



*Praveen Kulkarni¹, Xuezhu Cai¹, Thomas Morrison¹, Jennifer Honeycutt¹, Heather Brenhouse¹, Malav Trivedi², Kevin Gamber³, Mark Nedelman⁴, Craig F. Ferris¹

Department of Psychology¹, Northeastern University, Boston, MA; Pharmaceutical Sciences², Nova Southeastern University, Fort-Lauderdale, FL; Horizon Discovery³, St. Louis, St Louis MO; Ekam Imaging⁴, Boston, MA, USA.



Introduction

The etiology of Alzheimer's disease (AD) is unknown but considered to be the combination of genetic and environmental factors together with aging. The most prevalent genetic risk for sporadic Alzheimer's disease is the allele E4 of the apolipoprotein E4 (APOE E4). The many neuropathological findings that define AD are associated with APOE E4 carriers together with general cerebrovascular impairment and neuro-inflammation. There are reports from human imaging studies that suggest the apoE4 protein could affect neurodevelopment many years before the onset of AD. Indeed, brain structure alterations may precede overt cognitive impairment in AD by several decades. Early detection of these alterations holds inherent value for the development and evaluation of preventive treatment therapies. In collaboration with Horizon Discovery (St Louis) we characterized the brain and cognitive development of male and female APOE E4 knock-in (KI) rats, a preclinical model of Alzheimer's disease.

Materials & Methods

Experimental Design

Wild-type (WT) (n = 12) and APOE E4 knock in (n = 12), male and Female, rats were obtained from Horizon Discovery. Studies were performed on a Bruker BioSpec 7T / 20cm USR. Four types of scans were acquired 1) high resolution Anatomy scans for volumetric assessment 2) T2 map 3) Diffusion Tensor imaging 4) Functional Connectivity scans. All animals were studied at 4-5 months of age.

Diffusion Tensor Imaging

DTI was acquired with a diffusion-weighted (DW) spin-echo echo-planar-imaging (EPI) pulse sequence having the following parameters: TR/TE 500/20 ms, eight EPI segments, and 10 non-collinear gradient directions with a single b-value shell at 1000 s/mm² and one image with a b-value of 0 s/mm² (referred to as b0). Geometrical parameters were: 60 slices, each 0.313 mm thick (brain volume) and with in-plane resolution of 0.313x0.313 mm² (matrix size 96x96; FOV 30 mm²). The imaging protocol was repeated two times for signal averaging. Each DTI acquisition took 35 min and the entire MRI protocol lasted about 1 hour 10 min.

T2 relaxometry

Multi echo images were acquired using MSME slice sequence (TR= 5.4 sec and TE: 11, 22, 33, 44, 55, 66, 77, 88, 99, 110 msec.) Images were acquired with a field of view [FOV] 3 cm², data matrix = 128x128x20 slices, thickness = 1 mm. T2 measurements were computed using Paravision 5.1 software (Bruker, Billerica, Massachusetts) by fitting absolute signal at particular TE.

Image Analysis

Image analysis included DTI analysis of the DW-3D-EPI images to produce the FA, ADC and RA maps. DTI analysis was implemented with Matlab (©Mathworks, USA) and **MedINRIA (1.9.0; <http://www-sop.inria.fr/asclepios/software/MedINRIA/index.php>)** software. Because sporadic excessive breathing during DTI acquisition can lead to significant image motion artifacts that are apparent only in the slices sampled when motion occurred, each image (for each slice and each gradient direction) was automatically screened, prior to DTI analysis, for motion artifacts. Following the elimination of acquisition points with motion artifacts, the remaining acquisition points were corrected for linear (motion) and non-linear (eddy currents/susceptibility) artifacts using SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK).

Registration to 3D Atlas

Each subject was registered to a 3D segmented and annotated rat brain atlas (Ekam Solutions LLC, Boston MA.). The alignment process was facilitated by an interactive graphic user interface EVA. The affine registration involved translation, rotation, and scaling in all 3 dimensions independently. The matrices that transformed the subject's anatomy to the atlas space were used to embed each slice within the atlas. All transformed pixel locations of the anatomy images were tagged with the segmented atlas regions, creating a fully segmented representation of each subject. T1 parameter values for each ROI were computed based on each segmented map.

