Expression studies of specific genes

1. Purpose

The precise expression pattern for many genes expressed within brain tissue is still largely unknown especially for the specific genes within gene families. This information can be very important especially when studying gene function in specific regions of the brain and can determine or alter the path by which studies or priorities of these genes would take.

By taking advantage of knock-in mice carrying the IRES-lacZ reporter gene under the control of endogenous promoters, it is possible to perform a comprehensive analysis of the gene expression pattern in the adult brain tissue as well as developing CNS.

Since a number of reporter knock-in mouse lines generated by the G2C program whether it is by the use of gene trap (Sanger Team87 + Baygenomics) or gene targeting experiments have been significant, it will be very informative to carry out a systematic analysis of expression for these genes within the brain. This will help us to understand the differential biological pathways regulated by NMDA receptor and its interacting molecules.

This genetic method does not relay on the quality and specificity of the probes such as antibodies or oligonucleotides, as this only detects the signal driven by endogenous gene promoters.

G2C program will also create a number of conditional (Floxed) alleles these therefore would not have the reporter gene integrated. Therefore in these cases immunohistochemical methods will be required to detect the endogenous expression pattern as well as to score the level of gene activation. Antibodies will either be bought in from a supplier or specifically tailored to the gene (i.e. in case of studying family genes a lot can be very similar and therefore will require the generation of a specific Ab for each family member). For details on this method see Neuroanatomical studies.

2. Procedure

30µm free floating Sagital sections and Coronal sections are sectioned and every 3rd section is used to carry out this study.

This is carried out on initially ~3 mutant and 2 Wt mice. 1 brain can be used for sagital and coronal sections.

- Mix together the X-Gal staining solution mix just before use.
- Remove the PBS from the sectioned tissue using a fine tipped pastette and a brush to ensure the tissue is not taken up by the pastette. Or using a brush gently lift each section out of the well of PBS into another with the x-gal solution.
- Added about 2-2.5mls of staining mix to each appropriate well (this is based on 6 well plate).
- Covered plates in tinfoil and placed on belly dancer o/n at RT.
- Next day remove stain wash the sections in PBS and then place into fresh PBS or 4% paraformaldehyde fix until ready to counter stain or mount onto slides.
- Mounted sections onto positively charged slides and leave to dry o/n.

- Coverslip the slides the next day by: Dehydrating tissue on slides in EtOH (2 X 5mins) clearing tissue in Xylene (2x 5mins) and then Cover slip in a few drops of DPX solution. Leave to dry for 24-48hrs.

3. Materials

3.1: Preparation of X-Gal staining mix

- 22mls 0.1MPBS
- 25µl 1M $MgCl_2$
- 0.5mls 2% X-Gal (made up in DMF)-Melford MB1001
- 2.5mls Ferri/Ferrocyanide mix*

**82mg Kferricyanide (Sigma 393517) in 2.5mls DDW*
105mg Kferrocynanide (Sigma P3289) in 2.5mls DDW.
Mix both together.

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

3.3: DPX mountant for microscopy – VWR 360292F

4. Quality Control

- All animals which should be expressing the knock-in mice carrying the IRES-lacZ reporter gene (i.e knockout mice) are stained along side a wild type littermate mouse which will not have this reporter gene and therefore should give no stain.
- All the X-gal staining is carried out on 4 mutant and 4 wild type samples.

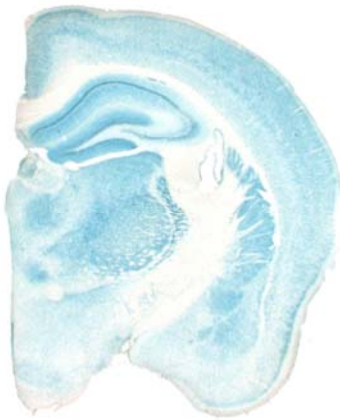
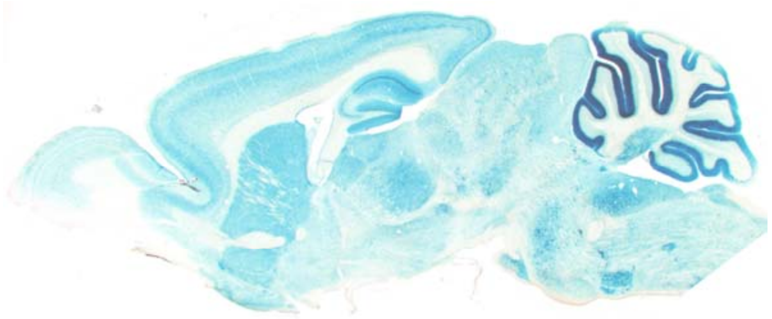
5. Examples of Data

Published work using the above method:

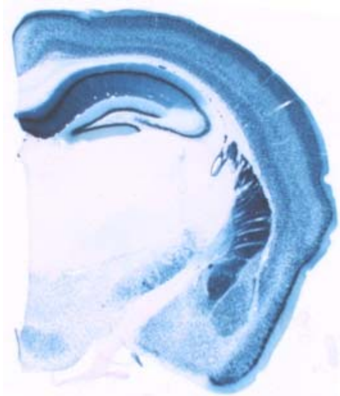
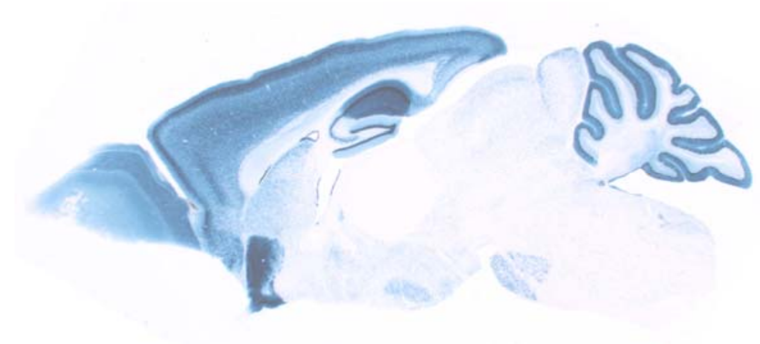
Barnett MW*, Vitalis T*, Porter K*, Watson RF, Komiyama NH, Grant S.G.N. & Kind P.C. (2005) SynGAP regulates pattern formation in the trigeminal system of mice. Submitted to Journal of Neuroscience.
** These authors contributed equally to this work.*

Porter K, Komiyama NH, Vitalis T, Kind PC and Grant SGN(2005). Differential expression of two NMDA receptor interacting proteins, PSD-95 and SynGAP during mouse development. European Journal of Neuroscience 21: 351-362. PMID: 15673435

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

Gene A

Gene B

Gene C

6. Supporting Information

7. Document History

This document was created by: Karen Porter 22.02.08