

Manganese-Enhanced MRI Reveals Multiple Cellular and Vascular Layers in Normal and Degenerated Retinas

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Introduction The neural retina is characterized histologically into six distinct and highly stratified layers (1). From the vitreo-retinal interface, these layers are the ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), and inner+outer photoreceptor-segment layer (IS+OS). Two flanking blood supplies nourish the retina (2). The *retinal* vasculature, located closest to the vitreous, exists predominantly within the ganglion cell layer but projects some capillaries deep into the IPL and INL. The *choroidal* vasculature, on the other hand, is external to the neural retina and is sandwiched between the retinal pigment epithelium and the sclera. If considered with the six histologically defined layers, the *choroidal* vasculature (CH) constitutes an additional (seventh) layer. The outer nuclear layer and the photoreceptor-segment layer are avascular.

In this study, we used manganese-enhanced MRI (MEMRI) to resolve rat retinal layers at 25 x 25 μm in-plane resolution. Seven distinct bands of alternating signal intensities were detected. Three sets of experiments were performed to corroborate layer assignments. First, Gd-DTPA enhanced MRI studies were used to visualize the two vascular layers bounding the retina. Second, standard histology was performed and histological layer thicknesses were obtained for cross-validation of MRI-derived layer assignments and laminar thicknesses. Finally, we investigated an established animal model of photoreceptor degeneration, the Royal-College-of-Surgeons (RCS) rats (3). RCS rats have a genetic defect that results in a spontaneous and complete degeneration of the photoreceptors by postnatal day 90 (P90), resulting in expected loss of the IS+OS and diminishment of the associated choroidal vasculature and projections into the OPL and ONL.

Methods MnCl₂ solution was intravitreally injected over 2 mins under anesthesia. To optimize the dosage for retinal contrast enhancement, 5 μL of isotonic 20, 30, 60 or 120 mM of MnCl₂ were injected intravitreally into the left eye of an initial group of animals (n = 12). Subsequent studies using the optimal dose (5 μL of a 30 mM) were carried out on two groups of animals: i) normal adult rats (n = 5) and ii) RCS rats at postnatal day 90 (P90) (n = 5) with complete photoreceptor degeneration.

MRI was performed ~24 hrs after injection on a 4.7T/40cm scanner. T₁-weighted MRI used a conventional gradient-echo pulse sequence with TR = 104 ms, TE = 8.5 ms, 0.8 mm slice thickness, 16 repetitions, matrix=256 x 256, and FOV = 6.4 x 6.4 mm, yielding 25 x 25 μm resolution. Gd-DTPA was administered iv to visualize the two vascular layers bounding the retina. Histology was obtained after MRI. Layer thicknesses were obtained using the half-height method (4).

Results & Discussion In normal retinas, Mn-enhanced MRI and signal intensity profiles showed a diffuse bright band closest to the vitreous (#1) and three bright bands (#3, #5 and #7) interspersed among three dark bands (#2, #4, and #6) (Fig 1). Following iv administration of Gd-DTPA, subtraction of pre and post Gd-DTPA images revealed enhancement on either side of the retina (band #1-3 and 7) (Fig 2). On the other hand, there were no significant Gd enhancements of the middle sections of the retina (layer #4-6) and the vitreous. MRI and histologic layer assignments and laminar thicknesses are summarized in Table 1.

In contrast, MEMRI of the P90 RCS retinas revealed only four bands of alternating hyper- and hypo-intensities plus a debris band (Fig 3A). The overall retinal thickness was markedly reduced. Comparison of the MEMRI intensity profiles clearly revealed the disappearance of bands #4-6, diminished intensity of band #3, the appearance of a debris layer, and a thinning of the total retinal thickness in the P90 RCS retinas (Fig 3B). Fig 3C shows the histological comparison of a normal and a P90 RCS retina. In the P90 RCS retina, only a thin debris layer was visible in place of the OPL, ONL and OS+IS and corresponding to band #4-6. The total thickness including the CH of the P90 RCS retinas was 212 ± 22 μm by MRI and 208 ± 15 by histology, significantly thinner than the normal retinas. Together, the P90 RCS rat data further corroborate the MRI layer assignments.