Diffusion Tensor Imaging

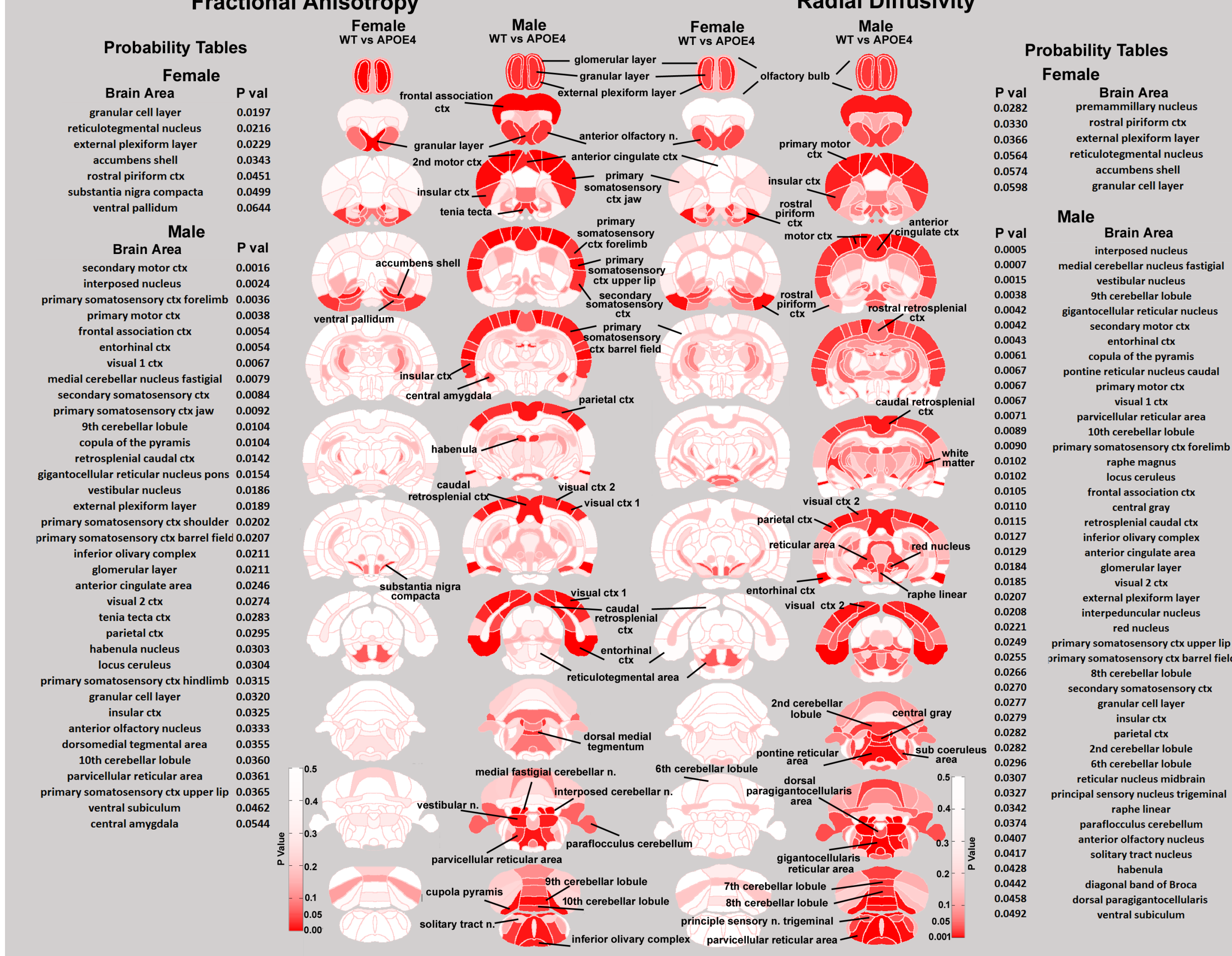
Indices of Anisotropy (IA)

- Fractional Anisotropy (FA)
- Apparent Diffusion Coefficient (ADC)
- Lambda 1
- Lambda 2
- Lambda 3
- Eigen Vectors
- Radial Diffusivity (RD)

