Hippocampal Cell Cultures

Preparation:	**Typical Schedule**
1. Place coverslips in staining racks (Thomas Scientific* #8542-E40) and rinse 4X1 minute in milliQ or comparable grade water. Clean in concentrated HNO_3 for 30 to 40 hrs. the standard coverslip we use is an 18 mm circle.	Mom. /sat
2. Rinse 2 X 1 hr, then 2X ½ hr. in H_2O. Tap off excess H_2O and dry in oven Wed./Mon.	
3. Place racks in beakers, cover with foil, and sterilize with dry heat (225° for 6 hrs.)	Wed./Mon.
4. Place coverslips in 60 mm. Plastic Petri dishes, 5 per dish (use microbiological dishes, such as Falcon* # 1007, not tissue culture grade). Apply 3 small drops of sterile, melted paraffin to the coverslips. These paraffin beads keep the conerslips from resting directly on the glial cells during co-culturing. Sterilize by UV irradiation for 30 min.	Thur/Tue.
5. Dissolve polylysine (Sigma* P2636), 1 mg/ml in borate buffer and filter sterilizer. You will need 1-2 mls. Per dish. Cover each coverslip with polylysine solution	Thur/Tue.

(about 6 drops will do) and leave over night (about 18 hours). When prepared properly, the coverslips will be hydrophilic so that the polylysine solution spreads over the surface rather than beading up.

6. Rinse coverslips with sterile H_2O, two changes, 2 hours each. Fri./Wed.

7. Remove final rinse and add 4 ml of MEM with 10% horse serum to the dishes containing the polylysine-treated coverslips. The hippocampal neurons will be plated into these dishes. Fri/Wed.

8. One day before you plan to prepare hippocampal cultures, select the dishes containing a confluent monolayer of glial cells to be used for co-culturing. Remove the medium from these dishes, add 6 ml. of N2.1, and return to incubator. Mon/Thur.

9. Set out cultures (see next page). Tues./Fri.

Comments: We typically set out cells twice a week, on Tuesdays and Fridays. Our typical schedule for preparations is in the column at the right. We have *not* rigorously tested all of the variables involved, and *do not* intend this as a definitive protocol for culturing hippocampal neurons. Rather it is the protocol currently in use in our lab.

DISSECTING AND PLATING:

1. Euthanize the pregnant rat with halothane or other approved anesthetic, remove uterus and place in a sterile petri dish. The ideal stage is 18 days of gestation when the fetus is about 25 mm from crown to rump. Measure length to confirm the accuracy of timing. (In our nomenclature, the day of sperm positivity is day 0. Some suppliers regard this day 1; in their nomenclature, we use animals at E19.)

The remaining steps are done in a laminar flow hood.

2. Decapitate fetuses and dissect out the brains.

3. Place the brains in BSS I small petri dishes. We fill the dishes with paraffin to make a better surface for dissection.

4. Remove the hippocampi under a dissecting microscope. Strip away the meninges, and then collect the dissected hippocampi in a small petri dish in BSS.

5. Place all of the hippocampi from one litter in a 15-ml. centrifuge tube. Remove the BSS and add 5 ml of .25% trypsin. Incubate at 37° for 15 minutes.

6. Remove trypsin, add 5 ml of BSS, and let stand for 5 minutes. Repeat this step twice more. Finally, bring the volume to 3 to 5 ml.

7. Dissociate the cells by pipetting up and down, first in a normal Pasture pipet, and then in a pipet whose tip has been fire polished to about half the normal diameter. Continue pipetting until no chunks of tissue remain.

8. Determine the density of cells in a hemacytometer. The yield should be 500,000 cells per hippocampus.

9. Determine the fraction of viable, trypan-blue excluding cells. We prepare a stock solution of 0.8% trypan-blue in 0.9% NaCl. This is then diluted 1:9 with BSS, mixed one to one with a few drops of

the cell suspension, and allowed to stand 4 minutes before counting the fraction of dye-excluding cells. When things are working well, 85 to 95% of the cells should exclude trypan-blue.

10. Add the desired number of viable cells to each of the dishes containing the polylysine-treated coverslips in MEM with 10% horse serum. Our standard plating density is 150,000 cells per 60 mm plastic petri dish.

11. After 3 to 4 hours, transfer the coverslips, with neurons attached, to dishes containing glial cells in N2.1 medium. Turn the coverslips over so the neurons are facing down, toward the glia.

12. To reduce glial proliferation, we usually add araC (5 X 10^{-6} M) after 2 to 3 days.

13. When feeding it is essential <u>not</u> to change the culture medium completely; the neurons depend on a "conditioning" of the medium by the glial cells for their long term survival. We routinely exchange about 2ml of medium once a week.