Ex-vivo culture of rodent hippocampus

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Protocol: Organotypic hippocampal cultures - mice

MATERIAL

Equipment:

Forceps, Scissors and Spatulas

ET-OH

60mm petri dishes

Tissue chopper (McIlwain)

Magnifying glass

6 Multiwell plate

Millicell Cell Culture Insert 0.4 μm pore, 30 mm diameter (Millipore corp - PICM03050)

Reagents:

Gey´s balanced salt solution, Sigma - G9779

10x D-glucose (0.250 M) autoclaved

Opti-MEM, Gibco BRL - 31985

Hank's BBS (HBSS), Gibco BRL - 24020-091

horse serum, Gibco BRL - 26050-070

Neurobasal medium, Gibco BRL - 21103-049

L-glutamine, Sigma - 25030-024

B27 supplement, Gibco BRL - 17504-010

L-Glutamic acid monosodium salt, Sigma G1626

Propidium Iodide, Sigma – P4864

Media

Gey´s balanced salt solution + 25 mM D-glucose (9 ml Gey's + 1 ml D-Glucose 0.250 M)

Culture medium (A) - 50% OPTI-MEM, 25% heat inactivated horse serum, 25% Hank's balanced salt solution, glucose 25 mM (7,5 ml OPTI-MEM + 3,75 ml HS + 2,25 ml Hank's + 1,5 ml glucose 0.25 mM)

Culture medium (B) - Neurobasal medium, glucose 25 mM, L-glutamine 1 mM, 2% B27 supplement (13 ml Neurobasal Medium + 1,5 ml D-Glucose 0.25 mM, Glutamine, 0.3 ml B27 supplement 50x)

Procedure

- Prepare Gey's medium + glucose and culture medium (A).
- Pre-heat culture medium (A) at 37ºC and add 1ml to each well of the MW6. Chill on ice Gey's medium and add 5 ml to two 60 mm plate.
- Sterilize the material in ET-OH.
- Mount the blade on the tissue chopper. Sterilize the equipment. Set the thickness to 350 uM and test cut orientation.

Organotypic Culture

- P6-P8 pups are anesthetized and sacrificed by decapitation
- The brain is carefully removed and the hippocampus dissected (both hemispheres).
- Cut the hippocampus in the tissue chopper" transversally at 350 μm thickness. The blade should be wet with Gey's + D-Glucose.
- Hippocampal sections are collected to a 60 mm petri dish with cold Gey's + D-Glucose and carefully separated with the help of a spatula.

- Hippocampal sections well defined and without damage are selected and transfered to the "insert" placed on a 6 well plate with 1 ml of culture medium (A) (OPTI-MEM/Hank's/Horse Serum). The plates should be pre-warmed. Add 0.1 ml of medium to the membrane of the insert. Culture 3-5 sections per insert, well separated of each other.
- The organotypic culture is incubated at 37ºC with 5% CO2 during 7 days, changing media every 2-3 days. Check for the health status.
- On the 7th day, hippocampal sections are changed to culture media (B) (Neurobasal/Glucose/L-Glutamine/B27).

Evaluation of unhealthy cells/neurons

- Propidium iodide is added to the culture medium to a final concentration of 2 uM. Add 1.3 ul of stock solution 1.5 mM. The sections are incubated with propidium iodide for 24h.
- For visualization, the sections are fixed under the hood with PFA 4% for 20 minutes and washed twice (5 minutes) with PBS.
- Neuronal Cell death is evaluated with FluorojadeB:

  - Rinse twice with dH2O for 2 minutes
  - Incubate slices in 0.06% potassium permanganate solution for 10 min
  - Rinse with dH2O for 2 min
  - Incubate the slices for 30 min in a 0.001% solution of Fluoro-Jade C dissolved in 0.1% acetic acid vehicle (Stock solution in the fridge 0.01% (use it within two hours of preparation)
  - Rinse three times with dH_2O for 1 min per change
  - Nuclei is stained with DAPI at 0.1 ug/ml (15 minutes)
  - Dry the slices at RT for 5-10 minutes
  - Clear slices in xylene for at least 1 min and then coverslip with DPX

- A fluorescence microscope equipped with different filters is used. Distinct hippocampal regions are analysed with a special focus to CA1, CA3 and dentate gyrus (DG).