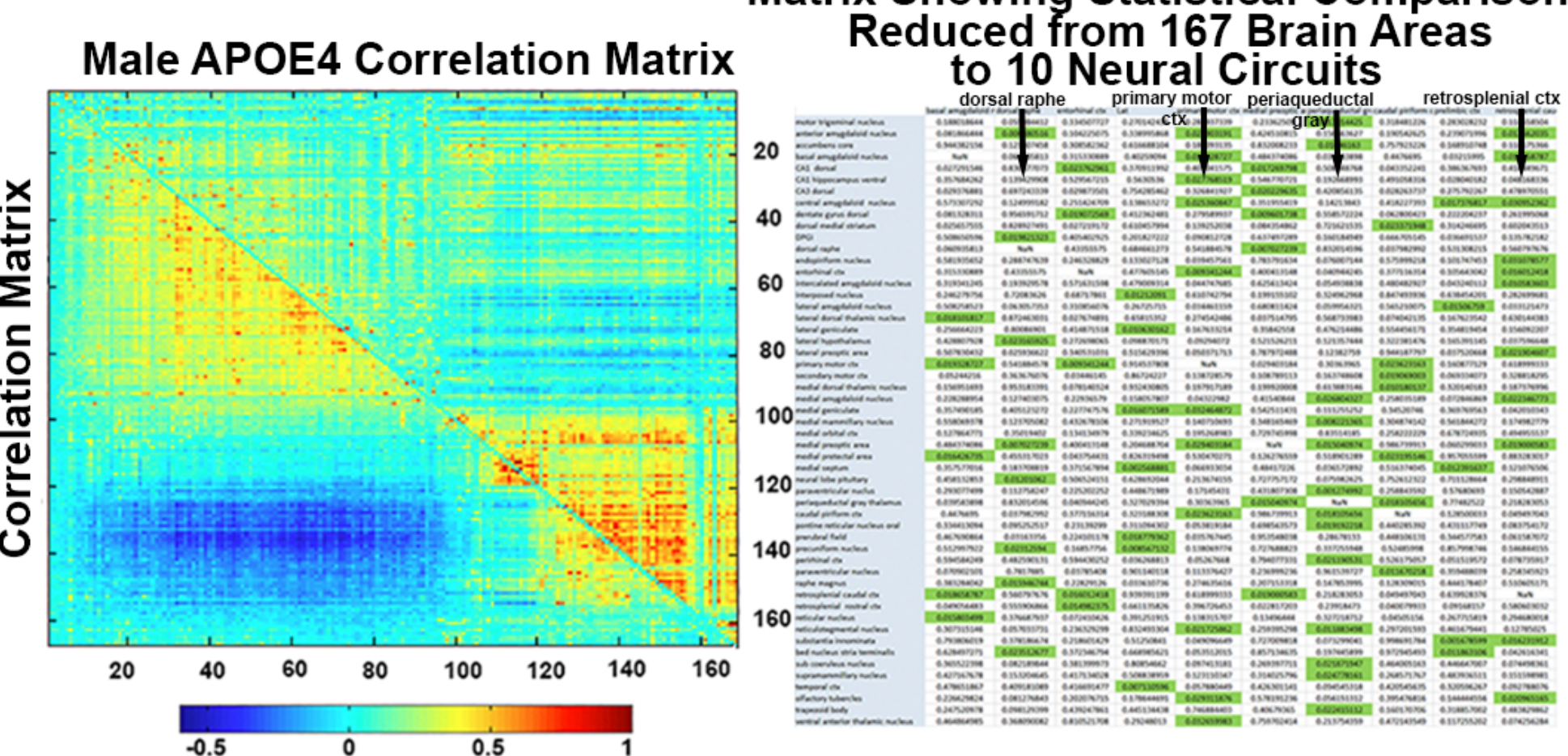
ca. 20,000 voxels are given a numerical measure of diffusion and registered to atlas and localized to one of 152 different brain areas

Main Results

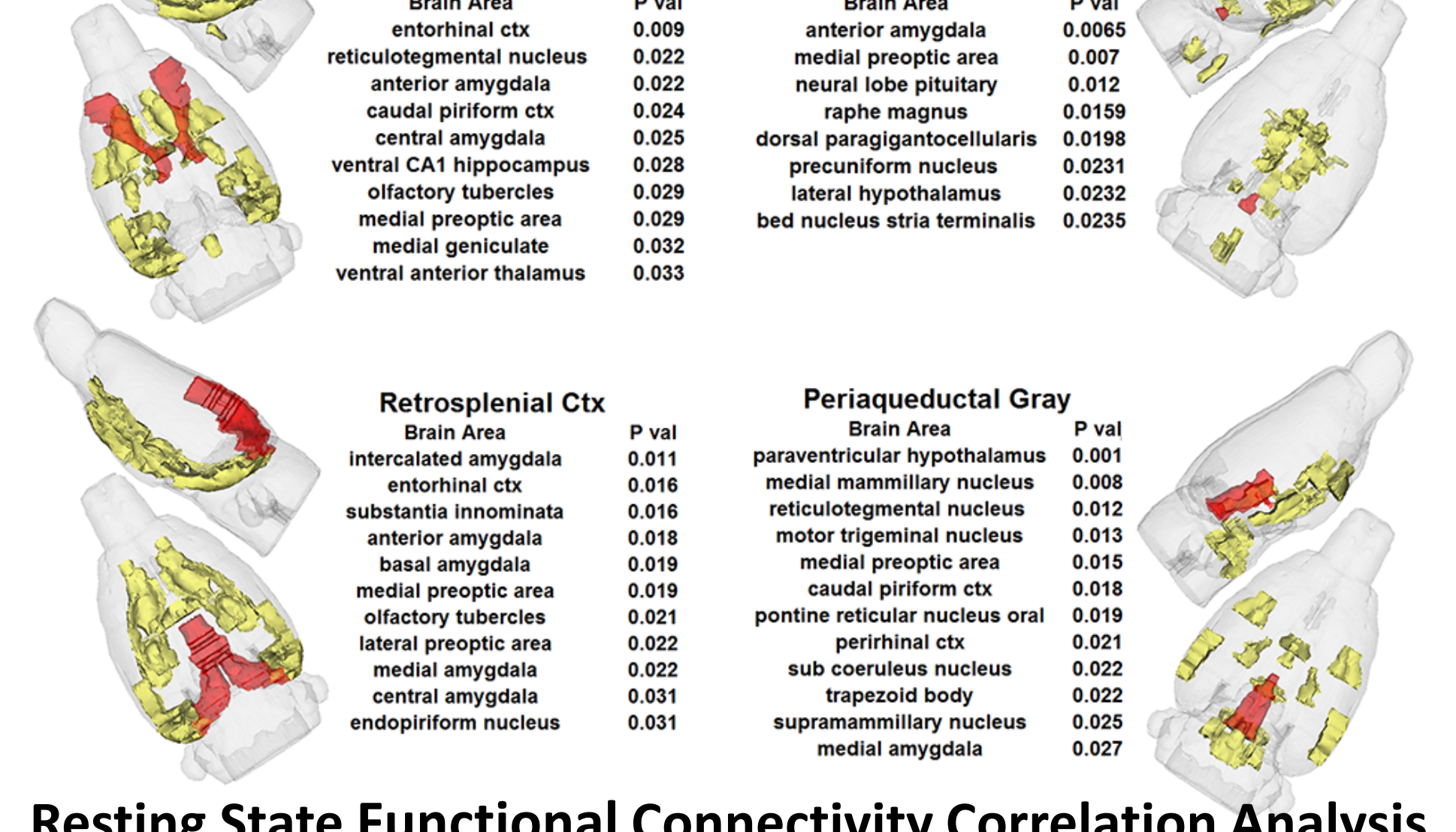
Probability Maps Comparing Female Wild-Type to Female APOE4 and Male WT to Male APOE4: Fractional Anisotropy and Radial Diffusivity



