Layer-Specific MRI of the Rat Retina with Intraocular Injection of Gadolinium-DTPA

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INTRODUCTION The retina can be divided into seven layers, including the ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), inner and outer segments (IS+OS), and the choroid vascular layer. A recent study demonstrated that MRI with intraocular (IO) manganese (Mn) injection markedly enhances the anatomical structure of rat retina, revealing 7 layers that are consistent with histological assignments (1). Mn, a potent T1 relaxation agent, is a calcium analog and is taken up by cells and trapped in the intracellular space. MRI with IO Mn injection should thus enhance nuclear layers of the retina more than synaptic layers.

In this study, we explored layer-specific MRI with IO Gadolinium-DTPA (Gd) injection in rats. Gd is another potent T1 relaxation agent, which localizes to the extracellular space because cell membranes are impermeable to Gd. Thus, we expected IO Gd injection to enhance the synaptic layers of the retina more than nuclear layers, assuming that synaptic layers would have larger extracellular space than the nuclear layers. To facilitate layer assignments, Gd was also injected intravenously (IV) in the same animals. Since the blood-retinal barrier and the retinal pigment epithelium are impermeable to IV injected Gd, this experiment would delineate the retinal and choroid vascular layers bounding the retina and thus help layer assignments. We performed these experiments on rats at 7T with 25x25 µm resolution.

METHODS Rats were imaged under ~1% isoflurane, paralyzed and mechanically ventilated. End-tidal CO2, heart rate, O2 saturation and rectal temperature were maintained within normal physiological ranges. MRI was performed on a 7T/30cm Bruker scanner using a small surface eye coil (ID = 1.0 cm). A 5-10 µl dose of 125-250 mM Gd was injected into the vitreous. MRI was then performed for up to 4 hours after injection. T1-weighted gradient-echo images were acquired with 200 ms TR, 6.5 ms TE, 6.4x6.4 FOV, 256x256 matrix (25x25 µm), and 3 slices of 500 µm thickness. Automated profile analysis was performed to obtain average profiles along the length of the retina (1). To help determine which layers of the retina are vascularized, additional Gd was given to rats intravenously (n=4) in the same animal.

RESULTS It took ~2 hours after IO injection for Gd-DTPA to uniformly distribute in the retina and provide stable contrast. SNR was markedly improved, allowing much higher resolution to be obtained. T1-weighted MRI revealed 6 layers in the retina (**Figure 1**). Group-averaged thicknesses of layers 1 to 6 were 41±3.5, 50±20, 39±5.2, 33±13, 40±3.5, and 54±3.5 µm (mean±SD, n=5). The 7th layer which was not visible in MRI, was likely mixed in with the strong vitreous signal, although this remains to be independently verified. The neural retina was measured to be 216±26 µm and the choroid was 41±3.5 µm by MRI (n=5). From histology of the rat, the neural retina is 279 µm and the choroid is 34 µm (2).

After IV Gd injection, layer 1 was strongly enhanced (**Figure 2A**), indicating it is the choroid bounding the retina. IV Gd enhancement of the retinal vascular layer was less apparent because of its low blood volume and intraocular Gd. The observed IV Gd enhancement of the vascular layers bounding the retina is in agreement with previous reports (1-3). Taken together, we assigned the 6 MRI layers to histological layers as detailed in **Figure 2B**. Nuclear layers were hyperintense and the synaptic layers were hypointense.

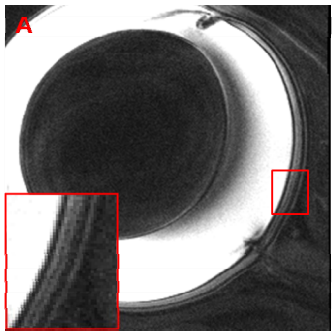
DISSCUSSION Intraocular Gd-DTPA injection markedly enhanced contrast in the retina, revealing 6 layers. The 7th layer which is believed to be mixed in with the strong vitreous signal is not resolved. Intravenous injection of Gd helped set the boundary and layer assignments.

The previous report of IO Mn injection yielded 7 layers in the rat retina (1). From the vitreous boundary, the bright, dark, bright, dark, bright, dark and bright layers were assigned to be GCL, IPL, INL, OPL, ONL, IS+OS, and choroid, where the nuclear layers were hyperintense. Surprisingly, the alternating bright and dark layers of the IO Gd data herein appeared to be in synchrony with Mn data, whereas the opposite was expected - that is IO Gd should yield hyperintense synaptic layers. Thicknesses derived from the two methods were also similar, albeit with limited spatial resolution, and both were consistent with histology (1).

This finding disproved our original hypothesis that IO Gd should enhance synaptic layers more than nuclear layers. A possible explanation is that T1-weighted intensity is a weighted average of both the intra- and extracellular signals. T1 relaxometry measurements could resolve this apparent discrepancy. In the presence of sufficient intraocular Mn or Gd concentration and compartmentalization, T1 relaxometry measurements could resolve intracellular and extracellular T1 values, which are expected to differ between IO Mn and Gd injections.

CONCLUSION This study demonstrates that intraocular Gd-DTPA injection markedly enhances contrast in the retina, revealing 6 anatomical layers at 25x25 µm MRI. This technique offers novel contrast to study the retina at very high spatial resolution. This could complement existing retinal imaging techniques and allow longitudinal, lamina-specific imaging of retinal diseases in animal models.

REFERENCE 1) Nair et al, ISMRM 2007, 2452. 2) Cheng et al, PNAS 2006, 103:17525. 3) Shen et al, JMRI 2006 23:465.



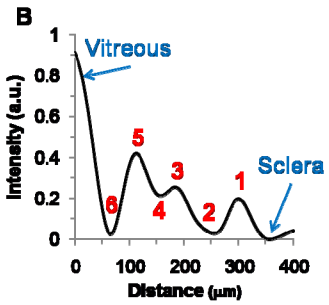
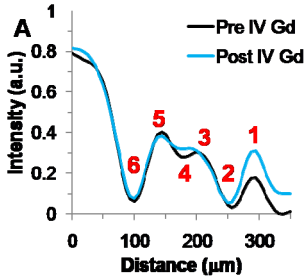


Figure 1. (A) Image of a rat retina at 25x25 µm acquired 2 hours after injection of 250 mM Gd. (B) Anatomy profile (in arbitrary units) along the retina showing 6 alternating dark-bright layers.



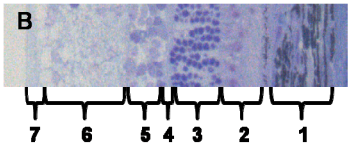


Figure 2. (A) Anatomy profile (in arbitrary units) from the retina before and after IV Gd administration showing large enhancement of layer 1, the choroid. (B) Histology of the retina with the proposed assignment of MRI layers to anatomy. Layer 1-choroid, 2-IS+OS, 3-ONL, 4-OPL, 5-INL, 6-IPL, 7-GCL. In MRI, layer 7 likely blends in with the vitreous.