New Techniques for 3D, High-Resolution, Whole Brain Mapping of Murine Vasculature

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INTRODUCTION: Knowledge of the 3D architecture of blood vessels is crucial for several reasons: (i) various neuropathologies ranging from alzheimers disease to brain tumors involve anomalous blood vessels [1, 2]. (ii) The physiological underpinnings of image contrast in functional MRI (fMRI) critically involve the microvasculature [3]. (iii) The cerebral vasculature is central to understanding drug delivery, and pharmacokinetics of novel therapies in the brain. Additionally, the widespread development of transgenic mouse models of disease has created the need to image and characterize the vasculature of the murine brain [4]. While histological techniques such as optical microscopy and corrosion casting are powerful tools for imaging microvasculature at submicron resolutions, they suffer from limited coverage, and information on the 3D blood vessel geometry once destroyed by sectioning requires complex reconstruction. Additionally, damage to small vessels due to cast brittleness, collapse of vessels when supporting tissue is removed during maceration, and problems with digitizing the cast are but some of the pitfalls of corrosion casting approaches. Here we describe two novel methods for wide-area-mapping of the murine brain vasculature using magnetic resonance microscopy (μMRI), a rapidly evolving type of 3D MRI that offers very high *ex vivo* imaging resolutions (currently ~20-30μm^3) [5]. μMRI has the advantage of generating digitized 3D images, simplifying subsequent image analyses. More importantly, μMRI is non-destructive, provides excellent soft tissue contrast, preserves tissue and blood vessel architecture, and one can obtain μMRI images with various kinds of "physiological stains" or image contrast, e.g. diffusion-weighted μMRI to visualize orientation of white matter fibers in the brain.

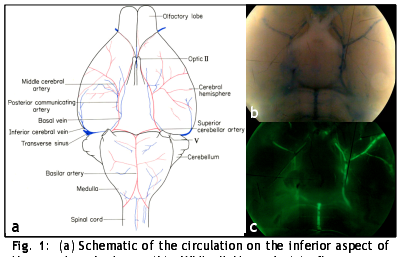

Fig. 1: (a) Schematic of the circulation on the inferior aspect of the murine brain. (b) White-light and (c) fluorescence microscopy views (10X) of a freshly excised, intact mouse brain perfused with the *gelatin* perfusate.

METHODS: We conducted a series of experiments to determine the feasibility of perfusing the murine cerebral vasculature with different perfusates with the goal of obtaining complete vascular filling, optical validation, repeatability and high contrast to noise ratio in the μMRI images. *Protocol I*: The first perfusate tested was 3% gelatin doped with 4% Evans blue and 30mg/ml of FITC-dextran for optical validation. Animals were anesthetized with a cocktail of ketamine/acepromazine, and perfused at 10ml/hr with a power injector via the left ventricle after making an incision in the right atrium. Following perfusion, the brain was excised and fixed in cold PFA for 24h, and imaged on the microscope. Once repeatable perfusion was achieved, Evans blue was replaced with 20mg/ml of BSA-GdDTPA, and the gelatin-filled brain imaged on a 400MHz spectrometer after 48h of fixation with the following parameters: T1-weighted (T1w) RARE, TE=10ms, TR=100ms, NA=12, resolution=31μm×37μm×30μm; T2-weighted (T2w) RARE, TE=75ms, TR=1000ms, NA=4 same resolution as T1w. *Protocol II*: The second perfusate tested was a silicone rubber compound called MICROFIL® (FlowTech Inc., MA), which is radio-opaque and light microscopy opaque. Animals were anesthetized with a cocktail of ketamine/acepromazine, heparinized and manually perfused via the left ventricle with 5ml of Microfil. Following perfusion, the brain was excised and fixed in cold PFA for 24h, and imaged on the microscope. Once repeatable perfusion was achieved, the Microfilled brain was imaged on a 400MHz spectrometer after 48h of fixation with the following parameters: T2*weighted (T2*w) multi-echo gradient echo, TE=5.19/11.49/17.79/24.09ms, TR=200ms, NA=8, resolution=37μm×66μm×37μ. Co-registered T1w/T2w images were also obtained as before. IDL was used to compute R2* on a voxelwise basis from multi-echo GE data.

