Variability of Automated Shape Analyses of Fixed Brain Mouse MRI

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Introduction

The study of genotype-phenotype relationships in the brain can be performed rapidly and accurately using high-resolution MRI on inbred mouse strains. Previous work has investigated the variability inherent in automated analyses of excised mouse brains scanned at 60µm isotropic resolution [1,2]. Recent advances have significantly improved the protocol and leaves the brain inside the skull to avoid deformations due to handling, while also increasing the MR 3D resolution to 32µm. The goal of this study was to ascertain the variability and statistical power of automatically detecting shape differences in the brain using this new protocol.

Methods

Six eight-month-old FVB/N (Charles River, Wilmington, MA) mice were anaesthetized with a combination of Ketamine (100 mg/kg) and Rompun (20 mg/kg) via intraperitoneal injection. Thoracic cavities were opened and animals were perfused through the left ventricle with 30 mL of phosphate buffered saline (PBS) (pH 7.4) at room temperature (25˚C). This was followed by infusion with 30 mL of iced 4% paraformaldehyde (PFA) in PBS. Following perfusion, the heads were removed along with the skin, lower jaw, ears and the cartilaginous nose tip. The remaining skull structures were allowed to postfix in 4% PFA at 4˚C for 12 hours. Following an incubation period of 5 days in PBS and 0.01% sodium azide at 15˚C, the skulls were transferred to a PBS and 2 mM Prohance® (Bracco Diagnostics Inc., Princeton, NJ) solution for at least 7 days at 15˚C. MR imaging occurred 12 to 21 days post-mortem.

A four-channel 7.0-T MR scanner (Varian Inc, Palo Alto USA) with a 6-cm inner bore diameter gradient set was used to acquire anatomical images of brains within skulls. Prior to imaging, the skulls were removed from the contrast agent solution and placed into plastic tubes filled with a proton-free susceptibility-matching fluid (Fluorinert FC-77, 3M Corp., St. Paul, MN). Custom-built, 12-mm over-wound uniform solenoid coils [3] were used to image 3 brains in parallel. The parameters used in the scans were optimized for grey/white matter contrast: T2-weighted, 3D fast spin-echo sequence, with TR/TE= 325/32 ms, four averages, field-of-view 12 x 12 x 25 mm and matrix size = 780 x 432 x 432 giving an image with 32 um isotropic voxels. The total imaging time was 14 hours.

The native MR images underwent an initial rigid body registration towards a standard model [1]. Upon completion a linear average was created by performing a 12 parameter (3 translations, 3 rotations, 3 scales, and 3 shears) registration of all possible pairs of mice. The non-linear atlas was then created by iteratively deforming each mouse towards the previous average, the first registration target being the linear average. Deformation magnitudes were then computed at every grid point for each mouse, and the standard deviation thereof estimated across all 6 mice. All analyses were performed using in-house tools based upon the ANIMAL package [1,4]. Positional variance was estimated using the standard deviation of the deformation magnitudes (SDDM) [1]:

$$SDDM = \sqrt{\frac{1}{N-1}\sum_{k}(x-\bar{x})^2 + (y-\bar{y})^2 + (z-\bar{z})}$$

Results

The mean SDDM across the brain was 121µm. Given two groups of 20 mice, a jacobian determinant change of 0.40 can on average be identified assuming a power of 0.95 and a significance level of 0.001 (equal to a False Discovery Rate of 0.05 in previous studies).

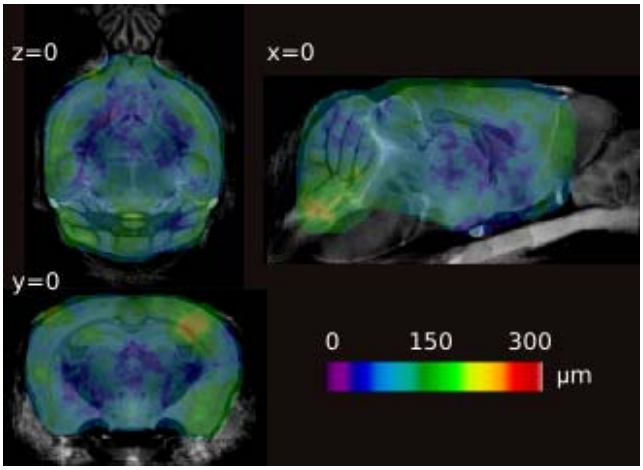

Figure 1Standard Deviation of the Deformation

Conclusions

The results show that the refined MR sequence and revised specimen processing protocol lead to reduced variability in an inbred murine model. The 121µm mean SDDM obtained compares favourably to previous results using excised brains (149µm in 129Sv inbred mice, 150µm in C57 mice, and 129µm in CD1 mice) [2], especially since, due to the fact that the brain was not removed from the skull, no global shape correction was required to correct for handling artefacts [5].

References

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