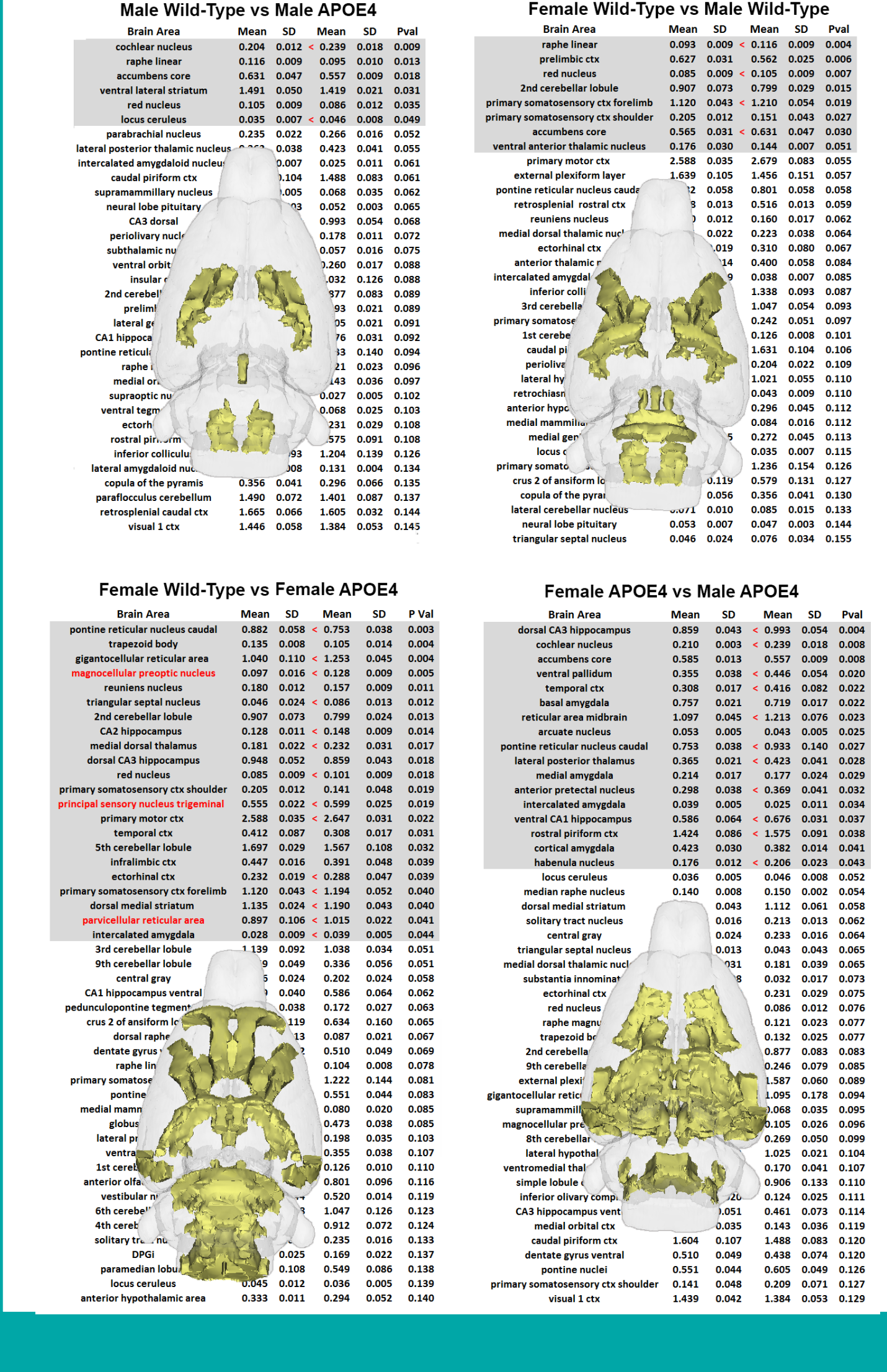
Resting State Functional Connectivity Correlation Analysis Comparing Wild-Type and APOE4 Males



