Neurological Study of Mouse Model of Fetal Alcohol Spectrum Disorders using Advanced Imaging Techniques

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Target audience: Neuroimaging scientists focusing on mouse model of human neurological diseases

Purpose: The term *Fetal Alcohol Spectrum Disorders* (FASD) refers to all mental and physical abnormalities that happen to a fetus under maternal alcohol consumption[1]. These abnormalities can be observed from embryonic stage to adulthood[2]. Previous studies have reported brain malformations and malfunctions in *ex vivo* mouse models of acute high dose exposure using Magnetic Resonance (MR) based techniques[3]. This study focuses on chronic alcohol exposure pattern in mouse models, which is designed to match the first 3 – 4 weeks of pregnancy in humans. Super-resolution volumetric MRI[4] is used to evaluate developmental rate in brain regions longitudinally from adolescence (postnatal day, PD, 28) to adulthood (PD80) and Computed Tomography (CT) is used to evaluate skull formation in FASD and control groups *ex vivo* at PD80.

Method: Specimens used are in-bred C57Bl/6J mice. 10% ethanol-water was provided *ad libitum* from gestational day (GD) 0 to GD8 to pregnant mice in FASD group, corresponding to the first 3 – 4 weeks in humans. Tap water was provided for the rest of the gestation in FASD group (N = 17), and throughout the gestation in control group (N = 7). Only male offspring were studied. MRI scans were performed using Bruker Biospin 9.4T Biospec equipped with mouse brain cryoprobe. Volumetric MRI was done using proton density 2D gradient echo FLASH (Fast Low Angle Shot) sequence with recycling delay (TR) of 850 ms, TE of 5.5 ms, flip angle of 30 degrees and matrix size of 229 × 229, resulting in a resolution of 0.07 × 0.07 × 0.21 mm^3, with 2 scan averaging. Each mouse underwent three sets of these 2D scans with the space position being shifted dorso-ventrally by 0.07 mm to enable 3D super-resolution reconstruction using Matlab script, resulting in isotropic 70 µm 3D MR images. Tensor-based morphometry (TBM) was done at each time point including the following steps: A template for all specimens was generated using Advanced Normalization Toolkits (ANTS)[5]. Jacobian maps that indicate volume contractions or expansions relative to the template were calculated for each subject from the deformation information. Statistical Parametric Mapping (SPM5)[6] $p<0.05$ calculated the statistically significant regions of volume differences between FASD and control groups.

CT images were acquired using Siemens Inveon microCT scanner. Due to the amount of radiation exposure for high-resolution imaging, *ex vivo* samples were prepared post PD80 MRI to obtain isotropic effective voxel resolution at 27.8 µm. The scans were done at 80 kV and 350 µA with 360 rotation steps, medium-high magnification and binning factor of 2. The exposure time was 1300 ms. A similar TBM procedure was performed for the analysis of CT images.

Results: Super-resolution reconstruction produced images with superior image contrast, SNR and lower artifacts compared to the original individual images. TBM analyses of high-resolution anatomical MR images showed that changes in the adolescent PD28 FASD brains could be detected in the lateral ventricle, hypothalamic and thalamic regions. These changes are different in adult PD80 brains, where changes are concentrated in the dentate gyrus of the hippocampus. The differences in PD80 skull morphology are limited to small areas near the front part of the cortex and the lambda region (white arrows) (Figure 1).

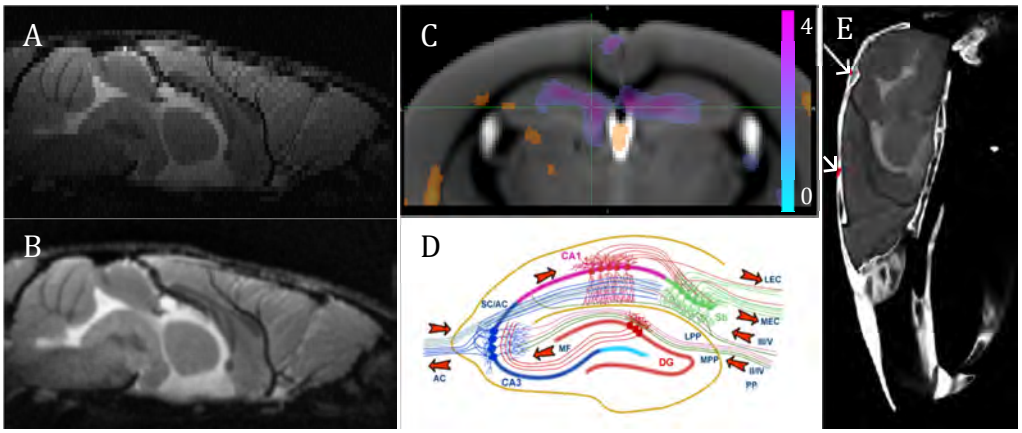

Figure 1. (A) One of a set of three-shifted MR images showing low SNR and pixelated images in the slice direction. (B) Super-resolution reconstruction produced from the image dataset A. (C) TBM analysis showing specific hippocampal region with volumetric contractions in FASD relative to control subjects at PD80. (D) Contracting region can be mapped to the hippocampus dentate gyrus, a region normally containing a high density of neurons and important for memory and learning[7]. (E) TBM analysis showing two regions of volume expansion (white arrows) in the skull of FASD subjects relative to control subjects.

Discussion: Using TBM, brain regions affected with FASD abnormal development can be detected. Currently, there appears to be no spatial correlation between subtle changes detected in the brain and in the skull areas. Progress is being made to acquire more data in both groups to increase the statistical power and accuracy.

Conclusion: This study demonstrates that *in utero* chronic alcohol exposure at moderate dose in mice can result in abnormal brain development in the offspring, which can be monitored from adolescent to adult age using high-resolution MRI and CT.

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