Custom-Fit, 3D-Printed Marmoset Brain Holders for Comparison of Histology with MRI

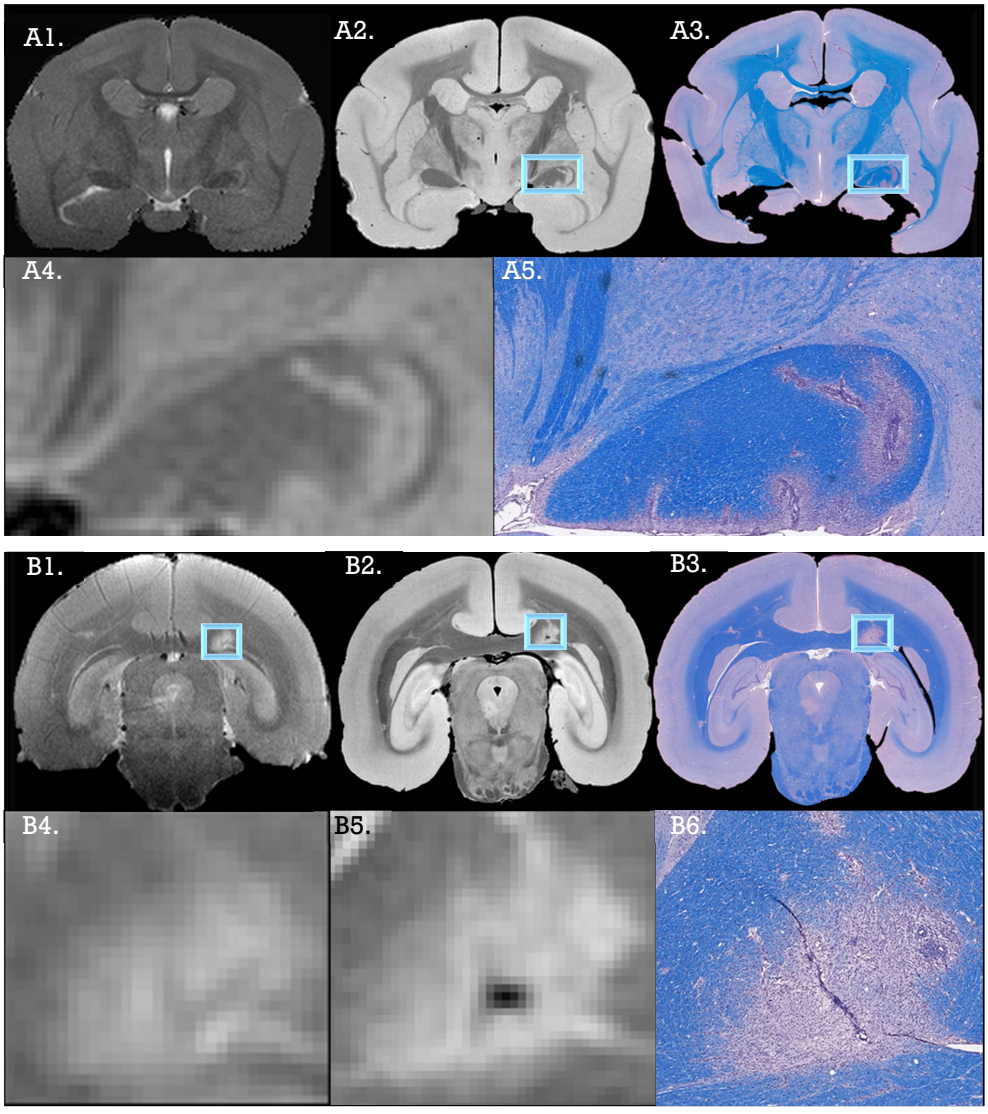
Joseph Guy[1,2], Pascal Sati[1], Steven Jacobson[3], Afonso C Silva[4], and Daniel S Reich[1]