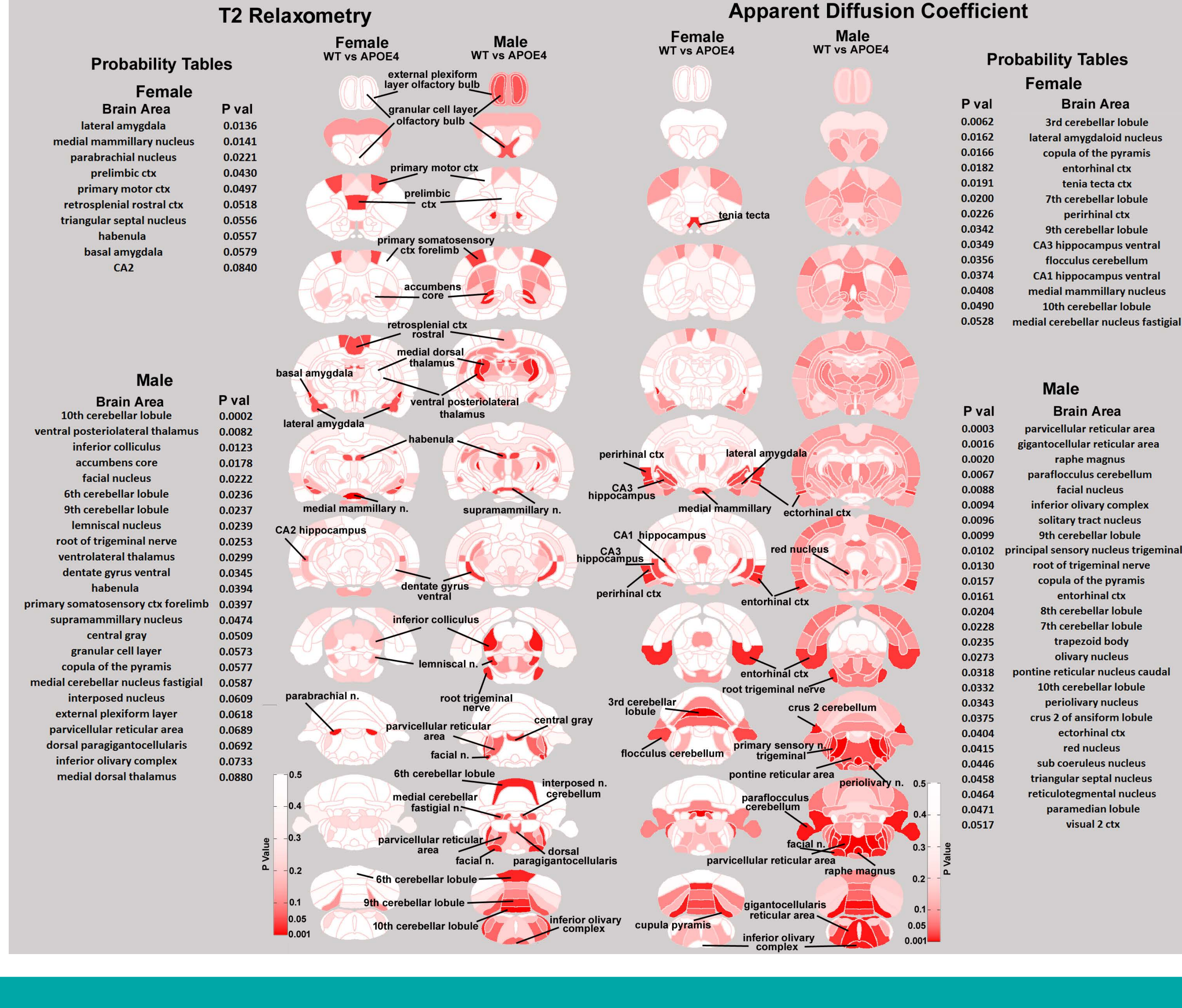
Resting State Neural Circuits in APOE4 Males



