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Serial Sectioning of Mouse Brain

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ABSTRACT

This protocol highlights sectioning 40 micron mouse brain tissue to be used for histology experiments.

GUIDELINES

Make sure to punch the hole in the non-injected side of the brain. That will allow to quantify the staining much easier later. If injected bilateral, then do not punch the hole. The hole location will be different for different parts of the brain: for striatum it will be in the cortex, and for SNpC in the middle.

MATERIALS

- Sliding microtome HM450 (Thermo Scientific)
- Microtome blades (Thermo Scientific , e.g. Epredia MX35 Premier Microtome Blades)
- Microtome metal stage
- Two buckets of dry ice with cover (one for crushed dry ice, and one for the regular dry ice)
- Dry ice
- Foil
- Paint brush
- Large Petri dish with phosphate Buffered Saline (PBS)
- 30% sucrose
- 50% glycerol in phosphate buffered saline (PBS) (antifreeze solution)
- 24 or 12 well plates (for sectioning 1/6 or 1/12 of the brain)

MANUSCRIPT CITATION:
Williams GP, Schonhoff AM, Jurkuvenaite A, Gallups NJ, Standaert DG, Harms AS. CD4 T cells mediate brain inflammation and neurodegeneration in a mouse model of Parkinson's disease. Brain. 2021 Aug 17;144(7):2047-2059. doi: 10.1093/brain/awab103. PMID: 33704423; PMCID: PMC8370411.

Schonhoff, A.M., Figge, D.A., Williams, G.P. *et al.* Border-associated macrophages mediate the neuroinflammatory response in an alpha-synuclein model of Parkinson disease. *Nat Commun* **14**, 3754 (2023). https://doi.org/10.1038/s41467-023-39060-w

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We use this protocol and it's working

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PROCEDURE

1 Label 24 or 12 well plate with appropriate identifiers (date, project, region to be sectioned etc.) and fill each well ⅔ full with anti-freeze solution (50% glycerol in PBS).

2 Freeze microtome stage on the dry ice.

3 Crush dry ice for the sprinkling the stage later

4 When ready place the stage into place, level it. Put the dry ice into it to keep it cold while working.

5 Put the microtome blade into the place, keep it covered not to get injured.

6 Place few drops of sucrose on the cold stage and put your brain on it. The brains bottom will be hindbrain, and the top of the brain will be the forebrain. The brain lateral side will be facing you, and dorsal away from you. Put few more drops of sucrose to support the brain. Sprinkle crushed dry ice around the brain and cover the whole stage with foul for 2-3 min, to fully freeze.

7 Prior to sectioning, adjust the thickness setting, all sections will be 40 microns thick, and trimming has to be 80 microns. This knob is found on the side of the microtome.

8 Slowly move the blade towards you, slicing a section of the brain. To prevent the sections from folding and breaking, slice slowly and in a single continuous motion.

9 Gently scoop the brain section away from the blade, using a thin painting brush. Slowly place the brush

into antifreeze solution. Gently move the brush to allow the section to unfold in the liquid. Make sure the sections go into appropriate wells.

10 Continue slicing until you have sliced the entirety of the brain.

11 Secure the well plate with tape. Store brain sections in antifreeze solution at -20C.