Layer-Specific Anatomical MRI of the Retina with Balanced Steady State Free Precession with and without Manganese Enhancement

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INTRODUCTION The retina consists of highly structured cellular and synaptic layers. Anatomical imaging of the retina has reported 3 layers in rats using gradient echo (GE) MRI at 60x60 μm (1) and 4 layers in mice using spin-echo MRI at 47x47 μm (2). Manganese-enhanced MRI (MEMRI) with GE in rat retinas following intraocular injection of manganese (Mn) showed 7 layers (3). To improve upon previous anatomical MRI studies of the retina, we employed balanced steady state free precession (bSSFP) MRI at 35x35x200 μm. We compared GE and bSSFP imaging with and without Mn injection in the mouse retina. bSSFP in principle should offer high SNR per unit time with high spatial and temporal resolutions.

METHODS Male C57BL/6 mice (6-10 weeks) were imaged under isoflurane (~0.8%) and xylazine (~5 mg/kg/hr) and spontaneous breathing conditions. Respiration rate, heart rate, oxygen saturation, and rectal temperature were monitored and maintained. MRI was performed on a 7T/30cm Bruker scanner with a 150 G/cm gradient using a small surface coil (ID = 0.6 cm). bSSFP images were acquired with a 4.48x5x5 mm FOV, 128x144x25 matrix (35x35x200 μm) zero-filled to 256x288x25, and TE/TR of 4.22/8.44 ms (n=4). FASTMAP shimming was used to reduce banding and the RF phase cycling angle was adjusted in each subject to minimize banding near the retina. T1-weighted, spoiled GE images were acquired with a 4.48x4.48 mm FOV, 128x128 matrix (35x35 μm) zero-filled to 256x256, three 200 μm slices, and TE/TR of 5.5/200 ms (n=3). Averages were acquired so the total acquisition time for bSSFP and GE were the same. Automated profile analysis (1) was performed to align the retina and obtain average profiles along the length of the retina. In addition, similar studies were performed on 3 rats after intraocular injection with 3-5 μl of 30 mM MnCl2 and imaged 5-8 hours after injection.

RESULTS **Figure 1** shows a bSSFP image at 35x35x200 μm and its profile from a mouse. Seven alternating bright-dark layers were visible. Thicknesses of layer 1 to 7 by bSSFP were 44, 41, 32, 30, 35, 46, and 45 μm (n=4). **Figure 2** shows a GE image at 35x35x200 μm. Only the bright choroid layer (labeled as 1) was clearly detected. SNR's in the retina of bSSFP and GE were 27.3±4.0 and 20.6±0.62, respectively. **Figure 3A-B** shows MEMRI with GE and bSSFP. Seven layers were detected by both sequences. Taken together, we tentatively assigned the 7 MRI layers to correspond to the histological layers depicted in **Figure 3C**.

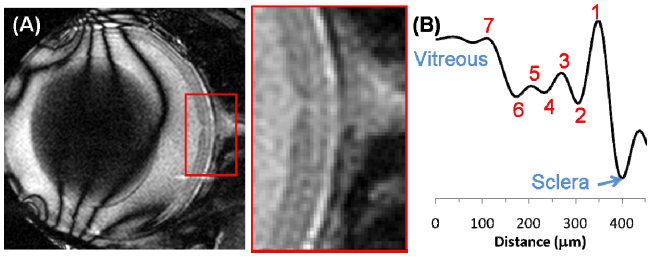

Figure 1. (A) bSSFP image of a mouse retina at 35x35x200 μm. (B) Average profile along the retina showing 7 alternating dark-bright layers.

