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Primary cortical neuron isolation and culture

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Shiyi Wang[1]

[1]Duke University

ASAP Collaborative Rese…

Shiyi Wang
Duke University

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Abstract

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1. Cortices from P1 rat pups of both sexes (Sprague Dawley, Charles River Laboratories, SD-001) were micro-dissected.

2. Digested the cortices in papain (~7.5 units/ml) at 33°C for 45 minutes

3. Triturated the cortices in low and high ovomucoid solutions and resuspended in panning buffer (DPBS (GIBCO 14287) supplemented with BSA and insulin) and passed through a 20 µm mesh filter (Elko Filtering 03-20/14).

4. Filtered cells were incubated on negative panning dishes coated with Bandeiraea Simplicifolia Lectin 1 (x2), followed by goat anti-mouse IgG+IgM (H+L) (Jackson ImmunoResearch 115-005-044), and goat anti-rat IgG+IgM (H+L) (Jackson ImmunoResearch 112-005-044) antibodies, then incubated on positive panning dishes coated with mouse anti-L1 (ASCS4, Developmental Studies Hybridoma Bank, Univ. Iowa) to bind cortical neurons.

5. Adherent cells were collected by forceful pipetting with a P1000 pipette. Isolated neurons were pelleted (11 minutes at 200 g) and resuspended in serum-free neuron growth media (NGM; Neurobasal, B27 supplement, 2 mM L-Glutamine, 100 U/ml Pen/Strep, 1 mM sodium pyruvate, 4.2 µg/ml Forskolin, 50 ng/mL BDNF, and 10 ng/mL CNTF). 70,000 neurons were plated onto 12 mm glass coverslips coated with 10 µg/ml poly-D-lysine (PDL, Sigma P6407) and 2 µg/ml laminin and incubated at 37°C in 10% CO_2.

6. On day in-vitro (DIV) 2, half of the media was replaced with NGM Plus (Neurobasal Plus, B27 Plus, 100 U/mL Pen/Strep, 1 mM sodium pyruvate, 4.2 µg/ml Forskolin, 50 ng/ml, BDNF, and 10 ng/ml CNTF) and AraC (10 µM) was added to stop the growth of proliferating contaminating cells.

7. On DIV 3, all the media was replaced with NGM Plus. In experiments involving lentivirus infection, 100 µl of supernatant containing lentivirus plus polybrene (1 µg/ml) was added to the AraC NGM mixture on DIV 2 and completely washed out on DIV 3 and replaced with NGM Plus containing 100 ng/ml BDNF.

8. Neurons were fed on DIV 6 and DIV 9 by replacing half of the media with NGM Plus.