[1]**, then incubate the plate at 37 degrees Celsius, 5% carbon dioxide, and high humidity for 48 hours **[2]**.

4.2.1. Talent adding growth factors to the wells, with both growth factor containers in the shot and labeled. **TEXT: 1 X growth factor concentration** *Videographer: Obtain multiple usable takes because this will be reused in 4.3.3.*

4.2.2. Talent putting the plate in the incubator and closing the door.

4.3. After the incubation, remove half of the culture medium **[1]** and replace it with fresh B27 medium supplemented with double concentration of the growth factors. Repeat this process every third day **[2]**. On days when it is not necessary to change the culture medium, add growth factors to a final concentration of 1 X **[3]**.

4.3.1. Talent removing half of the culture medium.

4.3.2. Talent adding fresh medium, with the medium container in the shot.

4.3.3. *Use 4.2.1.*

Results

5. Results: Neurospheres Derived from Neurogenic Niches of the Adult Prairie Vole

5.1. Neurospheres were formed from neural stem cells isolated from the subventricular zone and dentate gyrus of both female and male adult prairie voles **[1]**. Although there was debris in the primary culture **[2]**, only neurospheres were present after the first passage **[3]**.

5.1.1. LAB MEDIA: Figure 3.

5.1.2. LAB MEDIA: Figure 3. *Video Editor: Emphasize A.*

5.1.3. LAB MEDIA: Figure 3. *Video Editor: Emphasize B.*

5.2. A higher number of neurospheres were obtained from the female subventricular zone than from the male subventricular zone or the dentate gyrus of both females and males **[1]**, suggesting that the number of neurospheres obtained depends on the proliferative zone and the vole sex **[2]**.

5.2.1. LAB MEDIA: Figure 4 A. *Video Editor: Emphasize the female VZ bar.*

5.2.2. LAB MEDIA: Figure 4 A.

5.3. The diameter of the neurospheres was measured on days 8, 11, and 14 **[1]**. It increased progressively for male and female voles in both neuronal regions, but neurospheres derived from the male brains were smaller in comparison to those derived from the female brains **[2]**.

5.3.1. LAB MEDIA: Figure 4 B.

5.3.2. LAB MEDIA: Figure 4 B. *Video Editor: Emphasize the male data in both plots.*

5.4. Adhered neurospheres were characterized on day 6 in the presence of growth factors **[1]** or on day 15 without growth factors **[2]**. At Day 6 under undifferentiated conditions, the neurosphere-derived cells expressed nestin, a marker for neural progenitors **[3]**.

5.4.1. LAB MEDIA: Figure 5. *Video Editor: Emphasize A.*

5.4.2. LAB MEDIA: Figure 5. *Video Editor: Emphasize B.*

5.4.3. LAB MEDIA: Figure 6.

5.5. It was also possible to identify doublecortin-positive cells and the proliferation marker Ki67 *(pronounce 'kee-sixty-seven')*, which indicate the presence of either neuronal precursors or immature neurons **[1]**. However, the lack of colocalization of Ki67 with DCX suggested the presence of postmitotic neuroblasts **[2]**.

5.5.1. LAB MEDIA: Figure 7.

5.5.2. LAB MEDIA: Figure 7. *Video Editor: Emphasize the MERGE images.*

5.6. At day 15 under differentiation conditions, mature neurons **[1]** and cells with the glial phenotype were found **[2]**, demonstrating the differentiation potential of the isolated cells **[3]**.

5.6.1. LAB MEDIA: Figure 8. *Video Editor: Emphasize the MAP2 images.*

5.6.2. LAB MEDIA: Figure 8. *Video Editor: Emphasize the GFAP images.*

5.6.3. LAB MEDIA: Figure 8.

Conclusion

6. Conclusion Interview Statements

6.1. **Wendy Portillo:** When attempting this protocol, keep in mind that being able to identify neuroanatomic regions and having previous practice in microdissection is fundamental to isolate only cells from the neurogenic niches.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.2.2, 2.3.1, 2.4.1, 2.4.2.*

6.2. **Daniela Ávila-González:** Following this protocol, experiments can be designed to identify mechanisms involved in proliferation, differentiation and survival of the neural stem and progenitor cells, processes that are still unknown in the prairie vole.

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