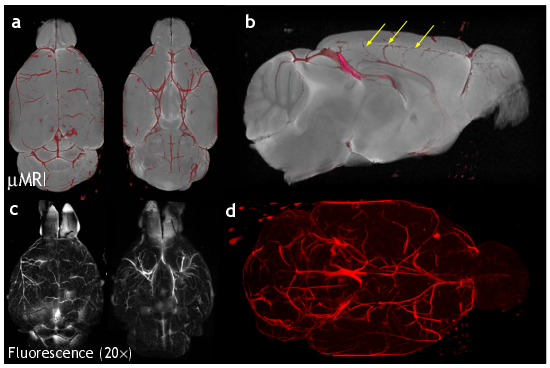

Fig. 2: Imaging results obtained with the gelatin perfusate. (a) 3D rendering of the T1w μMRI volume showing vessels in red, coregistered with the T2w μMRI volume. (b) 3D volume rendering of the datasets in (a), cut-away at the mid-sagittal plane, wherein one can clearly visualize the cortical blood vessels (arrows). (c) Fluorescent-images of the same intact brain independently corroborate the wide-area vessel map in (a). (d) MIP of the T1w dataset showing the vasculature of the entire murine brain.

RESULTS: **Fig. 1** demonstrates the feasibility perfusing the murine brain with *Protocol I* and shows two independent readouts of perfusion quality i.e. white-light and fluorescence imaging of intact whole brain. **Fig. 2** shows μMRI of the brain using *Protocol I*, and its optical validation. **Fig. 3** shows μMRI of the brain using *Protocol II*, and its optical validation. While both protocols enable whole brain mapping of the murine vasculature, the Microfil® technique is superior to the gelatin technique in terms of vascular coverage.

DISCUSSION: The data presented here demonstrate two novel techniques for whole brain mapping of the murine vasculature. The first is a method that makes vessels conspicuous by virtue of positive contrast due to T1 relaxation induced by BSA-GdDTPA. However, sub-voxel sized vessels are not visible using this method. The second method exploits the susceptibility difference introduced between the vessels and background tissue by the presence of Microfil®. While this T2* contrast mechanism makes sub-voxel sized vessels visible, they are "scaled" versions of the true architecture. We are currently in the process of morphometrically characterizing the cerebral vasculature obtained by both methods in terms of vessel size, blood volume etc. We are also in the process of characterizing vascular and cortical remodeling, and response to antiangiogenic therapy in a murine brain tumor model.

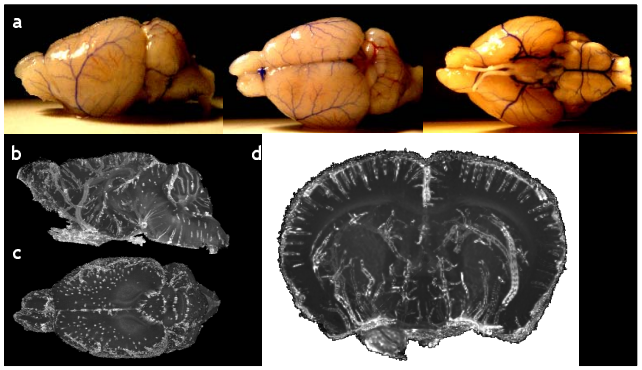

Fig. 3: Imaging results obtained with the ***Microfil®*** perfusate. (a) Views of the lateral, superior and inferior aspects of the microfilled (blue) brain. (b) 3D R2* maps of the brain shown in (a). Cut-away views in the: (b) sagittal, (c) transverse and (d) coronal planes, respectively. One can visualize the cortical blood vessels in exquisite detail in each view.

CONCLUSIONS: These preliminary data indicate that μMRI using novel contrast mechanisms has the potential to yield a plethora of information regarding vascular remodeling in a wide range of pre-clinical disease models.

REFERERENCES: **1.** Iadecola, *Nat Rev Neurosci*, May;5(5):347-60, 2004. **2**. Jain et al., *Nat Rev Neurosci*, Aug;8(8):610-22, 2007. **3**. Moonen et al, *Science*, Oct 5;250(4977):53-61, 1990. **4**. Raman et al. *NMR Biomed*, May;20(3):186-99, 2007. **5**. Johnson et al. *Ann N Y Acad Sci*, 30;820:139-47, 1997.

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