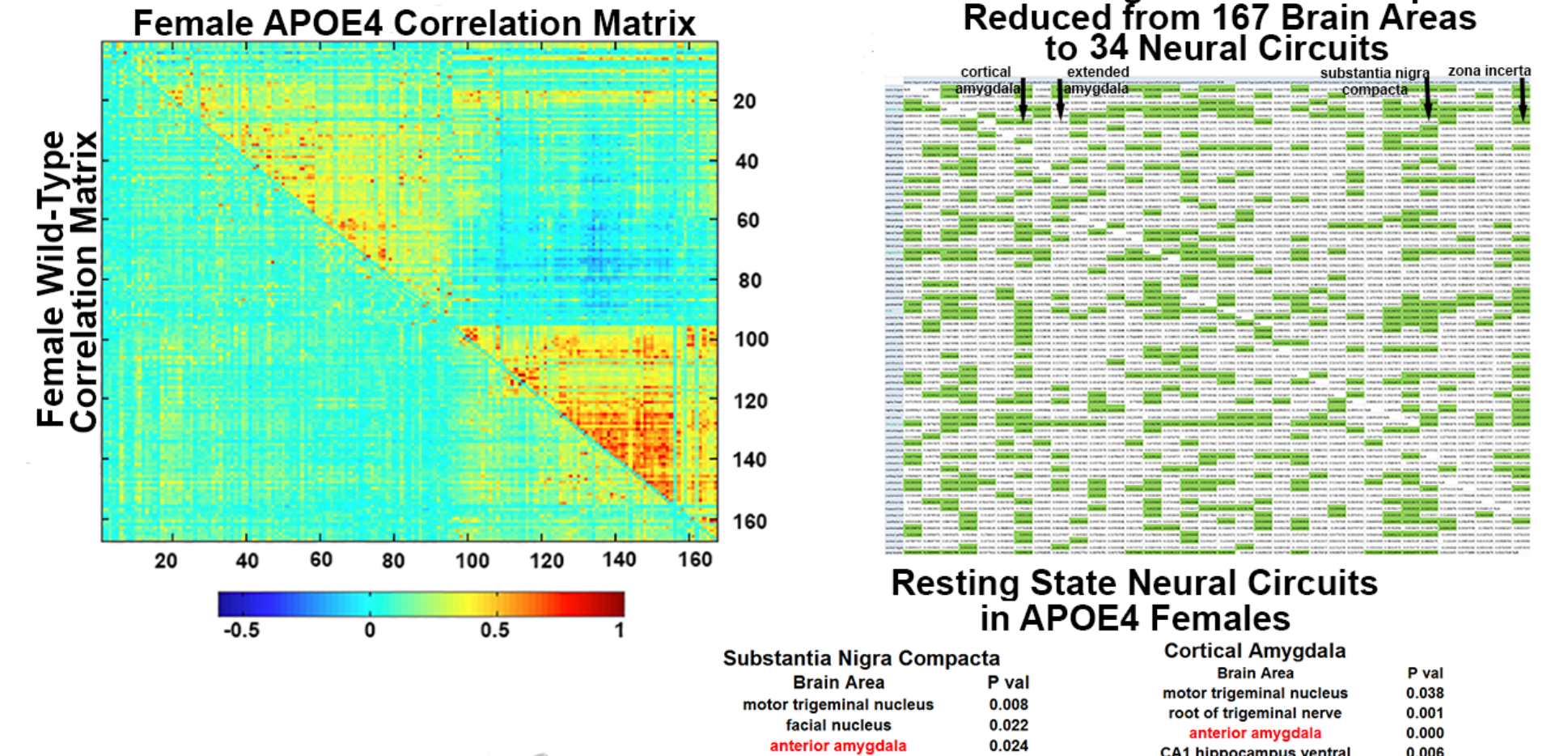
Quantitative Volumetric Analysis of Brain Regions



