A DTI investigation of neuroanatomical differences in a mouse model of early life neglect

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INTRODUCTION. Early life neglect and abuse is a common problem in the USA with little discrimination for race, gender or socio-economical status and the situation has not improved much over time. There is growing recognition that changes in white matter (WM) are associated with a variety of psychiatric conditions. Diffusion tensor imaging (DTI) has shown reduced fractional anisotropy in the medial and posterior corpus callosum of maltreated children with post-traumatic stress disorder (PTSD) [1] and in the anterior limb of the internal capsule, a fiber tract with important projections to the PFC, of macaques with disrupted mother-infant attachment, but not the posterior limb or occipital white matter [2]. Recently, a novel mouse model of early life neglect based on maternal separation with early weaning (MSEW) was developed [3]. In the present work we used DTI to examine the consequences of MSEW with regard to neuroanatomical structure.

MATERIALS AND METHODS. ***Animal Preparation.*** The animals were anesthetized with chloral hydrate (1500 mg/kg IP), followed by intracardiac perfusion with ice cold PBS followed by ice cold 4% paraformaldehyde (PFA) in 0.01 M phosphate buffered saline (PBS) (pH=7.4). After perfusion, the brains were harvested maintaining integrity and stored in 4% PFA in PBS at 4 °C for 2 weeks. One hour before the DTI scans, the brains were soaked 3 times for 10 minutes each time in 10 mL PBS to remove the PFA solution. Then, the brains were placed into a custom built MRI compatible tube, filled with Fluorinert, an MRI susceptibility-matching fluid (Sigma-Aldrich, Inc., St. Louis, MO). ***DTI experiments.*** The DTI datasets were obtained on a 9.4 T horizontal bore magnet (Bruker, Billerica, MA, U.S.A.) with a custom-made ^{1}H radio frequency coil. The DTI experiments were performed using the Stejskal-Tanner spin-echo diffusion-weighted sequence with a diffusion gradient of 5ms and a delay between the two diffusion gradients of 15 ms. 20 contiguous coronal slices of 0.5mm thickness were acquired. The 128×64 images were zero-filled to 256×256, resulting in an in-plane resolution of 100μm×100μm. Sixteen different images were acquired for each slice, fifteen corresponding to various noncollinear diffusion weighting directions with the same b = 1000 s/mm^2 and one with no diffusion weighting. ***DTI data processing and analysis.*** The six elements of the diffusion tensor were calculated for each voxel from the intensities of the 16 diffusion-weighted images. The tensor eigenvalues and the corresponding eigenvectors were obtained by matrix diagonalization [4-5]. The fractional anisotropy (FA) was calculated and the composite FA images were generated using BioImage Suite (http://www.bioimagesuite.org) as follows. First smoothed versions of the FA images (Gausian smoothing sigma=0.2 mm) were non-rigidly registered to a single smoothed FA map using a non-linear intensity-based warping parameterized in terms of a tensor b-spline grid with uniform control point spacing of 3mm [6] as was done in previous work [7]. Next the unsmoothed FA maps were warped to this common coordinate space and resampled to isotropic resolution (0.1mm); following which average FA maps for each group were computed (Fig. 1).

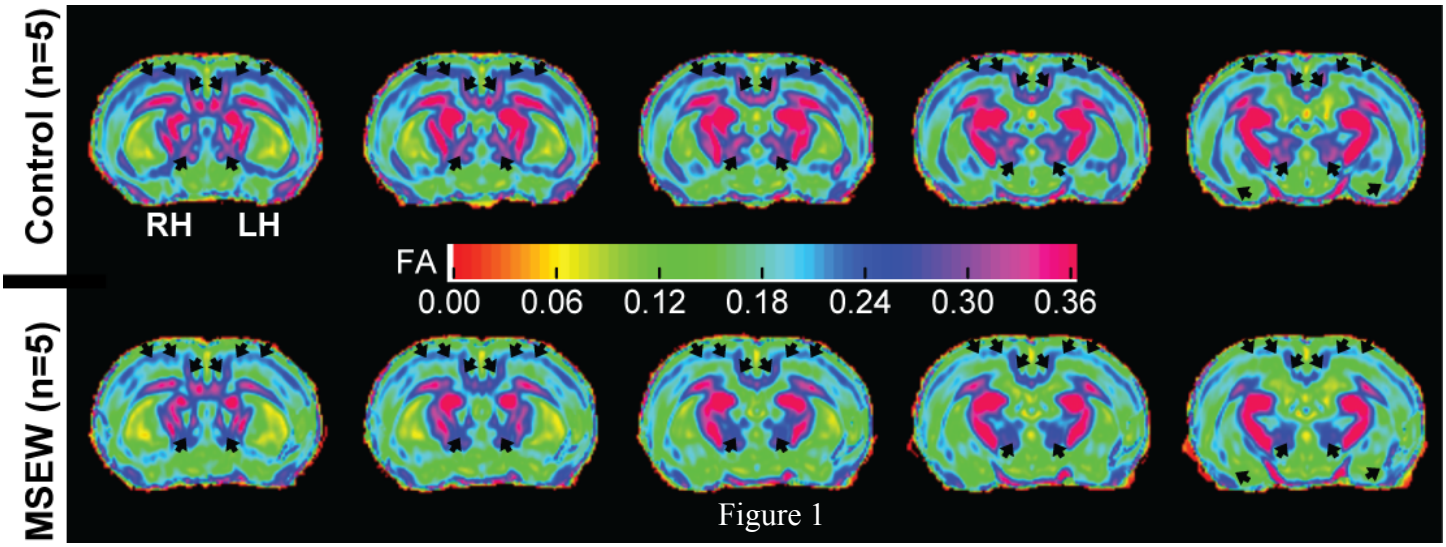

Figure 1

RESULTS. Coronal optical slices spaced 100 μm and representing mean averages for each condition show reduced FA in different brain areas of the MSEW animals compared with the controls (Fig. 1, black arrowheads). MSEW animals showed decreased FA in several white matter fiber tracks including the cingulum, corpus callosum, anterior commissure and septofimbria. We also observed decreased FA in multiple gray matter regions including the cingulate gyrus, basolateral amygdala, thalamus, and middle and deeper cortical layers.

DISCUSSION. DTI imaging clearly demonstrated that both hemispheres of MSEW mice are abnormal. MSEW animals showed decreased FA in some white matter fiber tracks consistent with findings based on histology. These regions included the cingulum and the corpus callosum among others. Although both hemispheres were affected, asymmetric involvement of the white matter, for example in the corpus callosum and white matter near the basolateral amygdala, were evident (Fig. 1). In addition, histological studies reveal slight left-right asymmetry in MSEW vs control. Future DTI analysis should co-register each hemisphere of each group independently to highlight such asymmetry. Although we did not conduct detailed analyses of the regions, DTI revealed significantly decreased FA in multiple gray matter regions middle and deeper cortical layers, and these changes warrant further investigation in future studies.

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