White Matter Alterations in Mouse Models of Alzheimer's Disease

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Introduction

Alzheimer's disease (AD) is the only leading cause of death that is still on the rise[1]. Approximately 500,000 people die every year because they have the disease[1]. While AD is most strongly associated with plaques and tangles, myelin has been shown to be disrupted in patients with AD[2]. Still accounting for Aβ plaques and tau, the myelin-model explains the former as a secondary effects. The enzymes BACE1 and γ-secretase cleaves are known to cleave APP, producing Aβ. BACE1 may increase in quantity, and γ-secretase may increase in efficiency as a response to myelin breakdown, as the enzymes also cleave neuregulin which signals oligodendrocytes to myelinate[3]. Neuregulin mediates activity from cyclin dependent kinase5 and GSK3b, which increase tau phosphorylation[3]. Thus, myelin disruption is tied into the pathology of AD.

In order to understand the pathology and progression of AD, our lab has developed a mouse model (APP/SwDI/NOS2-/- ; CVN-AD) that recreates AD-like pathology. Producing a mutant human Abeta peptide on the NOS2 knockout background, mirroring the NO level in humans, this mouse demonstrates the 4 major pathologies of AD – amyloid deposition, neuron loss, neurofibrillary tangle formation, and behavioral deficits. We have used this model to study changes in myelinated brain regions during the development of an AD-like disease process.

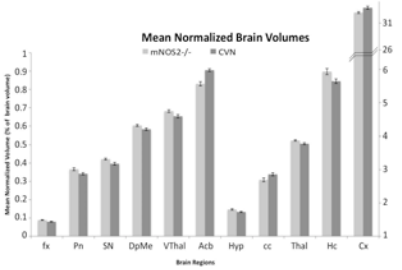
Thus, the **objective** was to characterize the white matter of the CVN-AD models, through volume changes and fractional anistropy (FA).

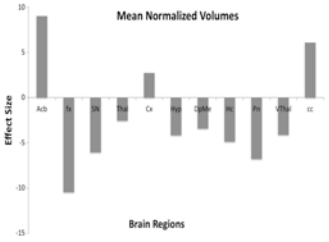
Methods

- High field magnetic resonance histology of the mouse brain was done using a 9.4T magnet, and a diffusion tensors imaging protocol using 12 diffusion directions, and reconstructed at 55 um isotropic resolution.
- A reference mouse brain atlas was used for providing priors for automated segmentation of 38 regions (Johnson 2011- Waxholm reference). We used advanced normalization tools (ANTs, Avants 2011) for image registration using the DWI and FA contrasts. SurfStat (Worsley, Chung, 2009) was used for voxelwise statistics)
- Statistics were done using MATLAB and SurfStata, fitting a general linear model to examine differences by genotype in local volume, and individual DTI parametric images.

Results: Volume

Significant changes ($p<0.05$) in the mean normalized volume of several brain regions were observed in CVN-AD models of AD relative to mNOS2$^{-/-}$ controls. Of interest, the CVN-AD have a decreased fornix, decreased hippocampus, and increased corpus callosum. Regional volumes are expressed as a percentage of total brain volume, thus normalized.





The effect sizes for the significant volume changes are presented as a percent difference of the CVN-AD relative to the mNOS2$^{-/-}$ controls. Among regions with significant differences in CVN-AD mice, the fornix (fx) is 10.4% smaller, the hippocampus (Hc) is 4.9% smaller, and the corpus callosum (cc) is 6.1% larger relative to the mNOS2$^{-/-}$.

Among structures of particular interest in AD, the CVN-AD relative to mNOS2-/- shows significant differences in:

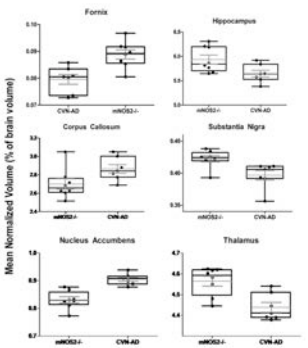
Gray Matter

- Smaller hippocampus (5.65±0.07% of brain volume, p< 0.03, effects size -4.86%) (compared to 5.94± 0.09%)
- Smaller Substantia Nigra (0.397±0.007%, p<0.05, effects size -6.0%) (compared to 0.422 ±0.005%)
- Smaller Thalamus (4.438±0.024, p<0.05, effects size -2.78) (compared to 4.508 ±0.024%)
- Larger nucleus accumbens (0.905±0.008%, p<0.001, effects size 9.0%) (compared to 0.831±0.011%)

White matter

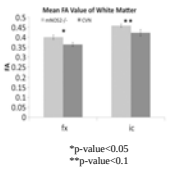
- Smaller fornix (0.080±0.002%, p< 0.01, effects size -10.44%) (compared to 0.089±0.002%)
- Larger corpus callosum (2.86±0.05%, p<0.05, effects size 6.07%) (compared to 2.70±0.06%)

Box Plots of Volume Changes in Particular Regions

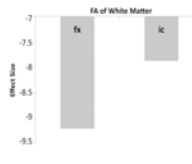


Note: The black error bars represent the range, the gray error bars represent SEM, the black line represents the median, and the gray line represents the mean. Percentages are of brain volume.

