Brain Histopathology

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Brain histopathology collaboration. Originally started with Jonathan Flint, Wellcome Trust Centre for Human Genetics, Oxford, UK (details below), but transitioned to Binnaz Yalcin, Université de Bourgogne, France:

At the end of the pipeline, brains were collected from 3 male mutant and wild-type mice and drop fixed in 10% neutral buffered formalin for 48h whilst shipped to our collaborator. Brains were transferred to 70% ethanol. Brains were cut at a thickness of 5µm on a sliding microtome (HM450, Microm, France) on symmetrical and stereotaxic planes to obtain coronal sections matching Bregma +0.98mm and Bregma -1.34 mm or at Lat +0.6mm for parasagittal sections according to the Allen Mouse Brain Atlas. The sections were then stained with 0.1% Luxol Fast Blue (Solvent Blue 38; Sigma-Aldrich) and 0.1% Cresyl violet acetate (Sigma-Aldrich) and scanned using Nanozoomer 2.0HT, C9600 series at 20× resolution. ImageJ freeware was used to quantify images. A full list of structures assessed and detailed protocol are available from:

- Collins, S. C. *et al.* A Method for Parasagittal Sectioning for Neuroanatomical Quantification of Brain Structures in the Adult Mouse. *Curr Protoc Mouse Biol* **8**, e48, doi:10.1002/cpmo.48 (2018).
- Mikhaleva, A., Kannan, M., Wagner, C. & Yalcin, B. Histomorphological Phenotyping of the Adult Mouse Brain. *Curr Protoc Mouse Biol* **6**, 307-332, doi:10.1002/cpmo.12 (2016).

Original methodology:

At the end of the pipeline, brains were collected from 3 male mutant and wild-type mice and drop fixed in 4% paraformaldehyde. On arrival in Oxford, brains were placed in a 30% sucrose phosphate buffered saline (PBS) solution overnight until they reached osmotic equilibrium. Brains were then sliced coronally at a thickness of 40µm. Sections were collected throughout the extent of the hippocampus. All sections were placed in 96 well plates in antifreeze solution (50% PBS, 25% ethylene glycol, 25% glycerol) and stored at -20°C. Stereotaxically matched hippocampal sections were placed in a dish containing PBS and mounted onto electrostatic charged glass slides which were then assembled in a vertical slide holder to dry for at least 30 minutes. Following a brief dip in water to remove dust they were placed in a 0.5%/0.06% cresyl violet acetate/acetic acid solution for approximately 1 minute. Slides were dipped again in water to remove excess staining solution and taken through a 50%, 75%, 95% and 100% alcohol series. They were then placed in Histoclear for 5 minutes, cover-slipped in DPX mounting medium and left to dry overnight. Slides were scanned at 6400dpi via an Epson Perfection V750 Pro scanner. ImageJ freeware was used to quantify images using a scale of 0.251pixels/µm. One section per brain was used for quantification and was stereotaxically matched using thalamic anatomical markers. Measurements assessed the hippocampus, cortex, ventricles and corpus callosum. In addition to quantified measures, sections were assessed qualitatively. This involved examining sections under light microscopy for evidence of cellular ectopias.

The age at which the mice were culled, the anaesthetic used and the diet the mice were fed was pipeline dependent.