3D Quantitative Micro-MRI Mapping of Alzheimer's Plaques in Transgenic Mice using Aβ1-42 Targeted-USPIOs

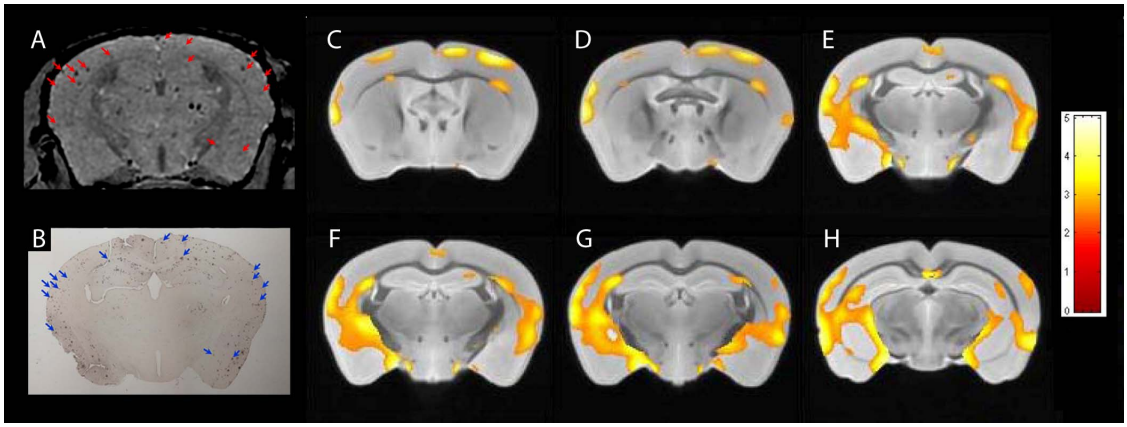
D. M. Hoang[1], J. Yang[2], L. K. Hill[1], W. Tsui[3], Y. Sun[2], Y. Li[2], M. De Leon[3], T. Wisniewski[2,3], and Y. Z. Wadghiri[1]
[1]Radiology, NYU School of Medicine, New York, NY, United States, [2]Neurology, NYU School of Medicine, New York, NY, United States, [3]Psychiatry, NYU School of Medicine, New York, NY, United States

Introduction: Amyloid β (Aβ) plaques are one of the pathological hallmarks of Alzheimer's disease (AD). Their visualization in the brain is very important to monitor AD progression and to evaluate the efficacy of therapeutic interventions. Numerous studies have investigated the visualization of Aβ plaques using MRI through endogenous detection both in human and in mouse brains. Only two groups have been so far successful in enhancing Aβ plaques using targeted contrast agent in transgenic mice [1,2]. Since these initial proof-of-concept studies, most of our own efforts have been devoted towards the development of more soluble and less toxic peptide-based MRI targeted probes while maintaining their affinity towards Aβ deposits [3]. Our approach has proven so far successful only with intra-carotid injection in order to enable effective delivery of the Aβ probes to the neighboring brain. This also required the co-injection of mannitol to increase their permeation across the blood brain barrier (BBB). In the present study, we examined whether the use of ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles, chemically coupled with Aβ1-42 peptide along with mannitol through femoral intravenous injection can be effective to detect individual plaques using *in vivo* μMRI and map the amyloid burden throughout the brain.

