Increased cortical volume revealed by atlas-based volumetry in a bigenic mouse model of Alzheimer's Disease

Kristof Govaerts[1], Janaki Raman Rangarajan[2], Tom Struys[3], Fred Van Leuven[4], Uwe Himmelreich[1], and Tom Dresselaers[1]

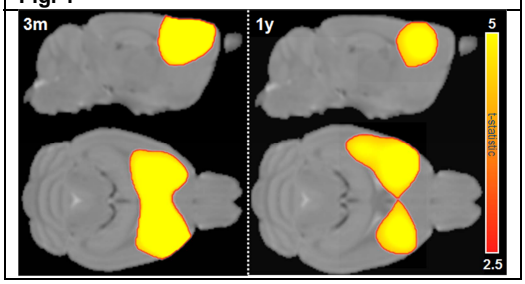
[1]Imaging & Pathology, KU Leuven, Leuven, Vlaams-Brabant, Belgium, [2]Electrical Engineering, KU Leuven, Leuven, Vlaams-Brabant, Belgium, [3]Morphology, Universiteit Hasselt, Hasselt, Limburg, Belgium, [4]Human Genetics, KU Leuven, Leuven, Vlaams-Brabant, Belgium

Target audience: This abstract is relevant for researchers interested in mouse models of Alzheimer's Disease as well as those interested in atlas-based registration and voxel-based morphometry in rodents.

Purpose

Although atrophy is a well-established MRI biomarker for Alzheimer's Disease (AD) in patients (1), results in mouse models of AD have been less consistent and depend on the model and modality used. Often, clear atrophy cannot be detected in amyloid models (2, 3, 4), while this does not seem to be the case for tauopathy models (5). In this study, we make use of the biAT mice, which combine the two types of AD pathology and also display an early behavioral phenotype representing prodromal AD (6). Atlas-based registration is a valuable tool in clinical settings, but is not yet consistently used in mouse research. Voxel-based morphometry in particular has not seen much use in murine research, likely because of the small differences expected in the early stages of AD and the limited resolution of in vivo mouse MRI. However, atrophy monitoring could provide crucial contributions to preclinical trials of novel AD treatments.

Fig. 1



Methods

- *MRI*: Double transgenic APP.V717I x Tau.P301L (biAT) and age-matched FVB control mice were imaged at 3 (biAT: 16, FVB: 16) and 12 (biAT: 18, FVB: 19) months of age. Imaging was performed on a 9.4T Biospin small animal MR system (20cm horizontal bore, Bruker Biospin, Ettlingen, GE), using a 7 cm linearly polarized resonator for transmission and an actively-decoupled mouse brain surface coil for receiving (Rapid Biomedical, Rimpar, GE). For anatomical imaging, we utilized a 3D T_2-weighted MRI protocol (TR 1 s, TE 36 ms, rare factor 10, zero-fill factor 1.33, 160x256x88 matrix, 1.5x2.4x0.83cm FOV). To assess reproducibility of regional volume measurements, a single FVB control animal was imaged five times on the same day, fixating the head and repositioning the coil each time.
- *Processing*: After bias field correction and intensity normalization using in-house developed Matlab scripts (The Mathworks, Natick, US) (7), data processing was performed using a nonrigid registration protocol incorporating NiftyReg (Centre for Medical Image Computing, University College London, London, UK). Atlas labels from the MDA2006 atlas were propagated to a study-specific FVB template created from the 3mo FVB scans, which was used as the reference for all subsequent registrations. Brain masks were propagated from the template image to individual floating images and corrected where necessary, after which bias field correction and registration was performed on brain masked images. Voxel-based morphometry was performed on the Jacobian maps in template space using SPM, and thresholded for a family-wise error rate of 5%. Regional volumes were corrected for whole brain volume and normalized to a brain volume of 500µl. Statistical analysis was performed using one-way ANOVA with Bonferroni's correction for multiple testing.
- *Histology*: 13mo animals were sacrificed by an i.p. overdose of Nembutal (300µl; Ceva) and subsequently perfused with 4% ice - cold paraformaldehyde (PFA) solution (Sigma - Aldrich). After overnight post fixation in 4% PFA, brain tissue was stored in a 0.1% sodium azide solution (Fluka, Sigma - Aldrich) at 4°C. Brains were embedded in paraffin, 7 µm sections were sliced and a Masson's trichrome staining was performed. Slices were scanned with a Mirax desk (Carl Zeiss, Oberkochen, Germany) and microscopic images were taken with the Mirax viewer software.