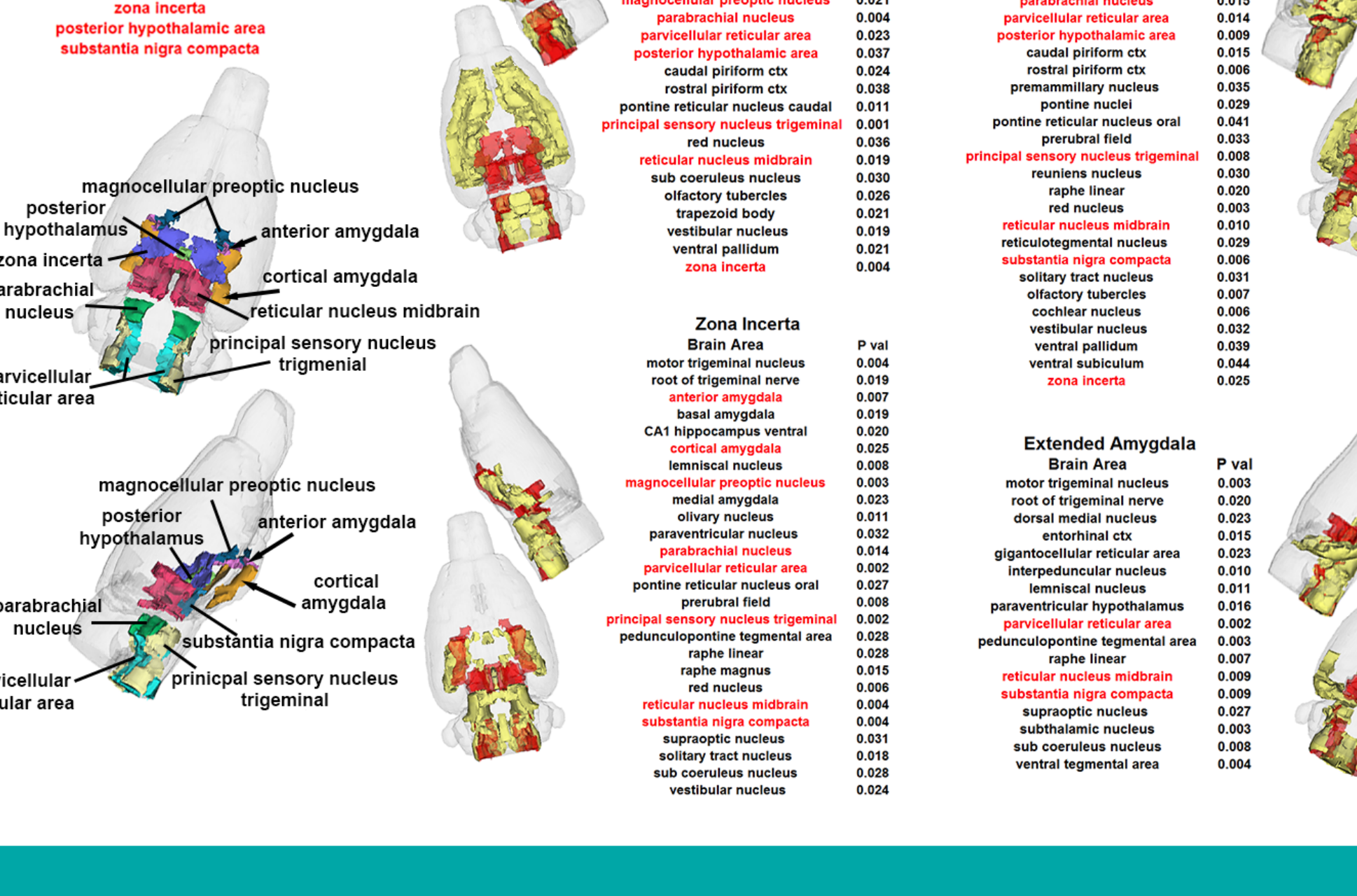
Probability Maps Comparing Female Wild-Type to Female APOE4 and Male WT to Male APOE4: T2 Relaxometry and Apparent Diffusion Coefficient



Resting State Functional Connectivity Correlation Analysis Comparing Wild-Type and APOE4 Females



Resting State Neural Circuits in APOE4 Females



Summary

- Male APOE4 rats show a deficit in cognitive function.
- This deficit is associated with microarchitectural changes in cerebral cortex, cerebellum and reticular activating system.
- Measures of oxidative stress are increased in cortex and cerebellum
- Functional connectivity is weak and altered between cerebral cortex and ventral brain areas e.g., amygdala, olfactory tubercles, preoptic areas
- There are few differences in brain volumes as compared to WT.
- Female APOE4 rats show normal cognitive function.
- Microarchitectural changes are associated with olfaction, ventral striatum and hippocampus.
- No post mortem measures were taken.
- Functional connectivity is strong with a cluster of brain areas e.g., principle sensory n. trigeminal, reticular activating system, amygdala and zona incerta, forming the core.
- There are many differences in brain volumes as compared to WT e.g., cortex, reticular activating system, and principle sensory n. trigeminal most of which show female APOE4 are larger the WT.

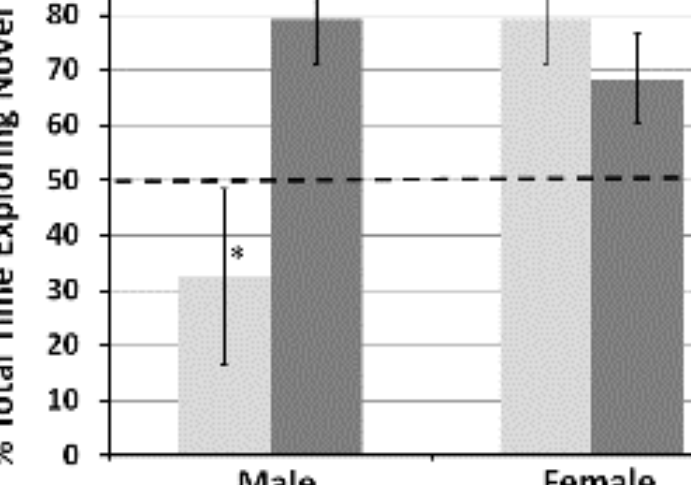
Speculation

Alzheimer's is a disease of aging. Male APOE4 rats, while adult at 5 months, are not old. Yet they show a deficit in cognition and alterations in brain microarchitecture, oxidative stress and connectivity. In contrast, female APOE4 rats of the same age have normal cognition, strong connectivity between core areas and many significantly enlarged brain areas as compared to WT.

