Comparing *in vivo* and *ex vivo* imaging in an Alzheimer's mouse model using tensor-based morphometry

+Joint first authors

INTRODUCTION Despite the growth of preclinical MRI applied to transgenic mice, there is limited information as to whether it is best to scan a mouse *in vivo* or *ex vivo* to maximise sensitivity and specificity to morphological differences. On one hand, *ex vivo* scans may be significantly longer; the use of contrast agents in high concentrations is permitted, and motion is entirely obviated, which can increase SNR/CNR to support advanced computation approaches. Conversely, living animals do not suffer distortions and dehydration from fixation, or artefacts from contrast agents, in addition to permitting longitudinal imaging, which may both increase statistical power and elucidate developmental or progressing degenerative changes [1]. The tradeoff between *in vivo* and *ex vivo* imaging has previously been discussed by Lerch et al [1], where they found that *ex vivo* imaging provides greater precision than *in vivo* imaging, and is preferable when multi-timepoint data is not required. However, when longitudinal data is desirable, it is still unclear to what extent the results are compromised in favour of the benefits of *in vivo* imaging. In this work, we sought to compare *in vivo* and *ex vivo* structural imaging in the rTg4510 mouse model of Alzheimer's disease (AD), an established model exhibiting neurodegeneration from 3 months of age [2]. By imaging a cohort of rTg4510 and age-matched wildtype control mice, we sought to investigate the morphological differences that can be automatically and objectively detected using *ex vivo* and *in vivo* structural MRI with tensor-based morphometry (TBM).

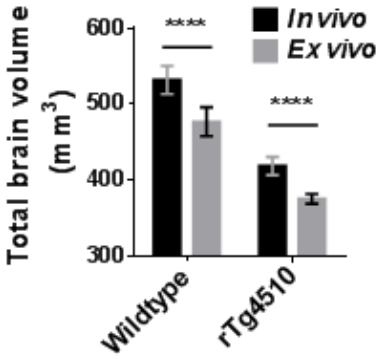

Figure 1 *Mean total brain volume for wildtype, rTg4510 and rTg4510(+DOX) animals. Error bars represent the standard error of the mean. **** = p<0.0001*

METHODS *Animals.* rTg4510 mice were bred as published previously [2]. 10 Tg4510s and 8 wildtype controls were imaged *in vivo* and *ex vivo* at 7.5 months. Prior to *in vivo* imaging, mice were secured in a cradle under anaesthesia with 1-2% isofluorine in 100% oxygen. Body temperature was maintained at 36–37.5 ^{o}C. *Image acquisition.* All scans were performed on an Agilent 9.4 T VNMRS 20 cm horizontal-bore system (Agilent Inc. Palo Alto, CA, USA). A 72 mm birdcage radiofrequency (RF) coil was used for RF transmission and a quadrature mouse brain surface coil (RAPID, Germany) was used for signal detection. A T2 weighted, 3D fast spin-echo sequence was implemented for structural imaging with the following parameters: FOV = 19.2 mm x 16.8 mm x 12.0 mm; resolution = 150 µm x 150 µm x 150 µm; TR = 2500 ms, TE_{eff} = 43 ms, ETL = 4; NSA = 1. Total imaging time was ~1h 30 mins. After *in vivo* imaging, the animal was immediately perfuse-fixed with 0.9% saline (15 – 20 mL) followed by 10% buffered formal saline (50 mL) doped with Magnevist (8 mM). A custom-build three brain holder was used to acquire high resolution *ex vivo* images. A 35L mm birdcage RF coil was used for RF transmission and signal detection using the following parameters: FOV = 32 mm x 25 mm x 25 mm; resolution = 40 µm x 40 µm x 40 µm; TR = 17 ms; TE = 4.54 ms; flip angle = 51^{o}; NSA= 6. Total imaging time was approx. 11 h. *Image processing*. TBM maps were generated using a fully automated pipeline [3] including groupwise, affine and non-rigid registration using NiftyReg [4] **.**

