Three Dimensional Stereotactic Atlas of Developing C57BL/6J Mouse Brains using Diffusion Tensor Microimaging and Micro-computed Tomography

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Introduction: The mouse is the most widely used model for studying mammalian brain development, and mouse brain atlases have a critical role in surgical interventions such as cell or electrode implantation. Stereotactic surgery relies on determining the exact location of structures within the brain based only on externally visible skull landmarks. High-resolution diffusion tensor microimaging provides detailed soft tissue anatomical information, which can be combined with superior bone contrasts attained by modern micro-computed tomography (microCT). Recently, the first CT-MRI combined atlas of the mouse brain was published[1]. Because the skull and brain undergo significant anatomical changes in early postnatal stages, the spatial relationship between brain structures and the overlying skull is not consistent during development. Therefore stereotactic atlases need to be constructed at each age. As previously shown[2], diffusion tensor imaging (DTI) can provide superb contrasts in neonatal and early postnatal brains, prior to myelin formation. In this study, a DTI-microCT combined stereotactic atlas of C57Bl/6J mice brains at six different developmental stages was developed. The atlas database contains high-resolution images with microCT and multiple MR contrasts (T2, diffusion-weighted (DW), apparent diffusion coefficient, diffusion anisotropy and orientation maps), that provide the exact stereotactic coordinates of any point within the brain relative to reference skull landmarks. The atlas will serve as an important resource for surgical interventions in mice models at these postnatal stages.

Methods: MRI of *ex vivo* perfusion-fixed brain specimens from C57BL/6J mice at six developmental stages: postnatal day 7 (P7), P14, P21, P28, P63 and P140, was performed on an 11.7 T NMR spectrometer. The brain samples were imaged within the skull to preserve brain morphology. DW images were acquired using a 3D diffusion-weighted multiple spin echo sequence (TE/TR = 35/700 ms, ETL = 4, NA = 2, b = 1000-1200 s/mm^2, six diffusion directions: [0.707, 0.707, 0], [0.707, 0, 0.707], [0, 0.707, 0.707], [-0.707, 0.707, 0], [0.707, 0, -0.707], [0, -0.707, 0.707]). The spectral data were apodized by a 10% trapezoidal function and zero-filled to give a nominal resolution of 62.5 x 62.5 x 62.5 µm^3. Total imaging time was about 24 h for each sample. Diffusion anisotropy was quantified using Westin's linear index (CL), and direction encoded color map (DEC) images were computed from the primary eigenvector and CL values. MicroCT of the samples was performed on a SkyScan 1172 microCT system at a resolution of 18 x 18 x 18 µm^3 (70 kV/141µA, rotation step of 0.4° through 180°, average of 6 frames, 58 ms exposure time). To construct the stereotactic atlas, microCT images of the mouse skull at each developmental stage were co-registered with the DTI brain images using a landmark-based rigid registration model with six degrees of freedom. The 3D viewing software "AtlasView" (H. Jiang, S. Mori, lbam.med.jhmi.edu) was used to provide user-interface for navigation through different imaging contrasts and specification of the stereotactic coordinates of any location within the brain.

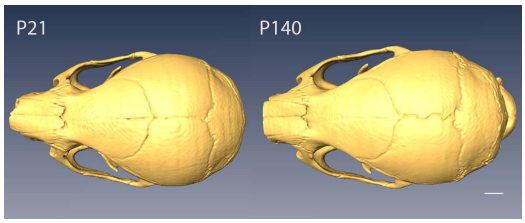

Fig. 1: 3D reconstruction of microCT images of P21 and P140 mouse skulls. Scale bar represents 2 mm.

Results and Discussion: High resolution microCT of the developing mouse skull enabled three-dimensional visualization of the bone structures and clear delineation of all cranial sutures and prominent skull landmarks. Fig. 1 shows the 3D surface reconstruction of microCT images of the mouse skull at P21 and P140 stages. The lambda and the bregma junctions were identified as the key landmarks to define the orientation of the stereotactic atlas. Fig. 2 shows the results of co-registration of the microCT and DTI images of mouse heads at P14 and P140. For each stage, the mid-sagittal section and the coronal section at the level of the bregma are shown, with the microCT image (displayed as a metallic color map) overlaid on the gray-scale DW image. Unlike conventional MR images, which often fail to generate satisfactory contrast during early postnatal stages due to incomplete myelination, DTI provides sharp contrasts between gray and white matter structures throughout development. The DEC images of the coronal section at the level of the bregma show white matter structures and fiber tracts that can be clearly delineated using the anisotropy contrast generated by DTI. Fig. 3 shows the coordinate system defined for the stereotactic surgical atlas. The y-axis was defined along the lambda-bregma line, with the origin of the coordinate system defined at the bregma junction. The exact stereotactic coordinates of different structures within the brain can be computed relative to the position of the bregma. The atlas database will prove useful for performing stereotactic operations on developing mouse brains with greater precision. It will also be useful for studying the interactions between the growing brain and the overlying skull, as well as comparing the morphological phenotype of mutant mice with cranial development disorders.

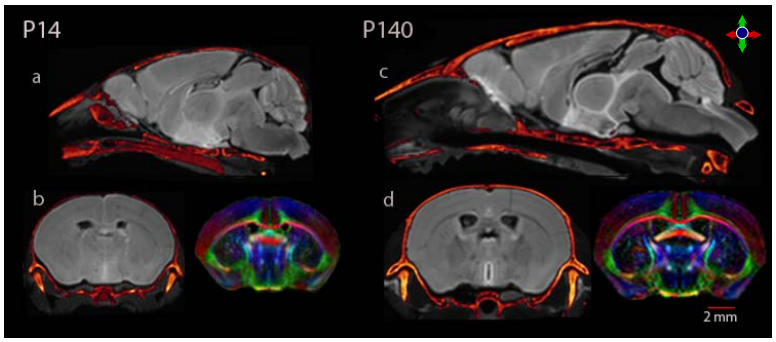

Fig. 2: Co-registration of microCT (metallic color map) and DTI data (gray-scale DW images) of P14 and P140 mouse brains. a,c) Mid-sagittal sections. b,d) Coronal sections at the level of the bregma, showing the microCT, DW and DEC contrasts.

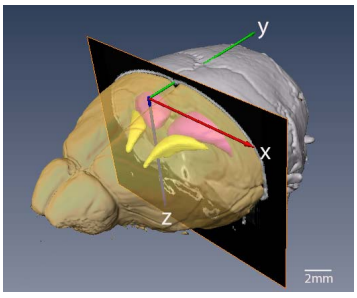

Fig. 3: Coordinate system defined for the stereotactic atlas. Cut-away view of the microCT skull image overlaid on the MR brain volume. Pink:hippocampus, yellow: lateral ventricles. Origin of the coordinate system is at the bregma landmark.

References: [1] E. Chan *et al*., Neuroscience 144: 604-15 (2007). [2] J. Zhang *et al*., Neuroimage 26: 1042-51 (2005).