Preparation of Primary Neurons from Rat Median Preoptic Nucleus (MnPO)

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[Abstract] Studying cell physiology is really important to understand their function and to determine action mechanisms taking place in these cells. Using brain slices can be sometime difficult to directly access to cells like neurons. Even if it more steps are needed to get cultured cells, this type of preparation allow a better access to neurons and provide a good way to study their basal properties. Here we describe a protocol of a short term primary neurons culture from MnPO, allowing to kept neurons in good condition to perform electrophysiological recordings.

Materials and Reagents

1. Wistar rats (3 weeks old) (Charles River Laboratories International)
2. ketamine-xylasine mixture
3. Pronase (Sigma-Aldrich, catalog number: 9036-06-0)
4. Thermolysin (Sigma-Aldrich, catalog number: 9073-78-3)
5. Bovine Serum Albumin (BSA) (Life Technologies, catalog number: 15561020)
6. Laminin (Sigma-Aldrich, catalog number: 114956-81-9)
7. Ruthenium red
8. KCl (Sigma-Aldrich, catalogue number: 7447-40-7)
9. $CaCl_2$ (Sigma-Aldrich, catalogue number: 10043-52-4)
10. $MgCl_2$ (Sigma-Aldrich, catalogue number:7786-30-3)
11. $NaHCO_3$ (Sigma-Aldrich, catalogue number: 144-55-8)
12. NaH_2PO_4 (Sigma-Aldrich, catalogue number: 7558-80-7)
13. NaCl (Sigma-Aldrich, catalogue number: 7647-14-5)
14. Sucrose (Sigma-Aldrich, catalogue number: 57-50-1)
15. HEPES (Sigma-Aldrich, catalogue number: 7365-45-9)
16. D-Glucose (Sigma-Aldrich, catalogue number: 50-99-7)
17. Dissection solution (see Recipes)
18. Artificial Cerebrospinal Fluid (aCSF) (see Recipes)

Equipment

1. Bath
2. Vibratome
3. 0.45 µm sterilize filter
4. 350 µm Sagittal slice containing MnPO
5. Needle
6. Pasteur pipettes
7. Centrifuge
8. 37 °C, 95% O_2-5% CO_2 Cell culture incubator
9. 6 hole petri dish (Thermo Fisher Scientific, catalog number: 50-341-84)
10. Micro-cover glasses (Thermo Fisher Scientific, catalogue number: NC9216450)
11. 15 ml Falcon tubes
12. Oxygen and Carbogen tank
13. Binocular

Procedure

1. Micro-cover glass is coated with laminin (5 µg/ml) and incubated at 37 °C, 95% O_2-5% CO_2 Cell culture incubator, 3 h before culture preparation.
2. Wistar rats is deeply anesthetized by intraperitoneal injection of a ketamine-xylasine mixture (87.5 and 12.5 ml/kg, respectively) and decapitated.
3. The brain is removed from the skull and immersed in oxygenated (95% O_2-5% CO_2) ice-cold (2 °C) dissection solution. Oxygenation system consist on a 95% O_2-5% CO_2 tank connected to a bath with a line transmitting a gentle bubbling. Then the brain is fixed with glue on a plate which will be place in the Vibratome tub containing oxygenated dissection solution.
4. A sagittal slice of 350 µm containing the MnPO is prepared using Vibratome (Figure 1).

Figure 1. Use Vibratome to do brain slices

5. Using binocular, the ventral region of the MnPO is punched (about 3mm ⌀) out with a curve needle (Figure 2), and placed in 1 ml aCSF solution (in falcon) containing 0.1 mg/ml of

pronase for 10 min at 37 °C and oxygenated.

Note: Curve needle is homemade from an aluminum needle tip 3 mm diameter, gently curved at 2/3 of length with a soldering and fixed to a 10 ml syringe.

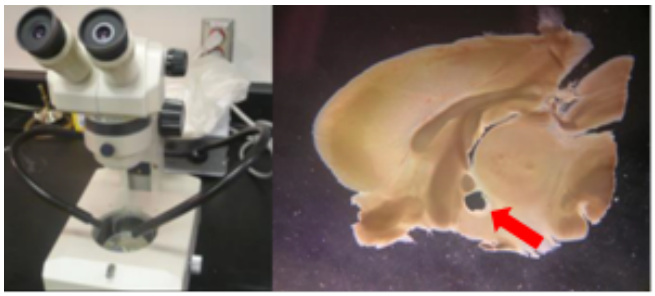

Figure 2. Use Binocular to realize MnPO micropunch

6. Micropunch is transferred to a new falcon tube with 1 ml aCSF solution containing 2 mg/ml of BSA for 15 min at 37 °C and oxygenated.
7. Micropunch is transferred to a new falcon tube with 1 ml aCSF solution containing 0.1 mg/ml of thermolysin for 10 min at 37 °C and oxygenated.
8. Transfer solution containing tissue in a 1.5 ml Eppendorf tube.
9. The tissue is then mechanically dissociated by successive trituration with glass Pasteur pipettes (Figure 3).

   Note: Use 4 Pasteur pipettes whose diameter has previously been gradually reduced with flame from no change to 50 µm. This step is done at room temperature. The come and go flow should be strong enough to separate cells, but also enough gentle to not break cell membrane. The size of micropunch should reduce from pipette to another, and completely disappear using the last pipette.

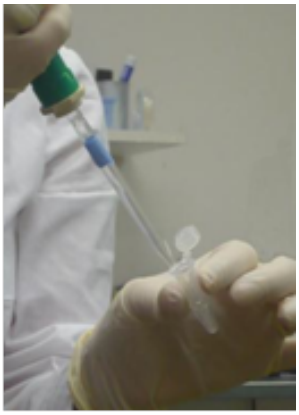

Figure 3. Mechanic trituration with Pasteur pipette

10. Transfer solution containing cells in a 1.5 ml Eppendorf tube.
11. Solution containing cell is centrifuged at 1500 *x g* during 2 min at room temperature.

Note: Sometime pellet is really small and difficult to see, but there are cells on the wall of the tube.

12. The supernatant is removed and cells are re-suspended in 50 µl aCSF.
    Note: Slow agitation for re-suspension to not break the membranes.
13. The aCSF solution containing cells (50 µl) is directly platted on micro cover glasses beforehand treated with laminine, then disposed in 6 holes petri dish, and incubated 1 h at 37 °C, 95% O_2-5% CO_2, before patch-clamp recording.
    Notes:
    a. *There is no culture media, cells remains during 1 h in aCSF solution. Do not exceed 1 h of incubation. Density of cell is relatively low, about 100 cells on 1 micro cover glass.*
    b. *Be sure that the solution stays on the micro-cover glass in bead, if not preparation will be dry and unusable.*

Recipes

1. Dissection solution
   200 mM sucrose
   10 mM D-Glucose
   2 mM KCl
   1 mM $CaCl_2$
   3 mM $MgCl_2$
   26 mM $NaHCO_3$
   1.25 mM NaH_2PO_4, pH 7.4
   Adjusted with CO_2 bubbling and verified with Ruthenium red
   Filter sterilize (0.45 µm)
   Stored at 4 °C
2. Artificial cerebrospinal fluid/aCSF
   140 mM NaCl
   3.1 mM KCl
   2.4 mM $CaCl_2$
   1.3 mM $MgCl_2$
   10 mM HEPES
   10 mM D-Glucose, pH 7.4 adjusted with KOH, osmolarity 300 MOsm
   Filter sterilize (0.45 µm)
   Stored at 4 °C

Acknowledgments

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References

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