Material and Methods: Animals: 13 to 17-month old APP/PS1 transgenic mice [4] and age-matched wild-type (C57Bl/6J) control mice were used in these studies. All mice used in these studies were maintained according to the protocols approved by the IACUC at the NYU School of Medicine. Targeted contrast agents: The USPIO nanoparticles (10mgFe/ml, Ocean Nanotech) were linked to Aβ1-42 peptide using standard EDC/NHS coupling methods, according to the manufacturer's instructions. MRI: All MRI scans were performed on a 7T micro-MRI system consisting of a 7-Telsa 200-mm horizontal bore magnet (Magnex Scientific, UK) equipped with an actively shielded gradient coil (Bruker BGA-9S; ID 90-mm, 750-mT/m gradient strength, 100-μs rise time) interfaced to a Bruker Biospec console. All mice were scanned 6-hrs after intravenous injection of the contrast agent to ensure maximum plaque detection with minimal nonspecific labeling of blood vessels [**1**]. For in vivo imaging the mice were anesthetized with 2.5% isoflurane in 75% NO2 plus 22% O2. For maintenance of anesthesia the isoflurane was reduced to 1%. Body temperature of the mice was maintained at 37ºC using a warm-water blanket. A 3D multi-Gradient Echo sequence was used to provide quantitative R2* measurements based on a four-echo train datasets with 100 micron isotropic spatial resolution. The advantage of the isotropic 3D-imaging approach is that the image set can be reprocessed in any desired slice orientation facilitating image comparison during co-registration with histology. The third echo was used to generate a T2*-weighted (TE=12.3 ms) image subset for both plaque visualization and voxel-based analysis [3]. For ex vivo imaging, a dedicated apparatus was designed to scan up to four brains simultaneously overnight using a Quadrature Birdcage Coil (ID= 28mm, length 29mm) designed in-house to fit a 35 cc syringe (ID = 24 mm) containing Fomblin (Solvay Solexis Inc., Thorofare, NJ). Histological Studies: After ex vivo MRI, serial coronal sections (40 μm) were cut and every third section were stained with a combination of 6E10 and 4G8, both monoclonal anti-Aβ antibodies (Covance, Emeryville, CA), as previously described [1]. Voxel-based morphometry (VBM): was performed as an additional quantitative analysis based on SPM5 (Wellcome Dept of Clinical Neurology, London) with SPMMouse toolbox [5]. Regional specific differences were assessed statistically using the GLM/univariate analysis with a one-tailed T statistics, showing voxels of lower intensity in APP/PS1 Tg mice compared to wild-type mice. We considered $p < 0.01$ (uncorrected for multiple comparisons) to indicate statistical significance for individual voxels, with a minimum cluster size of 500 voxels.

Results and Discussion: In comparison to WT mice (data not shown), figure (A) depicts an example of *in vivo* T2*-weighted MRI of a 14 month-old APP/PS1 mouse showing multiple dark spots throughout the brain, 6-h following USPIO-Aβ1-42 injection. These dark enhancements closely match the larger plaques (arrowheads) confirmed by immunohistochemistry (B). The regional differences seen in VBM comparing USPIO-Aβ1-42 injected APP/PS1 and WT mice correlated (C-H) with the amyloid plaque distribution observed histologically, contrasting with no differences between the two groups of mice without contrast agent injection in regions of the brain where amyloid deposition is expected (data not shown). Furthermore, comparison of absolute R2* quantitation assessed in various brain regions between APP/PS1 Tg mice and wild-type control both contrast injected showed significant differences in the cortex and the hippocampus (** $p<0.01$) but not in the cerebellum as expected in this mouse model.

Conclusion: Our results demonstrate that USPIO-based Aβ probes can help visualize individual large plaques after femoral injection. Combination of our targeting approach with quantitative methods help identify statistically differences between AD transgenic mice and wild type mice. The feasibility of using less invasive intravenous femoral injections for Aβ plaque detection in AD transgenic mice facilitates using this method for longitudinal studies in the pathogenesis of AD.



Acknowledgments. We thank S.J. Sawiak for providing the SPM mouse brain atlas. This work was supported by NIH grants AG20245, NS073502 and AG008051 to T.W., the American Health Assistance Foundation ADR (A2008-155) to Y.Z.W. and the Alzheimer Association (IIRG-08-91618) to Y.Z.W.

References: [1] Wadghiri YZ *et al.*, MRM, 50, 293 (2003). **[2]** Poduslo *et al.*, Neurobiol Dis. 11(2) 315 (2002). **[3]** Sigurdsson *et al.*, Neurobiol Aging 29(6), 836 (2008). **[4]** Holcomb L *et al.*, Nature Med., 4, 97 (1998). **[5]** Sawiak SJ *et al.*, Neurobiol Dis., 33,20 (2009).