Sex differences in the frontal lobe of the developing mouse brain

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Introduction: The frontal lobe is part of the limbic system, which controls many important functions including emotion, motivation, reward, and memory. Frontal lobe maturation and refinement continues into early adulthood in both humans and rodents, though the development of the frontal lobe is different between males and females (1). Diffusion tensor imaging (DTI) can be used to determine structural connectivity, a measure of the physical connections within and between brain regions, in both humans and animals. A comprehensive study measured the structural connectivity of the developing human brain from age 8 to 22, revealing higher connectivity within and between the frontal lobe to other brain regions in females compared to males (1). However, this study showed little structural connectivity between brain regions in young children (1). The developing frontal lobe undergoes structural changes from synapse overproduction during childhood followed by pruning in adolescence (2,3). This process occurs between postnatal day (P) 20 and P30 in rodents. Studying structural connectivity during postnatal frontal lobe development in mice will show connections formed at specific ages and may contribute to the understanding of the developing human brain structure at young ages. This project will test the hypothesis that the developing frontal lobe in the female mouse contains greater structural connectivity than the male mouse.

Materials and Methods: C57Bl/6J mice were bred at the University of Maryland, Baltimore. The Institutional Animal Care and Use Committee at the University of Maryland, Baltimore approved the protocols of this study. Food and water were given ad libitum. All mice were weaned at day 21. At P20 and P30 mice were anesthetized with 4% isoflourane and transcardially perfused with phosphate buffered saline (PBS) followed by 4% paraformaldehyde (PFA). The fixed brain was placed in a customized conical tube filled with Fluroinert (3M, St. Paul, MN) to decrease background signal intensity during scanning. Four male and female mice were used at P20 and five male and two female mice were used at P30.

Ex vivo MRI: All experiments were performed on a Bruker Biospec 7.0 Tesla 30 cm horizontal bore scanner using Paravision 5.1 software (Bruker Biospin MRI, Ettlingen, Germany). A four-channel Bruker ^{1}H surface array coil was used as the receiver and a Bruker 72-mm linear-volume coil as the transmitter. 3D diffusion tensor images were acquired with the spin echo sequence in the axial plane. 64 diffusion directions were applied at b=2000 s/mm^2. Five images at b=0 s/mm^2 were acquired. The field of view was 1.50cm^2, with isotropic resolution 150μm^3, TR/TE 500/31msec, 3D slab thickness of 15mm, and one average.

Data Analysis: Structural connectivity was inferred from 3D DTI (5) using a probabilistic tractography model to determine likely fiber pathways within and between brain regions (5,6). ROIs drawn included the frontal association (**FrA**), and cingulate (**Cg**) cortices as well as the white matter tracts of anterior commensure (**AC**) and corpus callosum (**CC**). The FMRIB Software Library View (FSL v5.0, Analysis Group. FMRIB, Oxford, UK) program was used to manually draw ROIs using the anatomical image as reference. The FSL Diffusion Toolkit (FSL v5.0, Analysis Group. FMRIB, Oxford, UK) generated DTI maps and estimated crossing fibers based on Bayesian estimation of the probability distribution of the tensor orientation within each voxel (6). One representative P30 male brain was taken as an atlas template; with all ROIs carefully drawn on the template. The rest of brains and tractography results were registered to the template through affine registration within FSL for further analysis. The probabilistic tractography output and structural connectivity values were extracted.

Results: Greater structural connectivity were observed from the Cg to FrA at P20 in female mice brains compared to males, whereas PFC to Cg showed higher structural connectivity in males. White matter connectivity did not change much between males and females or with age. At P30 greater structural connectivity were observed in male mice brain between Cg to PFC and FrA than females. The Cg to FrA connectivity increased from P20 to P30 in males, while the connectivity within the Cg decreased in females at P30. On the other hand, DTI values indicated no difference between males and females at P20 and P30 with MD and FA in white matter regions of the CC, AC, and the frontal lobe gray matter regions of FrA, PFC and Cg.

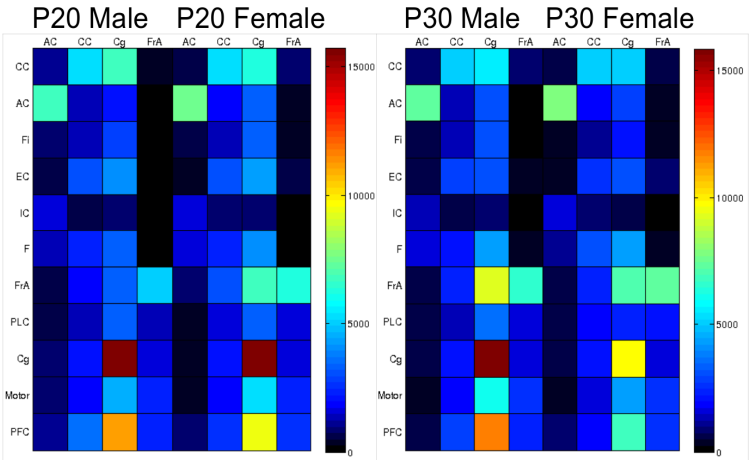

Figure 1 (right): Averaged structural connectivity maps from a) P20 and b) P30 mice brains in the CC, AC, FrA, and Cg. The structural connectivity of these four regions were calculated to the CC, AC, fimbria (**Fi**), external capsule (**EC**), internal capsule (**IC**), fornix (**F**), FrA, prelimbic (**PLC**), Cg, **motor**, and prefrontal (**PFC**) cortices. Red to blue represents stronger to weaker, and black shows no connectivity.

Conclusion: Using *ex vivo* samples we were able to show more structural connectivity in the frontal lobe of the developing mouse than was reported in children (1). While greater structural connectivity was observed in females at P20 compared to males from the FrA to Cg, the developing male brain showed higher connectivity between other frontal lobe regions compared to female frontal lobe. The highest connectivity was found in the cingulate cortex of both male and female mice at P20 revealing connections made between the Cg to PFC, FrA and motor cortices and the CC. The connectivity within the Cg and from Cg to PFC reduced in female at P30, suggesting the process of pruning may occur earlier in females than males. Structural connectivity within the AC and CC showed no differences between males and females and could be reflected by MD and FA. However, FrA, PFC and Cg showed connectivity differences that were not reflected by diffusivity, potentially due to the heterogeneity of the tissue. These findings indicate that probabilistic tractography may reveal subtle network connectivity changes not accompanying apparent structure changes from DTI. This project have shown that structural connectivity in developing female mouse brain is different from males, with surprisingly greater connectivity between many frontal lobe regions in males than females.

References:1. Ingalhalikar M, Smith A, Parker D, et al. *Proc Natl Acad Sci U S A*. 2014;111(2):823-828. 2. Andersen SL. *Neurosci Biobehav Rev*. 2003;27(1-2):3-18. 3. Semple BD, Blomgren K, Gimlin K, Ferriero DM, Noble-Haeusslein LJ. *Prog Neurobiol*. 2013;106-107:1-16. 4. McCutcheon JE, Marinelli M. *Eur J Neurosci*. 2009;29(5):997-1014. 5. Behrens TE, Woolrich MW, Jenkinson M, et al. *Magn Reson Med*. 2003;50(5):1077-1088. 6. Behrens TE, Berg HJ, Jbabdi S, Rushworth MF, Woolrich MW. *Neuroimage*. 2007;34(1):144-155.