Characterizing the Effect of Fetal Ethanol Exposure on Subsequent Morphological Development of Neurons in the Cerebral Cortex by Diffusion Tensor Imaging

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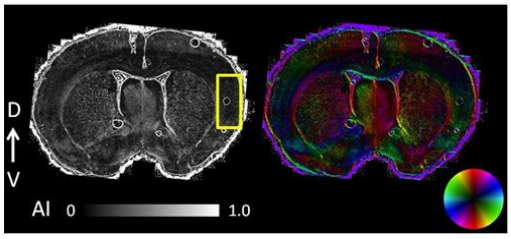
Introduction: Due to the major impact that prenatal exposure to ethanol has on the developing brain, and the proposed consequences that these effects have on behavioral and cognitive symptoms associated with fetal alcohol spectrum disorders (FASD), a great deal of research has focused on identifying developmental cortical abnormalities associated with FASD. Histological studies have provided abundant evidence of disrupted cortical formation, including simplified cerebral cortical neuronal morphology. However, detecting these abnormalities in vivo at early stages of human development is not feasible through histological methods.

Diffusion tensor imaging (DTI) is a quantitative, non-invasive method that is particularly well suited to study the cellular-level basis of tissue changes associated with development and pathology. The aim of this research was to investigate the effects of prenatal exposure to ethanol on DTI measurements in the developing cerebral cortex, and directly compare histological evidence of disrupted neuronal morphology in a rat model of FASD. The expectation is that DTI may be sensitive to cortical abnormalities in FASD and may serve as a non-invasive diagnosis measure and method to monitor therapeutic interventions in FASD.

Methods: Pregnant rat dams were untreated (C) or gavaged with either 4.5 g/kg, 25% ethanol (E) or calorie matched maltose-dextrin (M/D) from gestational day 0 through 20. At 3 postnatal time points (P0, P3, P6), pups from these dams were sacrificed, and their brains collected for analysis (n ≈ 4/age/group) (1).

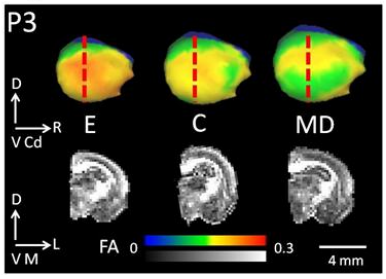
Figure 1



DTI data, specifically FA (a measurement of the degree of cellular-scale order in tissue), were acquired for right hemispheres on an 11.7 Tesla magnet (Bruker, Germany) interfaced with a 9 cm inner-diameter magnetic field gradient coil insert. Scan parameters were as follows: TR = 4000 – 5000, TE = 42.7 ms, FOV = 5.20 x 1.28 cm, Matrix = 250 x 64, Voxel size = 0.2 mm^3, Averages=6, Scan time = 12 hours). The b value was 2500 s/mm^2, δ = 12 ms and Δ = 20.958 ms. Two scans were acquired with b=0.

Subsequent to MRI, histological measures of anisotropy were derived from sections stained with the lipophylic dye DiI according to the protocol described by Budde et al. (2). Briefly, hemispheres were sectioned coronally on a vibratome at a thickness of 200 µm and imaged in their entirety at 10X magnification. A measure of in-plane anisotropy (anisotropy index, AI) was derived using structure tensor analysis, and regions of interest (ROIs) were manually drawn on the histological maps of anisotropy. Figure 1 displays a coronal AI image (left) and the same image color-coded by orientation (right) of a normal adult rat to demonstrate an example region of interest (yellow box).

Figure 2

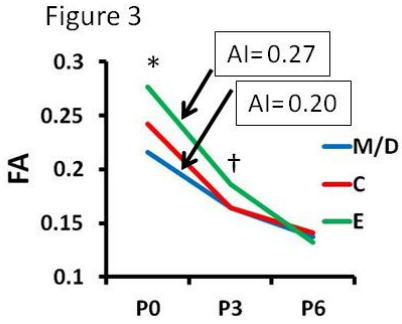


Results: Figure 2 shows the overall patterns that were observed among the groups. FA was generally highest in the E group, and lowest in the M/D group. Lateral cortical surfaces (top) and coronal FA maps (bottom; red lines in top panels indicate location) are shown for one subject per group at P3 for demonstration.

As seen in Figure 3, mean cortical FA was highest in the E group, and lowest in the M/D group. FA differed among the age groups at P0 (*, $p<0.05$) and P3 (†, $p = 0.08$), but not P6. Additionally, AI values taken from an ROI similar to that shown in Figure 1 on one P0 E and one P0 M/D brain (0.27 and 0.20, respectively) corresponded to FA values measured via DTI. (D = Dorsal, V = Ventral, Cd = Caudal, R = Rostral, M = Medial, L = Lateral)

Conclusions: This is the first evidence that there are disruptions in cortical developmental FA patterns seen in response to prenatal exposure to ethanol, and that these disruptions are related to neuronal morphology and differentiation. FA was higher in the E group at P0 and P3, corresponding to the period of early neuronal differentiation in the cerebral cortex. Additionally, histological estimates of anisotropy obtained through DiI staining and quantitative analysis corresponded to FA measured via DTI. Together, these data indicate that prenatal exposure to ethanol is related to microstructural abnormalities in the developing cerebral cortex that may reflect abnormal neuronal arborization or differentiation.

Figure 3



Given the sensitivity of DTI to detect abnormalities caused by ethanol exposure during gestation, and the relationship between abnormal FA measurements and abnormal neuronal differentiation, DTI is introduced as a potential diagnostic methodology for FASD.

References: [1] Leigland et al. ACER in press, [2] Budde et al. Neuroimage (63) 2012.