Lamina-specific anatomical MRI of the rat retina

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Introduction The retina is highly structured and is composed of three major cell layers (Fig 1): an outer photoreceptor layer, a middle bipolar cell layer, and an inner ganglion cell layer. The retina is nourished by the retinal and choroidal vasculatures [1] located on either side of the retina. The retinal vasculature exists primarily within the ganglion cell layer, but does project a deep planar capillary bed into the bipolar cell layer. The choroidal vasculature does not exist within any retinal layer, but rather is located directly beneath the photoreceptor layer, sandwiched between the retinal pigment epithelium and the sclera. The photoreceptor layer is thus avascular [2]. The ability to non-invasively resolve retinal tissue layers, including the two distinct vascular layers, could have many important applications.

Oxygenation changes (ΔPO_2 technique) in the vitreous humor near the retina had been reported by measuring T_1 changes [3] exploiting the paramagnetic property of dissolved O_2. Blood-oxygenation-level-dependent (BOLD) functional fMRI of the retina associated with visual stimuli and physiological challenges had been reported [4]. In a recent study [5], we showed the visual resolution of three tissue "layers" using T_2-weighted and diffusion-weighted MRI and two vascular layers using Gd-DTPA contrast-enhanced MRI in the cat retina (blood-retina barrier is impermeable to Gd-DTPA). The goal of this study was to extend laminar-specific anatomical MRI to the rat retina at higher spatial resolution. Layer structures were validated by histology. Structural differences between rat and cat retinas were compared.

Methods Ten male rats (400-500g) were imaged under ~1.5% isoflurane. End-tidal CO2, O_2 saturation, heart rate, respiration rate, and rectal temperature was monitored and regulated. MRI was performed on a 4.7T/40cm Bruker scanner using a custom-made single-loop coil (0.8 cm) for the left eye for improved sensitivity and reduced FOV. T_1-weighted imaging was acquired with FLASH acquisition, TR = 100 ms, TE = 4 ms, slice thickness = 0.8 mm, FOV = 8 mm x 8 mm, matrix = 128 x 128 (62 μx 62 μm), and 16 averages. Gd-DTPA was administered intravenously and pre- and post-Gd images were acquired with the same MRI parameters.

On a separate group of rats (n=10), eyes were fixed, embedded in epoxy resin, and sectioned at 0.5 μm, using a histo-diamond knife on an ultramicrotome, and stained with toluidine blue. Each histological section was photographed and thicknesses of different layers were derived automatically using Image Pro (Cybernetics).

Results Figure 1 shows a histological section of the rat retina and tissue layer assignments. The histological thickness of the *neural* retina was 148±25μm and of the choroid vascular layer was 37±12μm, totaling 185±35μm, in reasonable agreement with literature [6]. **Figure 2** shows T_1-weighted images (dark bands across the lens resulted from RF inhomogeneity). Three "layers" were observed in the retina, indicated by the alternating bright, dark and bright strips. The red arrows indicate the inner and outer strips. The inner strip appeared thicker than the middle and outer strip. These laminar structures converged in and around the optic nerve head which appeared slightly protruded. Laminar structure of the posterior part was clearer than that of the anterior part of the retina. Cognizant of partial voluming, the MRI thickness of different layers are summarized in Table 1.

TABLE 1. Retinal thickness

Inner layer	163 ± 25 μm
Middle layer	92 ± 19 μm
Outer layer	96 ± 18 μm
Total:	**307 ± 29 um**

T_1-weighted images post Gd-DTPA showed marked enhancement of the anterior segment of the eye due to the high permeability of the ciliary body (**Figure 3**). Extra-ocular signal enhancement was also observed (green arrow). Subtraction of post- and pre-contrast T_1-weighted images showed significant signal enhancement on either side of the retina, with the outer strip being markedly more enhanced than the inner strip, consistent with the higher choroidal blood flow. The Gd-DTPA enhanced strips overlaid with the T_1-weighted images before Gd-DTPA.

Discussion This study demonstrates the visual resolution of multiple distinct tissue and vascular "layers" in the rat retina using multiple MRI contrast mechanisms. Three major strips were interpreted as followed. The inner strip nearest to the vitreous, which appeared relatively brighter on T_1-weighted images and was enhanced by Gd-DTPA, likely overlapped the ganglion and bipolar cell layer and the embedded retinal vessels. The middle strip, which appeared relatively darker on T_1-weighted images and was not enhanced by Gd-DTPA, likely overlapped the photoreceptor layer, inner and outer segments of the photoreceptors. The outer strip, which appeared relatively brighter on T_1-weighted images and was also enhanced by Gd-DTPA, corresponded to the choroid vascular layer (Fig 1). The short TR used likely resulted in some blood flow weighting; however this does not change the overall interpretation of the layer structures.

MRI-derived neural retina (inner and middle "layers") and the choroidal vascular layer (outer "layer") retina were significantly larger than the corresponding histology. Limited MRI spatial resolution and/or shrinkage associated with the fixative processes could be factors for the observed discrepancy. Shrinkage is likely to yield a lower limit on thickness, underscoring the importance of *in vivo* measurements.

The rat MRI data are in good agreement with the cat retina data [5]. In the cat retina, three tissue "layers" were detected using T_2-weighted and diffusion-weighted MRI, and two vascular layers were detected using Gd-DTPA contrast-enhanced MRI. These assignments were also validated by histology. The rat retina was however thinner than the cat retina (358±13μm by MRI and 319±77μm by histology, both included the choroidal vascular layer [5]). This is because the cat has a tapetum [7] which serves to improve vision in low light condition and is sandwiched between the retinal pigment epithelium and the choroidal vascular layer. The tapetum (86±35 μm [5]), is vascularized by the choroidal vasculature and was enhanced by Gd-DTPA [5]. Thus, the cat retina was slightly thicker than the rat retina as expected.

Conclusion To the best of our knowledge, this is the first study demonstrating unequivocal laminar structures in the rat retina. Further improvement in spatial resolution is expected. Diffusion, T_1 and T_2 measurements are being analyzed to further characterize the laminar structures in the retina. MRI has the potential to provide lamina-specific anatomical, physiological (such as tissue blood flow and oxygenation) and functional information on the retina in a single setting without depth limitation and, thus, it could complement existing techniques to study the retina and the entire visual pathway.

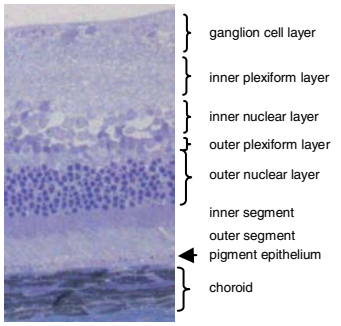

Fig 1. Histology

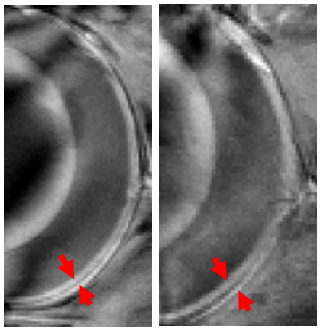

Fig 2. Lamina-specific anatomical MRI

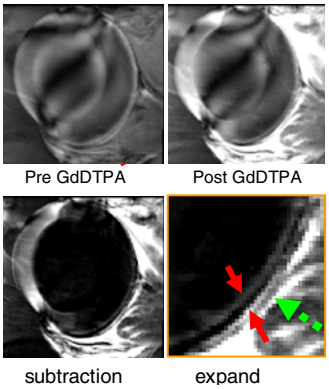

Fig 3. Gd-DTPA enhanced MRI

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