Neuroanatomical studies using Nissl staining

1. Purpose

The purpose is to study and look for any gross neuroanatomical phenotypes in the mutants compared to wild types. This method uses cresyl violet for the detection of Nissl body in the cytoplasm of neurons. The Nissl body will be stained purple-blue.

This stain commonly performed to identify the basic neuronal anatomy of several area's of the brain allowing us to visually and by cellular counts and statistical analysis make assessments as to whether the structures are altered.

2. Procedure

Every 3rd frozen 30µm free floating Sagital and Coronal section is used to carry out this study.

This is carried out on initially 4 mutant and 4 Wt mice. 1 brain can be used for sagital and coronal sections.

- The serial sections are mounted into positively charged slides and left to dry o/n.
- The next day the slides are washed with PBS.
- They are then place in Nissl Stain for 5-15mins.
- The tissue on the slides are then dehydrated by washes of 30%, 50%, 70% and 90% EtOH for 1min each.
- Then dehydrate in 100% EtOH for 5mins.
- Clear in Xylene for 5mins x 2 washes.
- Remove from Xylene and immediately mount (do not let dry) in of few drops of DPX solution.
- Leave o/n (24-48hrs) @ RT to dry.

3. Materials

3.1: Preparation of Acetate Buffer pH4.45

Nissl stain is made up in acetate Buffer pH4.45

0.2M Acetic Acid: 1.7mls Acetic Acid + 148.3mls 25% MeOH
0.2M Na Acetate: Make IM Stock (4.1g in 50mls DDW). Mix 20mls IM Na Acetate + 80mls 25% MeOH

3.2: Preparation of Nissl stain

- Mix 120mls of 0.2M Acetic Acid with 80mls of 0.2M Na Acetate.
- Add 1 g Nissl (Cresyl Violet) powder

Supplier: Sigma – C5042 Nissl (Cresyl Violet)

3.3: Dulbecco's phosphate buffered saline (PBS).

3.4: DPX moountant for microscopy – VWR 360292F

3.5: Staining dishes and racks.

4. Quality Control

- Visually you will be able to tell whether the staining has worked well or not.
- All the staining is carried out on 4 mutant and 4 wild type samples.

5. Examples of Data

Published work using the above method:

Barnett MW*, Vitalis T*, Porter K*, Watson RF, Komiyama NH, Grant S.G.N. & Kind P.C. (2005) SynGAP regulates pattern formation in the trigeminal system of mice. Submitted, Journal of Neuroscience.
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Komiyama N, Watabe AM, Carlisle HJ, Porter K, Charlesworth P, Monti J, Strathdee DJC, O'Carroll CM, Martin SJ, Morris RGM, O'Dell T & Grant SGN. (2002) SynGAP Regulates ERK/MAPK Signaling, Synaptic Plasticity, and Learning in the Complex with Postsynaptic Density 95 and NMDA Receptor Journal of Neuroscience 22: 9721-9732 PMID: 12427827

6. Supporting Information

7. Document History

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