In conclusion, high-resolution MEMRI resolves seven lamina-specific structures in the rat retina *in vivo* and these layers are consistent with histology.

References: 1. H. Wassle, B. B. Boycott, *Physiol Rev* **1**, 447 (1991). 2. A. Harris, L. Kagemann, G. A. Cioffi, *Survey of Ophthalmol* **42**, 509 (1998). 3. S. Wang *et al.*, *Current Eye Res* **27**, 183 (2003). 4. H. Cheng *et al.*, *Proc Natl Acad Sci USA* **103**, 17525 (2006).

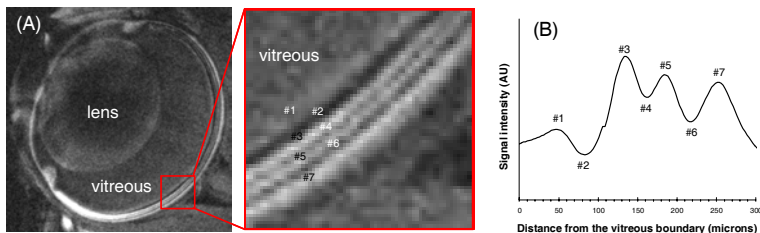


Fig 1. (A) Mn-enhanced MRI at 25 x 25 μm resolution and **(B)** the intensity profile. Seven distinct bands of alternating bright and dark signal intensities are visible.

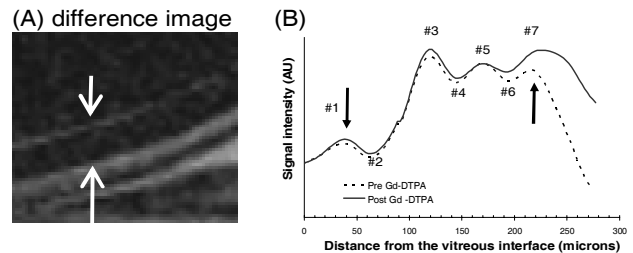


Figure 2. (A) Post- and pre-Gd-DTPA “difference” image and **(B)** signal intensity profiles. Arrows indicate Gd-DTPA enhancements.

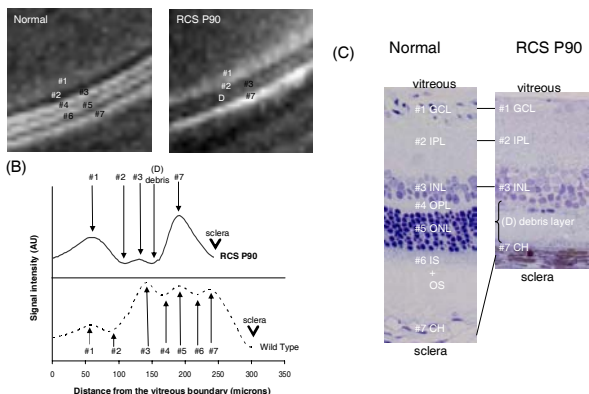


Figure 3. (A) Mn-enhanced MRI, **(B)** intensity profiles, **(C)** histology of a normal and a P90 RCS retina. In the P90 RCS retina, band #4, #5 and #6 appeared missing and band #3 showed reduced signal intensity whereas band #1 was slightly more enhanced. Histology showed bands #4-6 in the normal retina is replaced by a debris band (letter D) in RCS retina. The arrowhead in B indicates the sclera. CH: choroidal vascular layer.

TABLE 1. The layer assignments and laminar thicknesses of normal adult retinas (post natal day 90-120) as determined by MRI and histology (μm, mean ± SD, n = 5).

Band	MRI	Histology	Assignment
#1	56 ± 26	31 ± 6	GCL
#2	41 ± 9	61 ± 4	IPL
#3	39 ± 6	39 ± 6	INL
#4	30 ± 5	14 ± 3	OPL
#5	32 ± 9	53 ± 8	ONL
#6	29 ± 4	53 ± 8	IS + OS
#7	46 ± 8 ^a	18 ± 3 ^a	CH
Total	271 ± 21	252 ± 5	