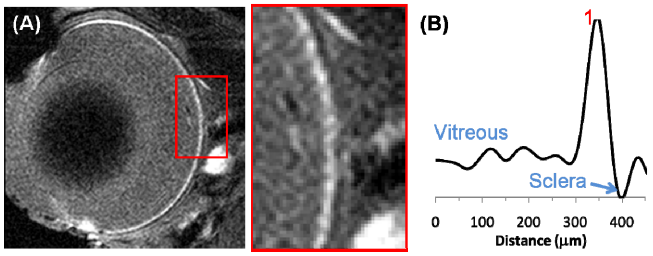

Figure 2. (A) GE image of a mouse retina at 35x35x200 μm. (B) Average profile along the retina.

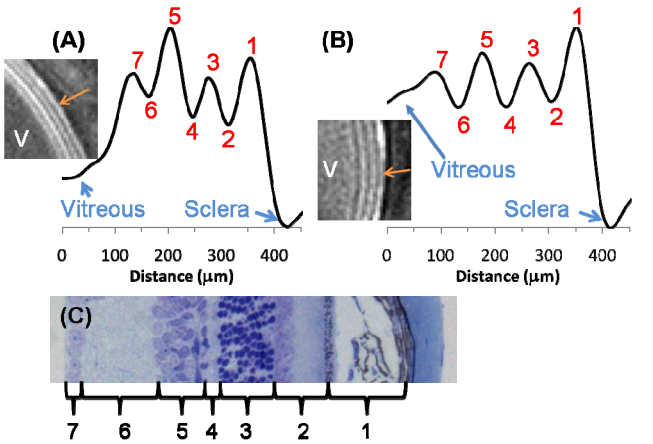

Figure 3. Profiles of the rat retina at 35x35 μm after intraocular Mn injection obtained by (A) GE and (B) bSSFP. V: vitreous. Orange arrows: choroid. (C) Histology of a mouse retina with the proposed assignment of MRI layers to anatomy. Layer 1-choroid, 2-inner and outer segments, 3-outer nuclear layer, 4-outer plexiform layer, 5-inner nuclear layer, 6-inner plexiform layer, 7-ganglion cell layer and nerve fiber layer.

DISSCUSSION bSSFP indeed provided better contrast in the retina and SNR per unit time. bSSFP revealed 7 layers in the mouse retina at 35x35x200 μm without Mn, whereas GE could not detect multiple layers. MEMRI by GE and bSSFP reliably detected 7 layers.

A potential problem with bSSFP is the banding artifacts as seen around the lens. With shimming and adjustment of the phase cycling angle, bands were moved away from the retina. Alternatively, bSSFP with multiple phase cycling angles could be used to remove banding artifacts.

The key finding is that bSSFP revealed 7 layers without Mn, compared to 3-4 layers previously reported (1,2). This could be because bSSFP provides better SNR per unit time and unique contrast which is dependent on T2/T1 ratio. The 7 layers in bSSFP are consistent with the 7 layers obtained with MEMRI by GE or bSSFP. In bSSFP without Mn, layer 7 was less clearly resolved likely due to partial voluming by the bright signal of the vitreous. MEMRI enhances this layer, raising its signal above the vitreous signal.

The tentative assignment of the 7 MRI layers to the histological layers was based on the notion that Mn accumulates intracellularly, which enhances cell body layers more significantly than the synaptic layers because cell bodies have larger intracellular volumes, as previously described (3). Further tests are needed to validate these assignments.

The neural retina and choroid thicknesses respectively, were 229 and 44 μm by bSSFP MRI (n=4) and 176-223 (4,5) and 15 μm (5) by histology. The neural retina thickness by MRI was similar to histology, but the choroid by MRI was thicker. Similar differences between histology and *in vivo* techniques have been noted in rats (1) and humans (6,7), likely due to collapse of choroid vessels after removal from the systemic circulation for histology.

CONCLUSION This study demonstrates a novel application of high-resolution bSSFP MRI to image the rodent retina, revealing 7 layers without using contrast agent. Layer assignments of bSSFP were consistent with those of MEMRI and histology. This approach could be used to investigate changes in laminar thickness and signal contrasts in retinal diseases in animal models.

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