Meyer Lab Hippocampal Prep, 9/07/01

PREPARATION OF COVERSLIPS

1) Use 25mm round Assistant brand coverslips (Carolina Biological, No. 1).
2) Sterilize coverslips by soaking in ethanol followed by flaming, and place coverslips in 6-well dishes (Falcon).
3) Prepare 0.1 mg/ml solution of Poly-L-Lysine (Sigma) in water. Pass through 0.22 filter to sterilize.
4) Immerse coverslips with the Poly-L-Lysine solution overnight at 37°C.
5) Next day, wash coverslips 3 times (for at least 30 minutes) with sterile water.
6) Before the dissection, remove the water and let the coverslips air-dry.

SOLUTIONS:

PLATING (for 200 ml total)
178 ml MEM (Earle's salts w/o L-glutamine, but with phenol red)
10 ml FBS (heat inactivated)
4 ml B-27
200 ul "Serum Extender" (Becton Dickinson; Cat. # 355006)
4 ml glucose (1 M stock in MEM)
0.5 ml Pen/Strep (Gibco, Liquid, 10000 u/1 ml)
1 ml Na-pyruvate (100 mM stock in MEM, obtained from Gibco)
2 ml HEPES (1 M stock in MEM)

Prepare solution no sooner than the day before the plating. Pass through 0.22 um filter.

DISSECTION
Earle's Balanced Salt Solution (Hanks is a reasonable substitute) + 10 mM HEPES, pH 7.4.

Pass solutions through 0.22 um filter. Stocks of trypsin (2.5%; Gibco) and Dnase I (Sigma) are stored frozen, to be added to the solution immediately before use.

RAT HIPPOCAMPAL NEURON PREP

1) Use 1-2 day old pups. Survivability is compromised at older ages. Typical yield is about 300-500K cells per hippocampus, and we want to plate 25-40K on each coverslip.
2) Dissect out CA1-CA3 hippocampus on ice-chilled dissection media. Store each hippocampus on ice until ready for digestion.
3) Cut the hippocampi into 7-10 pieces each.
4) Add 600 ul of trypsin stock and 6 ug of DNAse stock to the dissection media (with minced hippocampi) to a total of 5 ml solution. Shake gently for 10-15 minutes at 37 ^{o}C.
5) Wash 3X in plating media.

6) Triturate in 4 ml of plating media with a 1000 ul pipette. Be careful not to exert too much force during trituration (don't make bubbles).
7) Count the density of cells. Take a small sample of the cell suspension (10 ul) and add 10 ul Trypan Blue. Add 10 ul to each side of the hemocyometer. The number of cells in the 5x5 grid X 2 (dilution factor) X 10^4 = cells/ml.
8) Add enough of the cell suspension so that there are 25-30K cells per coverslip.
9) After 1-3 days, check to make sure that the glia have spread out. Add 45 ul of FUDR mixture to each coverslip (in 6-well dishes) to inhibit further glia proliferation.

FUDR Mixture
5 mg 5-fluoro-2-deoxyuridine (8 mM stock)
12.5 mg uridine (20 mM stock)
2.5 ml MEM