Preparation of Dissociated Hippocampal Culture

References

1. Ferreira, A., Sinjoanu, R. C., Nicholson, A., & Kleinschmidt, S. (2011). Aβ toxicity in primary cultured neurons. *Methods in molecular biology (Clifton, N.J.)*, *670*, 141–153. https://doi.org/10.1007/978-1-60761-744-0_11
2. Ferreira, A and Loomis, P.A. Isolation and culture of primary neural cells. In: "Cells: A laboratory Manual" Cold Spring Harbor Laboratory. Ed. By D. Spector, R. Goldman and L. Leinwand. Pp 9.1-9.13, 1998.
3. Banker, G and Goslin, K. (1998). Rat hippocampal neurons in low-density culture. In: Culturing nerve cells (Banker G, Goslin K, eds), pp339-370. Cambridge: MIT PS.

Solutions

1. Calcium and magnesium-free Hank's balanced salt solution (CMF-HBSS): 1X HBSS (Invitrogen), 9.9 mM HEPES and 1 U/100 μg of penicillin/streptomycin in water. Store solution at 4°C.
2. Neuronal maintenance medium (N2): 1X MEM (Invitrogen), 0.6% D-glucose, 0.1% ovalbumin, 1 mM sodium pyruvate, 5 μg/ml insulin, 20 nM progesterone, 100 μM putrescine, 30 nM selenium dioxide, 100 μg/ml apo-transferrin. Filter-sterilize and store at 4°C. Keep component stock solutions at -20°C.

Abbreviations

1. E18: Embryonic day 18
2. CMF-HBSS: Calcium and magnesium-free Hank's balanced salt solution
3. N2: Neuronal maintenance medium

Protocol

Dissection

1. Sacrifice a pregnant Sprague-Dawley rat, embryonic day 18 (E18; see Notes 1 and 2) using an approved method of euthanasia.
2. Saturate the abdomen of the rat with 75% ethanol before making an incision.
3. Using sterile forceps and scissors, remove the skin to expose the muscular abdominal wall by making a vertical incision from pelvis to thorax.
4. Soak the used instruments with ethanol to remove any hair (see Note 3).
5. Cutting through the abdominal wall, expose the uterus.
6. Remove the uterus of the rat and place it in a sterile 100 mm dish (see Note 4). Continue to work in a laminar flow hood.
7. Remove the fetuses from the uteri using sterile scissors and forceps (Dumont #3).
8. Decapitate the fetuses and transfer the heads to a 60 mm dish containing 3-4 ml CMF-HBSS.

9. To remove the brains, place a Dumont #3 forceps into the orbits to stabilize the head. Using a Dumont #5 forceps, peel away the skin and the skull to expose the brain.
10. Remove the brains and place them onto a drop of CMF-HBSS in the center of a 35 mm sterile dish coated with sterile paraplast (see Note 5).
11. Remove the meninges from each hemisphere (see Note 6).
12. Under a dissecting microscope, remove the meninges of each hemisphere and dissect the hippocampi (see ref. 5).
13. Place the hippocampi in a sterile 60 mm dish containing 3-4 ml CMF-HBSS. Make sure that the tissue is submerged in CMF-HBSS at all times.

Dissociation and Plating

1. Gently aspirate all dissected hippocampi with a sterile transfer pipette and transfer to a 15 ml sterile conical tube containing 5 ml freshly prepared 0.25% trypsin in CMF-HBSS.
2. Incubate in a water bath at 37°C for 15 min. Do not invert or mix the tube's contents.
3. After the incubation, gently remove the trypsin solution and add 5 ml of CMF-HBSS and let stand for 5 min at room temperature. Do not invert or mix the tube's contents.
4. Repeat the wash in step 3 twice more and finally add 0.5 ml of CMF-HBSS per embryo dissected. Do not invert or mix the tube's contents.
5. Using a Pasteur pipette followed by a fire-polished pipette, gently dissociate the cells. To prevent foaming, pipette against the tube wall.
6. Determine the density of the cells using a hemacytometer. The yield should be around 500,000 cells per hippocampus.
7. Add the desired number of cells to prepared coverslips or dishes. We plate the cells at high-density (500,000 cells/60 mm dish) and low-density (150,000 cells/60 mm dish containing coverslips).
8. Swirl the dishes to disperse the cells evenly.
9. Incubate the cells at 37°C with 5% CO_2.
10. After 2–4 h, examine the dishes to ensure that the neurons have attached.

Maintenance

After the neurons have attached (usually 2–4 h), change the media of high density–plated dishes to glia-conditioned N2 media. Transfer low density–plated coverslips, cell-side down, into dishes containing a monolayer of astrocytes that have been conditioned with N2 media (see Note 7 and 8). Keep the neurons for as long as 4 weeks without changing the media.

NOTES

1. We use E18 rat embryos because at this stage, the neuronal migration is complete and the hippocampus is formed. For mouse neuronal hippocampal cultures, we follow the same protocol, except the age of the embryos is 16 days.
2. For glial culture, we use the cortex from the E18 rats to be used for hippocampal culture preparations, to minimize the number of animals sacrificed for each culture. The same culture can be done using postnatal rat pups (1 or 2 days old; ref. 2).
3. Hair and skin are a source of fungal and bacterial contamination.

4. When removing the uterus, be cautious as not to pinch the nearby intestine, which is a source of bacterial contamination.
5. The 35 mm dishes used for dissection of the hippocampi are coated with sterile paraplast to better stabilize the brain hemispheres and to protect the delicate tips of our surgical instruments. We coat each dish with the same volume of paraplast to minimize changes in the focal plane of the dissecting microscope.
6. Meninges are a source of contamination with different cell types, which secrete toxins that do not support neuronal survival.
7. Use the glial cultures for conditioning of the N2 media once the cultures reach 40-60% confluence, usually in 10-14 days.
8. For the long-term survival of neurons, we use N2 media that has been glia-conditioned for 24 h.