[1]Translational Neuroradiology Unit, Neuroimmunology Branch, National Institute of Neurologic Disorders and Stroke, Bethesda, MD, United States, [2]Department of Biochemistry, University of Cambridge, Cambridge, Cambridgeshire, United Kingdom, [3]Viral Immunology Section, Neuroimmunology Branch, National Institute of Neurologic Disorders and Stroke, Bethesda, MD, United States, [4]Cerebral Microcirculation Unit, Laboratory of Functional and Molecular Imaging, National Institute of Neurologic Disorders and Stroke, Bethesda, MD, United States

Target Audience: Pre-clinical scientists and translational neuroscientists

Purpose: Direct comparison between MRI and histology is often difficult and imprecise. Our goal is to develop a robust method to improve our ability to compare MRI and histopathology, with high precision, in a marmoset model of multiple sclerosis (MS).

Method: We used 5 formalin-fixed brains from marmosets that previously participated in an experimental allergic encephalomyelitis (EAE) protocol[1] in which the animals were imaged *in vivo* at 7T/30 cm (Bruker Biospin) using T_2 weighted (T_2w) and PD weighted (PDw) sequences at 125 µm² in-plane resolution with 600 µm thick slices and a total scan time of 20 minutes. The fixed brains were first suspended in a 50 mL Falcon tube filled with an MR invisible media (Fomblin Solvay S.A.) and imaged at 7T using an *ex vivo* T_2w protocol (150 µm isotropic, 7.4 hours). 3D models of the brains' surfaces were extracted from these images using MIPAV (mipav.cit.nih.gov). These brain models were then imported into 3D-printing software (netfabb Professional) to generate 3D models of a brain cradle and a brain slicer unique to each brain. The brain cradles were then used to run an ultra-high-resolution T_2*w MRI protocol (100 µm isotropic, 32.7 hours) using the same setup as the first *ex vivo* MRI. After this final imaging, the brains were transferred to their respective brain slicers to be cut into 4 mm-thick slabs. The resulting tissue slabs were further cut into 10 µm-thick slices using a cryostat microtome. The slices were finally stained with hematoxylin and eosin (H&E), counterstained with Luxol fast blue (LFB), and digitalized using a Ventana iScan™ automated slide scanner.



Results: The digitalized histological brain slices were matched to the MRI images using large-scale anatomy as well as small-scale features (vessels and EAE lesions) for a more refined alignment. Representative results are illustrated in the figure. Two different brains (A, B) are displayed with their respective *in vivo* MRI (1), *ex vivo* MRI (2), and H&E+LFB staining (3). Magnified, upscaled views demonstrate the precision of the alignment (4-6). MRIs were rotated an average of 1.9° to match histology. EAE lesions scattered throughout the cerebral white matter consistently showed the same location and shape in both the MRI and histology images, despite being imaged with different resolutions, distortions, and artifacts, in all 155 histological samples investigated.

Conclusions: 3D printed brain holders/slicers were created in order to rigidly maintain brain orientation and alignment between *in vivo* and *ex vivo* MRI, and histological brain slices. This produced similar success to our technique for human brains.[2] This easy and relatively quick methodology increases the precision of registration of MRI with pathological staining of EAE lesions and strongly validates comparison and characterization of MRI-detected lesions. In future studies, this method will decrease labor, expense, and time required to compare in vivo imaging findings to post-mortem histology and validate MR biomarkers of lesion development in this invaluable animal model of MS.

References:

1. Gaitán MI, Maggi P, Wohler J, et al. Perivenular brain lesions in a primate multiple sclerosis model at 7-tesla magnetic resonance imaging. Mult Scler January 2014 vol. 20 no. 1 64-71

2. Absinta M, Nair G, Filippi M, et al. Postmortem magnetic resonance imaging to guide the pathologic cut: individualized, 3-dimensionally printed cutting boxes for fixed brains. J Neuropathol Exp Neurol. 2014 Aug;73(8):780-8