

## Fetal and Neonatal Brain Magnetic Resonance Imaging Methods

Anatomical and diffusion MRI data were collected on neonatal brains under Telazol sedation (Tiletamine/Zolazepam; 3mg/kg I.V.) using a circularly-polarized extremity transmit/receive wrist RF coil. A 3D T1-weighted MP-RAGE image, a turbo spin-echo dual-echo image and a diffusion tensor imaging data set were obtained at three time points: corrected term (167 dGA), term plus 1 month and term plus 6 months old. For the 3D T1-weighted MP-RAGE imaging sequence, pulse sequence parameters were TE/TR/TI = 3.86/2500/1100 ms, flip angle=12°, voxel sizes were 0.5mm isotropic and 128 slices were acquired. In-plane image sampling consisted of 256 and 216 data points in the phase-encode and readout directions, respectively. In the dual-echo imaging sequence, TE1/TE2=11/95 ms, TR=10640ms, echo train length=8, slice thickness=1 mm, pixel size = 0.5×0.5 mm, sampling matrix = 256×256 and 60 slices were acquired. In diffusion tensor imaging, a single-shot, echo-planar, T2-weighted sequence was used to acquire diffusion-weighted data with a spatial resolution of 1.0×1.0×1.0 mm. TE/TR =112/9500 ms, FOV=96mm and 40 slices were acquired, 35 isotropically distributed diffusion weighting directions were used to measure diffusion-weighted images with  $b=1000 \text{ s/mm}^2$  and six  $b=0$  images were additionally acquired.

All neonatal MRI data processing and analysis were carried out using the ANTS software suite (version 2.1; <http://stnava.github.io/ANTs/>), the FSL suite (version 5.0, <http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/>), Matlab R2016a software (MathWorks Inc., Natick, MA, USA) and SAS (SAS Institute Inc., Cary, NC, USA). To normalize the observed data into a reference space for group-level analysis, T1-weighted brain templates and neuromaps atlases were constructed from three additional control animals that were born from pregnancies that were carried to term (term controls), and underwent MRI scans at the same three time points.

Skull stripping: T1-weighted whole-head MP-RAGE images of a term control subject for each age were selected as references. At the beginning of the skull stripping process, intensity bias correction of all subject MP-RAGE images was implemented using ANTS “N4BiasFieldCorrection” tool. Next, the corrected individual MP-RAGE image was non-linearly aligned to the same age reference image using the ANTS b-spline registration tool (1). The transform parameters were obtained and were used to perform an inverse alignment of the reference brain mask to the individual using nearest neighbor interpolation. The inverse alignment result was the individual brain mask. For dual-echo and DTI images, the first echo image and distortion-corrected b=0 image were aligned to the T1-weighted MP-RAGE image obtained at the corresponding age separately using a 12-parameter affine registration implemented in ANTS by maximizing mutual information. Distortion-corrected diffusion-weighted images were obtained using the TOP-UP procedure implemented in FSL (2). Based on transformation parameters, the brain mask of the MP-RAGE image was inverse aligned to the dual-echo imaging and DTI space. To improve the skull stripping quality, all brain masks were visually inspected and modified manually if necessary. After skull-stripping, the brain proton density and T2 map was calculated from the dual-echo images using Matlab and FA and b0 (i.e. raw T2 signal with no diffusion weighting) maps were calculated using the FSL DTIFit tool. These maps were used for the future analysis of white matter maturation. For purposes of illustrating T2-weighted image contrast, image intensities were extrapolated to a TE value of 100 proton density and T2 images by assuming a monoexponential decay in signal intensity with TE.

Atlas mapping: To generate atlases for each subject brain image, label maps available in the INIA19 rhesus macaque brain atlas (3) were registered to T1-weighted template images from the set of term control subjects. The atlas mapping procedure is similar the one used for skull stripping, except that the whole-head image alignment was replaced

by T1-weighted brain images alignment. First, the subject's T1-weighted brain image was aligned to the reference brain image with the same age using b-spline registration method. Then, the subject labelmap was generated through inverse mapping of the reference atlas.

The T2 and FA maps were normalized to the corresponding T1-weighted image space and used the T1-weighted atlas for analysis. The first echo image in dual-echo images and b0 map were aligned to the corresponding T1-weighted brain image separately using ANTS mutual information 12-parameter affine registration tool. Based on the resulting transformation parameters, T2 and FA maps were mapped to the T1-weighted image space directly using linear interpolation.

Volumetric analysis was implemented based on the brain masks and parcellation atlases of T1-weighted image. The total brain volume (TBV), the white matter (WM), gray matter (GM), lateral ventricle, and hippocampal volumes at each age were calculated by Matlab. For further analyses, WM was subdivided into six regions according to the INIA19 labelmaps: occipital, parietal, internal capsule, external capsule, temporal, and frontal. The regional FA and T2 values in T1-weighted space were analyzed to investigate the maturation of WM. In each WM region, the mean value and standard deviation of FA and T2 were calculated separately. The repeat measures ANOVA method was used to analyze these results statistically using SAS.

## References

1. Avants, B.B., et al., *Symmetric diffeomorphic image registration with cross-correlation: evaluating automated labeling of elderly and neurodegenerative brain*. Med Image Anal, 2008. **12**(1): p. 26-41.
2. Smith, S.M., et al., *Advances in functional and structural MR image analysis and implementation as FSL*. Neuroimage, 2004. **23 Suppl 1**: p. S208-19.
3. Rohlfing, T., et al., *The INIA19 Template and NeuroMaps Atlas for Primate Brain Image Parcellation and Spatial Normalization*. Front Neuroinform, 2012. **6**: p. 27.