Generation of Brain Slices

(Modified from Hevner lab protocols)

1. Setup
   a. 2 hr before dissections:
      1. Prepare 1X Krebs (1000 ml) and cool on ice.
      2. Prepare 100 ml of *sterile filtered* Krebs buffer and cool on ice.
      3. Prepare 100 ml *serum–supplemented* medium, and place in incubator set at 37°C and 5% CO_2.
      4. Prepare 100 ml *serum–free* medium, and place in incubator set at 37°C and 5% CO_2.
   b. 30 min before dissections:
      1. Prepare 50 ml of 4% low melt agarose in PBS with glucose. To prepare: slowly add 2 gm low melt agarose to 50 ml PBS, while stirring rapidly with a stir bar. Remove stir bar and microwave to a boil. Add glucose (5 mg/ml). Place in 40°C water bath.
   c. 15 min before dissections:
      1. Set up vibratome area: sterile weighing spatulas, razor blades, scalpel, fine paintbrush, small ice bucket with sterile petri dish for collecting sections.
      2. Set up dissection area: absorbent pads, sterile petri dishes, sterile tools (scalpel, probe, small scissors, 2 small forceps, 1 #5 forceps), 800 ml of 1X Krebs on ice, plastic embedding molds pre-labeled with experiment and embryo identifying numbers.

2. Euthanize pregnant female by CO_2 followed by cervical dislocation.

3. Remove the embryos and place them in ice–cold Krebs buffer in a Petri dish on ice.

4. Working in ice–cold Krebs buffer, dissect the brains and embed them (coronal plane) in 4% low melting point agarose in plastic embedding molds. Place the embedding molds on ice to gel the agarose.

5. After the agarose hardens (~5 min), remove the embedded brain and agarose and trim for vibratome sectioning. To obtain sections with intact thalamic axons, the brain should be cut at an angle tilted ~30° forward from the coronal (i.e., top of plane more anterior than bottom of plane).

6. Fill Vibratome well with ice-cold Krebs buffer, and surround well with ice. (Change the Krebs buffer and add more ice between brains.)

7. Trim agarose to within ~2 mm of brain. Cut thick sections (250-300 μm) and collect sections with spatulas into serum–supplemented medium in a sterile Petri dish on ice.

Preparation for brain slice culture

8. Select sections that contain the brain region of interest, and transfer them onto polycarbonate membranes (shinny side up), on the surface of serum–supplemented medium (1 ml) in culture plates. Slices must be in incubator within two hours of initial dissection.

****Make sure top of membranes remain dry except for agarose and brain. If they become wet, they will submerge, with poor viability****

9. After incubating slices in serum–supplemented medium for 1-2 hrs, exchange for serum–free medium and place back in the incubator.

10. Slices can now be used for DiI injection, electroporation, or explant culture.

11. To prepare explants:
    a. Drain well of culture medium.
    b. Using a sterile scalpel, dissect tissue regions of interest. Agarose can also be cut free.
    c. Transfer tissues of interest to the same polycarbonate membrane, and orient appropriately.

12. Slices or explants can be maintained in vitro for 3-4 days, after which contamination and cell death become apparent.

Solutions and media

10X Krebs buffer (autoclave; can be stored at 4°C for 2-3 months)

NaCl	73.6 g/l (126 mM at 1X)
KCl	1.87 g/l (2.5 mM at 1X)
NaH2PO4•H2O	1.66 g/l (1.2 mM at 1X)
MgCl2	2.44 g/l (1.2 mM at 1X)
CaCl2	3.68 g/l (2.5 mM at 1X)

1X Krebs buffer (use same day as prepared)

10X Krebs	100 ml/l
Glucose	1.98 g/l (11 mM)
NaHCO3	2.1 g/l (25 mM)
Dilute to 1000 ml	

Sterile filtered Krebs buffer (use same day as prepared)

1X Krebs buffer	98 ml
HEPES 1M	1 ml
Pen/strep 100X	1 ml
gentamicin	200 μl

Sterile filter at pore size <1 μm

Serum–supplemented medium (use same day as prepared)

MEM with glutamine (MEM = modified Eagle's medium; Gibco)	88 ml
Fetal calf serum	10 ml
50% glucose	1 ml
Pen/strep 100X	1 ml
Gentamicin	200 μl

Serum–free medium (use same day as prepared)

Neurobasal medium	95 ml (Gibco)
B-27 supplement	2 ml (Gibco)
50% glucose	1 ml
100X glutamine	1 ml
Pen/strep 100X	1 ml
Gentamicin	200 μl

50% glucose (filter sterilize into 10 ml Falcon tube; store at 4°C for up to 1 wk)

5 g glucose

H_2O with stirring to 10 ml final volume.