Fig. 2

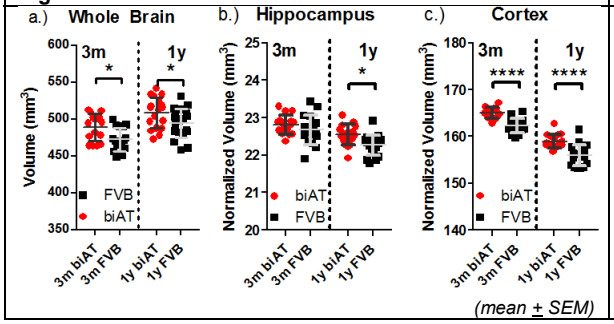
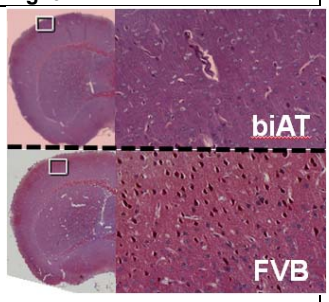


Fig. 3



Results

biAT animals displayed a modest but significant increase in whole brain volume at 3 and 12 months *vs*. FVB (**Fig. 2a:** 3m: 489 ± 17 vs. 472 ± 15 mm^3, p=0.011, 1y: 508 ± 20 *vs*. 494 ± 19 mm^3, p=0.038). Voxel-based morphometry (**Fig. 1**) revealed that these volume differences are largely localized to the region of the frontal cortex and basal ganglia, at both the 3 and 12 mo timepoints. Regional volumes normalized for brain volume revealed subtle but highly significant increases in volumes for the cerebral cortex of biAT mice vs FVB (**fig. 2c**, 3mo: 165.0 *vs.* 162.3mm^3, 1yo: 158.9 *vs*. 155.9 mm^3 for biAT and FVB,respectively; both p<0.0001). No significant differences in normalized volume were observed in the hippocampus at 3 mo, although a minor increase in normalized volume was found at 12 mo (**Fig. 2b,** 3mo: 22.8 *vs*. 22.7, n.s., 1yo: 22.6 *vs*. 22.3, p<0.05.). Reproducibility of volume measurements in a single animal scanned repeatedly was high: standard deviations of absolute measurements were on the order of 1% (whole brain: 1.11%, hippocampus: 1.05%, cortex: 0.79%), and for normalized measurements this was 1.5% (hippocampus: 1.54%, cortex: 1.40%). Histological analysis revealed regions with a reduced neuronal density in the frontal cortex (**fig. 3,** slice with anterior commissure).

Discussion

With our methods, we were able to reliably and reproducibly resolve volume differences of only a few percent. Increased cortical size is a counter-intuitive but not unprecedented finding in a mouse model for AD. Increased cortical thickness with a subsequent age-depended increase in cortical thinning has been reported in an APP model (2). Early and late increased cortical volume as well as late increased hippocampal volume has been reported in the TASTPM model (4). Other studies do not report clear trends suggesting global brain atrophy (3).

Considering the decreased neuronal density we observed in these regions, it is likely that the volume increases we report are the result of a secondary mechanism, most likely gliosis and/or edema. Even so, the absence of hippocampal volume changes suggests that the biAT model, although it displays many of the typical molecular and behavioral AD phenotypes (6), does not recapitulate atrophy-induced volume changes, or that the anatomical defects are too small and the methods lack the resolution and sensitivity to reveal the expected hippocampal atrophy.

References : 1. Jack et al., Lancet Neurol. 2010, 2. Hébert et al., Neurobiol. Aging 2013, 3. Lau et al., Neuroimage 2008, 4. Maheswaran et al., Brain Res. 2009, 5. Yang et al., Neuroimage 2010, 6. Terwel et al., Am J. Pathol. 2008, 7. Vande Velde et al., Neuroimage 2012