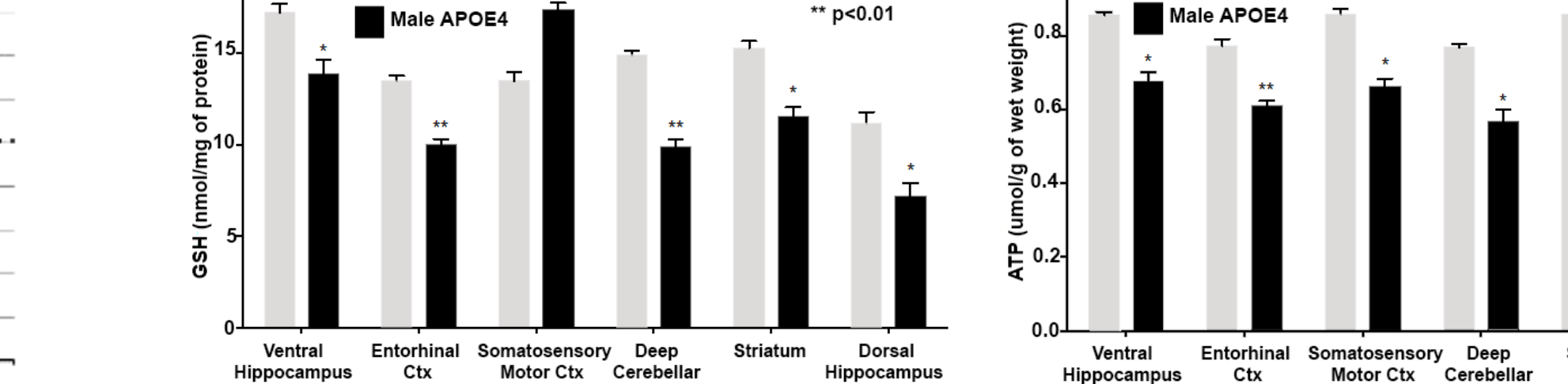
Does the APOE4 isoform confer some benefit to healthy adult females while they are still reproductively active?

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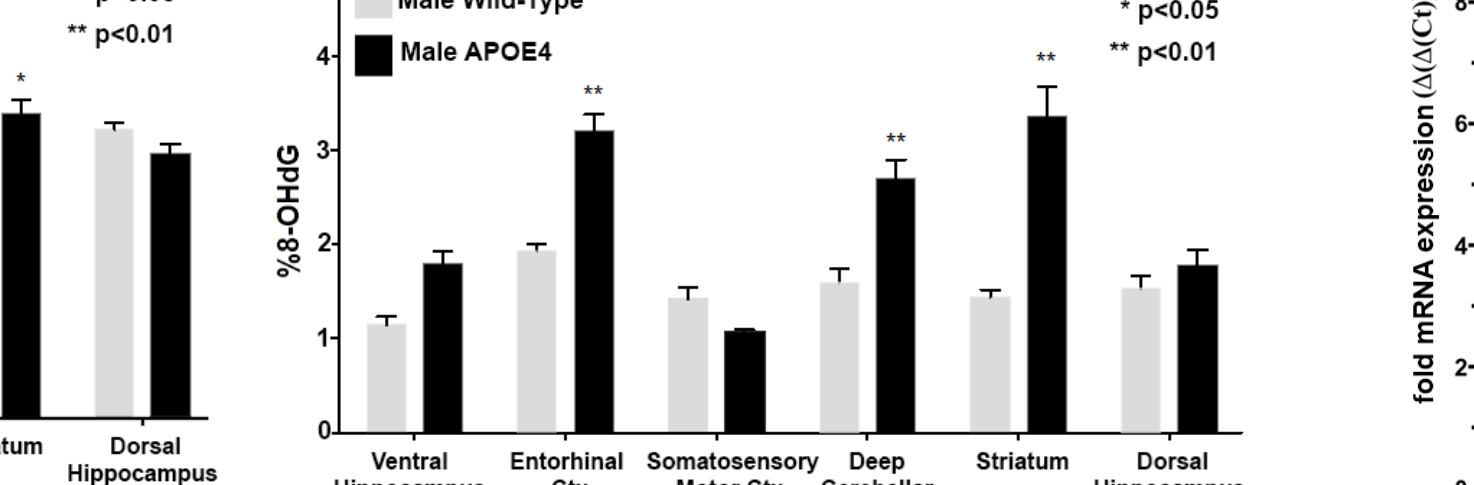
Cognitive Behavior



Region Specific Molecular Markers of Oxidative Stress



Changes in Apoptosis



Changes in Mitochondrial Genes

