Slice overlay assay

[as used in Polleux *et al.* (1998) Science 282:1904.]

Cortical slices

Embryonic day 18 (E18) to postnatal day 3 (PND3) rat brains were rapidly isolated in ice-cold HBSS and then embedded in 2.5 percent low-melting point agarose (diluted in HBSS) placed on ice to accelerate solidification. 250 microns thick coronal sections were performed using a vibratome, filled with HBSS.
Sections are collected using a droper (large opening flamed-tip Pasteur pipette) and platted onto a membrane insert of a 6-wells plate (Becton Dickinson-1 micron pore size). Put 1.8 mls of slice culture medium beneath the insert about 30 minutes before platting and leave the plate in a cell culture incubator (37°C-5 percent CO2).

Dissociated cells

After enzymatic dissociation of E18 rat cortex (see dissociation protocol), cells were labeled with the carbocyanine fluorescent dye DiI (Molecular Probes; diluted 10 mg/ml in 100 percent ethanol). Briefly, 10 microliters of the DiI stock solution (ultrasonicate right before using it) is added to 1 ml of cell suspension (5x106 cells/ml) and incubated for 15 minutes @ 37°C. Then the cell suspension is centrifuged gently (for 15 ml conical tubes, 1000 rpm-5 minutes @ room temperature) and the supernatant is then discarded. The pellet is gently resuspended in 5 mls of fresh, prewarmed cell culture medium. This washing step is repeated 3 more times to get rid of DiI microscopic crystals.
After the final wash, cells are resuspended @ a final concentration of 5x105 cells/ml and platted onto cortical slices prepared about an hour before the cells. The platting part can be tricky ! Delicately pipet about 400 microliters of the DiI labeled cells solution onto and around the slice. Don't drop the solution ! Shake the plate in X and Y axis gently three or four times and then put the plate in a cell culture incubator. The axon outgowth can be scored typically after 3-5 hours using live cell imaging.

Solutions

Hank's Balanced Salt Solution (HBSS)
10X HBSS (Gibco #310-4180) 50 ml
1 M Hepes (pH 7.4) 1.25 ml (=2.5mM)
1 M Glucose 15 ml (=6.5 mg/ml=35 mM)
100 mM CaCl2 5 ml (=1mM)
100 mM MgSO4 5 ml (=1mM)
1 M NaHCO3 2 ml (=4mM)
Add ddH2O to a total vol. of 500 ml. Filter sterile.

Slice culture medium for E18 to P7 rat brain.
For 50 mls:
34.5 mls Basal Medium Eagle (without L-glutamine)
12.5 mls HBSS (see above)
20 mM Glucose
1 mM L-glutamine
1mM Penicilin-Streptomycin
Filter sterile then add 5 percent Normal Horse Serum (heat inactivated).
For postnatal slice culture add kynurenic acid/Mg2+ (broad NMDA antagonist to prevent glutamate induced excitotoxicity) to the medium.

Cell culture medium for rat E18 culture.
See solutions for cell culture.