Sectioning of brain tissue for diagnostic work.

1. Purpose

The purpose of this method is to section brain slices frozen for later anatomical analysis. (Earlier method is explained in Tissue preparation method). The brains were removed, fixed a few hours in 4% paraformaldehyde and then cryoprotected overnight in 30% (w/v) sucrose. The brains were sectioned on a freezing microtome either coronally or sagitally @ 30 µM

2. Procedure

2.1 Setting up the machine

A sledge microtome (Leica) is used with a freezing stage.

- Turn on water and then turn on freezing stage – set temp by pressing the set button and then use ◄ the select field. Press ▲ or ▼ to increase or decrease temp setting Finally press the set button again to complete.
- Always set to -35°C when mounting tissue and freezing tissue then slice tissue at -20°C.
- With platform at -35°C, place a drop of sucrose onto platform and then overlay immediately with a small square of filter paper to make platform for tissue. (This stops the blade from coming down low enough to hit the metal stage and then damaging it).
- Knife is stored in oil to prevent rusting therefore wash and wipe with 70% EtOH before use.
- Handle at left of stage turns around while slicing, this can therefore be turned anticlockwise to lower and clockwise to heighten the stage depending on the tissue size, position..
- Side dial adjusts the slice thickness turn to adjust. (Normally slice about 30µM).
- Cut slices into PBS, usually use 3 wells slicing consecutive sections onto consecutive wells.

2.2 Slicing

- Remove brain from sucrose onto a flat surface.
- Using a sharp razor blade cut brain in half (one half slice coronal, other slice sagital). If adult brain cut of the brain stem when sectioning coronally before mounting.
- Dry off excess fluid with tissue.
- Platform already made and stage still set at -35°C, drop some sucrose onto platform and then brain straight into it positioning in orientation which you wish to slice. i.e. coronal straight up with olfactory bulbs pointing upwards.
- Spray rest of tissue with cryo spray. (Cryo spray- Bright 300ml bottle)
- N.B. Use as little sucrose as possible as this blunts the blade when slicing through it therefore use enough only for embedding tissue into orientation and use Cryo spray to freeze tissue which extremities are a distance from the platform, most of the tissue should freeze as the sucrose should be absorbed and through the tissue.
- Raise stage until tissue is just below blade.

- Change the settings for the stage to -20°C for slicing, should go down fairly fast.
- Move handle attached to blade back to set correct thickness (i.e. dial set at 30μM).

  N.B. When lowering stage using handle hold blade in place with handle as this sometimes moves also and could damage tissue.
- Slice through tissue, lift section off using a PBS wet brush (downward stroke), then wipe blade (downward stroke) with tissue to dry (this ensure the tissue does not get left over PBS frozen on top of it when moving the blade backwards across the tissue).
- Once completed slicing remove left over tissue from platform (leaving filter paper on platform for next tissue sample) using a blade. Freeze stage back down to -35°C and repeat mounting.

3. Materials

- Leica SM2000R sledge microtome with BFS-5MP freezing stage.
- Solid blade for use on the microtome.
- Dulbecco's phosphate buffered saline (PBS).
- Brushes, disposable blades for cutting the tissue and 6 well disposable plastic plates.

4. Quality Control

Visually you will be able to tell whether the sectioning is going well or not.

5. Examples of Data

Not applicable

6. Supporting Information

7. Document History

This document was created by Karen Porter on 25 February 2008