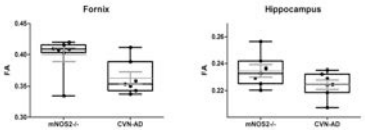
Results: Fractional Anistropy



*p-value<0.05
**p-value<0.1



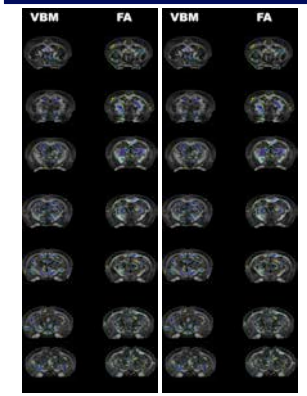
Box Plots of FA Changes in Particular Regions



Significant changes ($p<0.05$) in FA were observed in two white matter regions in CVN-AD models of AD relative to mNOS2$^{-/-}$ controls. The fornix has decreased FA in CVN-AD relative to mNOS2$^{-/-}$ controls, and the internal capsule approaches significance ($p<0.1$) reflecting poor white matter integrity. The effect size of white matter FA changes as a percent difference of CVN-AD relative to mNOS2$^{-/-}$ controls. Specifically, the fornix has 9.18% lower FA.

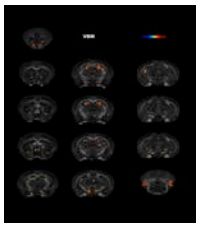
All of the brain regions have decreased FA in the AD models compared to mNOS2$^{-/-}$ controls, indicative of poor white matter integrity

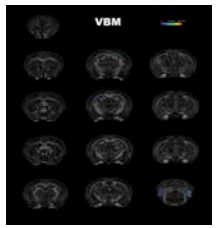
Results: Voxel Wise Analysis

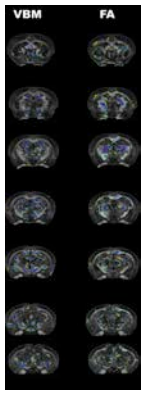


← One will be effect size panel comparing uncorrected jac and fa; will add scale bars on Monday

Results: Voxel Wise Analysis & E1

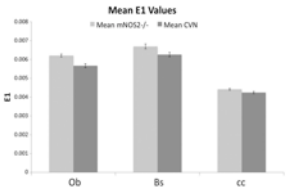






Corrected Volumes

← Rd and Adc uncorrected Volumes; discuss ventricles



Mean axial diffusivity (E1) values of the olfactory areas, brain stem, and corpus callosum are significantly smaller in the CVN-AD compared to mNOS2$^{-/-}$ controls (p-value < 0.05). This suggests poor axonal integrity for these white matter tracks.

Conclusion

- This small but significant difference in hippocampal volume (~5% smaller in CVN-AD mice) (as well as decreased FA) validates the role of hippocampal atrophy as an image based biomarker in the CVN-AD mouse.
- The changes in volume and FA of the fornix suggests the fornix plays a significant role as a biomarker in mouse models of AD.
- The results indicate a role of WM atrophy in the CVN-AD model of AD.
- More research using IHC, lipid stains, and electron microscopy will be conducted to further characterize the role of myelin breakdown in CVN-AD.

[1] Alzheimer's Facts & Statistics. June 2014. BrightFocus Foundation.
[2] Gold B.T., Johnson N.F., Powell D.K. & Smith C.D. (2012). White matter integrity and vulnerability to Alzheimer's disease: preliminary findings and future directions. Biochim Biophys Acta. 1822(3):416-22.
[3] Bartzokis G. (2011). Alzheimer's disease as homeostatic responses to age-related myelin breakdown. Neurobiol Aging; 32(8): 1341–1371.
[4] Wen Y, Planel E, Herman M, Figueroa HY, Wang L, Liu L, Lau LF, Yu WH & Duff KE. (2008). Interplay between cyclin-dependent kinase 5 and glycogen synthase kinase 3 beta mediated by neuregulin signaling leads to differential effects on tau phosphorylation and amyloid precursor protein processing. J Neurosci.28(10):2624-32.