RESULTS AND DISCUSSION In order to assess the global impact that formalin fixation has on brain volume, we extracted the total brain volume (TBV) for both *in vivo* and *ex vivo* specimens (Figure 1). Although we detected significant shrinkage from *in vivo* to *ex vivo* for both groups (p<0.0001), we found similar brain volume shrinkage between the wildtype (10.3%) and rTg4510 (10.4%) animals. We also observed striking consistency in TBV loss in the rTg4510 animals extracted from the *in vivo* (21.3%) and *ex vivo* (21.4%) data. Figure 2 shows TBM statistics overlaid on representative coronal slices of the group-averaged *in vivo* and *ex vivo* data. The *in vivo* results are remarkably reserved, despite the extensive atrophy highlighted by the TBV results (Figure 1). In vivo we observed discrete volume loss within the cortex and striata, in addition to expansion of the ventricles (Fig 2i-iii). *Ex vivo* analysis identified a similar but far more extensive pattern of change which more accurately reflects the known pathology in this model, including atrophy across all cortical regions and within the hippocampus [1]. Work is ongoing to uncover how closely the observed morphometric changes reflect the underlying tau-abnormalaties in this model.

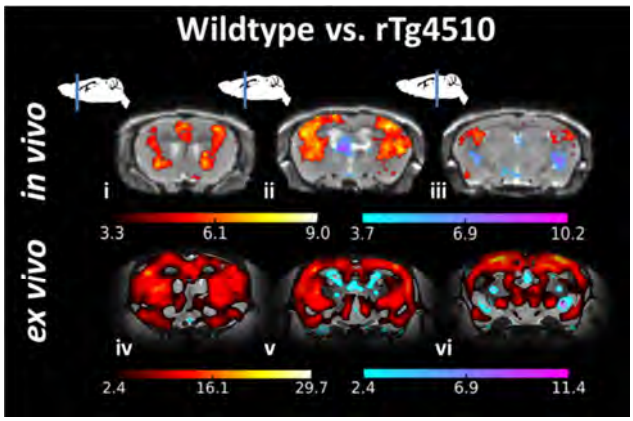

Figure 2 *Results from structural analysis of the in vivo (i-iii) and ex vivo (iv-vi) data, showing TBM statistical results overlaid on coronal slices of the final group average. Red: regions where the rTg4510 brains are relatively locally smaller than the average; blue: rTg4510 brains are locally larger. Based on FDR-corrected t-statistics (q=0.05).*

The obvious discrepancy between the *in vivo* and *ex vivo* results may be due to the high contrast afforded by *ex vivo* imaging, which enables the voxel-wise tests to highlight more extensive regions of difference between groups. In addition, the higher resolution enables improved localisation of volume change. Conversely, *in vivo* imaging suffers from relatively homogeneous grey matter contrast. As a result, the registration steps, which are crucial in determining the deformation fields, are unable to isolate deformations occurring sub-regionally within larger structures.

CONCLUSION We propose that, for longitudinal characterisation of a neurodegenerative or neurodevelopmental phenotype, *in vivo* imaging is suitable to broadly detect regions of morphometry. However, as proposed by Lerch et al, the precision and sensitivity of the results is greatly improved *ex vivo*. It is important to acknowledge that our observations are unique to the rTg4510 mouse, and may not represent every imaging paradigm for characterization of transgenic mice, due to the large brain voulme changes. Despite this limitation, we hope that our results may help guide other researchers working in the field of preclinical imaging when choosing whether an *in vivo* or *ex vivo* experiment is the most suitable.

REFERENCES [1] Nieman, B. J. et al *Frontiers in Neuroinformatics* (2012) **6**(6); [2] Santacruz et al *J Neuroscience* (2005) **25**(46); [3] Powell, N. *ESMRMB proceedings* (2013) **699**; [4] Biomedical Image Registration - Lecture Notes in Computer Science (2012) **7359**