Neuroanatomical studies using antibodies

1. Purpose

The purpose is to study and look for any gross neuroanatomical phenotypes in the mutants. The neuronal architecture in the hippocampus can be looked at using the synaptic-terminal marker synaptophysin, the dendritic marker MAP2 and a number of glutamate receptor antibodies.

The 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)

Immunohistochemistry was performed using the avidin-biotin-peroxidase complex method (ABC) (Vectastain Elite kit, Vector Laboratories, Burlingame, CA). This method was employed as it provides a simple and sensitive method to help localise antigens in the fixed tissue. This method involves the application of a biotin labelled secondary antibody followed by the application of avidin-biotin-peroxidase complex. This method exploits the high affinity avidin has for biotin and in addition avidin has four binding sites for biotin which in turn forms a web structure containing several peroxidise molecules. Therefore this results in an amplification of the antibody signal giving a high staining intensity. This method gives a superior result when compared to the unlabeled antibody method.

2. Procedure

30µm free floating Sagital sections are taken from Interaural 1-2 and Coronal sections are taken from around Interaural 2.74-0.52 (1.74 main view)

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

- Transfer sections into 24 well plate using Blue needle bent at end or a fine brush.
- Incubate sections in 200µl of appropriate primary Ab diluted in PBS with 0.5% Triton o/n at RT on belly dancer. Dilution varies depending on the Antibody.
- Remove solution from well using fine tipped pipette (sections stick to base). Repeat this throughout the method for each was or incubation.
- Wash 3 X 5mins in 300µl of PBS with 0.5% Triton.
- Incubate in 200µl of appropriate secondary Ab (Biotin labelled) diluted 1 in 200µl, in PBS with 0.5% Triton @ RT for ~2hrs. i.e. if primary is mouse then secondary has to be mouse-conjugated biotin etc.
- Wash 3 X 5mins in 300µl of PBS with 0.5% Triton.
- Incubate in ABC solution (add 2 drops of A into 10mls PBS with 0.5% Triton and 2 drops of B into same mix - solution made up 30mins before use) @ RT for 30mins.
- Wash 3 X 5mins in 300µl of PBS with 0.5% Triton.
- Make up DAB mix just before use. DAB treated for up to 20mins.
- Stop reaction using 0.1% Na Azide in PBS or by lifting sections out into PBS solution only.

- 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 ABC mix

Followed manufacturers method: Add 2 drops of A into 10mls PBS with 0.5% Triton, mix and then add 2 drops of B into same mix - solution is made up 30mins before use.

3.2 Preparation of DAB mix

1 tablet dissolved in appropriate amount i.e. some are for 15mls some for 5mls of DDW only or ½ DDW + ½ 0.2M PB. Add 0.003% H2O2 therefore added 1ml of diluted 10µl 30% H2O2 into 10mls.

Dulbecco's phosphate buffered saline (PBS).

Avidin-biotin-peroxidase complex kit (ABC) – PK-4000 (Vectastain Elite kit, Vector Laboratories, Burlingame, CA).

DAB tablets - Sigma Code: D5905 (3, 3'-diaminobenzidine tablets)

30% Hydrogen Peroxide solution (H_2O_2) Sigma Code: H1009.

4. Quality Control

- Controls are always run alongside the Mutant and Wild type samples. There is always a control with water only instead of primary antibody, the secondary antibody would be used with this. In addition there is always a control with primary antibody used as normal and then water only instead of the secondary antibody. This then checks for any none specific binding of either antibody.
- All the antibody staining is carried out on 4 mutant and 4 wild type samples.

5. Examples of Data

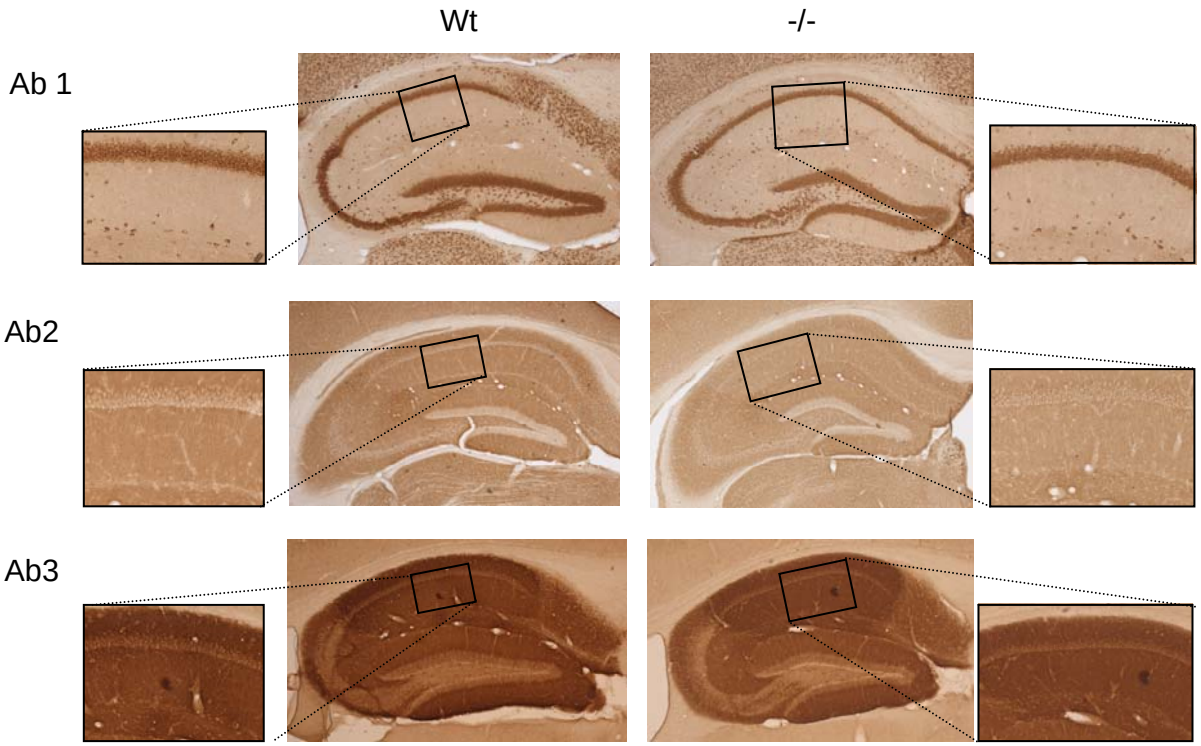

Figure 1: Various antibody staining on Coronal sections

The above image is taken from coronal sections and are x5 magnification of the hippocampus. The smaller inserts are x20 magnification of the CA1 region.

6. Supporting Information

7. Document History

This document was created by Karen Porter on 22 February 2008