Introduction

The cerebellum is involved in motor control and cognitive functions and is affected by numerous neurological diseases. The anatomy of the mouse cerebellum is well characterized and depicted in detailed paper-based atlases which use cytoarchitectural features to establish structural delineations in individual brains. More recently, magnetic resonance imaging (MRI), which permits the registration of multiple data sets, has been used to create digital atlases and assess anatomical variability. Existing MRI-based atlases contain a limited segmented cerebellar structures, limiting the level at which statistical and computational comparisons between individuals or groups can be performed. In this study we developed 1) a detailed protocol for segmenting the ex vivo C57BL/6J cerebellum on high-resolution MRI images and 2) a probabilistic atlas of the C57BL/6J cerebellum.

Methods

18 animals were perfused and fixed with 4% paraformaldehyde and 0.1% Magnetoj^®. Brains were extracted and incubated in 0.1% Magnetoj^® PB for 4 days, placed in Fomblin and imaged on a 1.5T (89mm) Bruker micro-imaging system using a 15 mm SAW coil. MRI data were acquired using a 3D gradient echo sequence with TR/TE/FA=50ms/12ms/30°, 32 KHz spectral bandwidth, and 8 excitations with an acquisition time of 5h 15mins to produce T_1/T_2*-weighted images at 30µm^3 isotropic resolution.

Images were placed in the stereotactic Waxholm space and a symmetric model was created using a recursive non-linear hierarchical fitting strategy. The final fitting step used a nonlinear transformation with a step size of 30µm. This resulted in a model with double the resolution of the original input data (15µm vs. 30µm).

The components of the cerebellum were then delineated, on the bases of differences in signal intensity and/or their location in reference to cerebral fissures, and partitioned using vector-based segmentation via a Cintiq tablet. Subsequently, surface smoothed three-dimensional surface reconstructions were created in Amira.

A probabilistic model was created using the same method as the ICBM152 model. Namely, the traced structures were nonlinearly transformed back to native space before a lower order nonlinear native space to model space transform was applied. In our case this was a grid transform with a step size of 4 times that of the voxel size (60µm).

Results & Conclusion

- Established a protocol for systematic delineation of the C57BL/6J mouse cerebellum in magnetic resonance images.
- The key to cerebellar segmentation is the identification of the fissures. The fissures separate the major vermal lobules and the parts of the cerebellar hemispheres (Fig.1).
- Delineated 38 cerebellar and cerebellar-related structures (Fig. 2,3,4).
- Calculated average region volumes and average T_2* intensities for each structure (Table 1).
- Created probabilistic maps for each structure (Fig. 5).
- The atlas will assist the segmentation of the cerebellum of novel mutants with C57BL/6J backgrounds and will permit the identification of altered morphologies.