Lamina-specific anatomical and functional imaging of retinal degeneration

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Introduction Retinitis pigmentosa (RP) (1) is a heterogeneous group of inherited diseases that cause progressive photoreceptor degeneration, affecting ~1.5 million people worldwide. Royal College of Surgeons (RCS) rat (2) which has a genetic defect shared by some patients with retinitis pigmentosa (3) is an accepted model of RP. Photoreceptors in the RCS rat retina progressively degenerate over the first three months of life due to a defect in the retinal pigmented epithelium that prevents proper phagocytosis of the shed photoreceptor outer segments.

We recently developed high resolution structural and functional (BOLD) MRI to resolve different layers in rat (4) and cat (5) retinas. In this study, we applied layer-specific structural and functional MRI to study retinal degeneration in the RCS rat retina at high spatial resolution.

Methods Two groups of rats were studied: 1) RCS rats of postnatal day 16 (P16) (n = 6) before photoreceptor degeneration (controls) and 2) RCS rats of P120 (n = 4) after photoreceptor degeneration. Rats were anesthetized with ~1% isoflurane, paralyzed and mechanically ventilated. End-tidal CO_2, heart rate, O_2 saturation and rectal temperature were maintained within normal ranges unless purposefully altered. To visualize the two vascular layers bounding the retina, Gd-DTPA was administered iv. To study fMRI responses, 100% O_2 and 5% CO_2 was administered.

MRI was performed on a 4.7T/40cm scanner using a single-loop coil for the left eye. Anatomical imaging was acquired with FLASH, TR=150ms, TE=3.5ms, slice thickness=0.5mm, FOV=8x8mm, matrix=128x128 (62x62µm), and NT=16. BOLD fMRI was acquired using two-shot spin-echo EPI with diffusion weighting to suppress the vitreous signal, TR=1s, TE=51ms, thickness=1 mm, FOV=1.1x1.1mm, matrix=128x128. Cross correlation analysis was used to derive BOLD % changes maps, BOLD percent changes and number of activated pixels for multiple layers.

Results & Discussion Anatomical MRI of P16 RCS rats (controls) before retinal degeneration showed three distinct bright-dark bright bands (**Fig 1A**). The layer assignments were cross validated previously (4): the inner band correlated with the combined ganglion cell layer/inner nuclear layer plus the embedded retinal vessels. The middle band, which appeared relatively hypointense and not enhanced by Gd-DTPA was assigned the avascular photoreceptor layer. The outer band which was enhanced by Gd-DTPA was assigned the choroidal vascular layer. In contrast, only a single band was visible at P120 (**Fig 1B**). Intravenous Gd-DTPA administration enhanced the entire retinal thickness in the P120 RCS rat (**Fig 1C**), consistent with the loss of the avascular photoreceptor layer. Intensity profiles revealed that the middle band was absent and there was an overall thinning of the P120 RCS retina (**Fig 1D**). The loss of the photoreceptor layer in P120 retinas was confirmed by histology (**Fig 1E**). The inner, middle and outer bands were 157 ± 6, 99 ± 17, and 95 ± 15 µm in the P16 retina by MRI. Only one band was detected in the P120 retina by MRI (169 ± 13 µm). The histological retinal thickness at P16 was 167 ± 31, 112 ± 20 and 34 ± 4 µm and at P120 was 101 ± 21, 29 ± 8, and 35 ± 1 µm.

Robust layer-specific BOLD fMRI responses were detected in the normal retina. Importantly, the two vascular layers responded differently to inhalation stimuli (**Fig 2**) as reported previously (4). Hyperoxia induced a larger BOLD response in the outer band (12 ± 2%) than the inner band (7 ± 2%, P < 0.01), whereas hypercapnia induced a smaller BOLD response in the outer band (1.6 ± 1%) than in the inner band (10 ± 2%, P < 0.01). In contrast, in the P120 RCS retinas, BOLD fMRI responses to hyperoxia in both vascular layers were attenuated (inner band: 4.2 ± 2.5 % and outer band: 8.7 ± 2.4%) and the BOLD fMRI responses to hypercapnia largely disappeared (inner band: -0.02 ± 6.5% and outer band: 0.003 ± 6%), suggesting perturbed vascular reactivity secondary to photoreceptor loss.

Conclusion Layer-specific MRI correctly identifies the disappearance of the photoreceptors, further corroborating MRI layer assignments. Moreover, MRI revealed perturbed vascular reactivity in P120 RCS retinas. Diminished BOLD response in the choroidal vasculature was not surprising because the choroid supplies predominantly the outer nuclear layer. The reduced BOLD response in the retinal vascular layer could be secondary to photoreceptor degeneration (i.e., thinning of the inner band). The application of the anatomical and functional MRI to the retina may reveal important neuronal and vascular changes of clinical diseases that will increase our understanding of the disease process and guide future treatment strategies.

References 1. E. L. Berson, *Invest Ophthalmo Vis Sci* **34**, 1659 (1993). 2. J. E. Dowling, R. L. Sidman, *J Cell Bio* **14**, 73 (1962). 3. A. Gal *et al.*, *Nat Genet* **26**, 270 (2000). 4. H. Cheng *et al.*, *Proc Natl Acad Sci USA* **103**, 17525 (2006). 5. Q. Shen *et al.*, *J Magn Reson Imaging* **23**, 465 (2006). Funded by NEI/NIH.
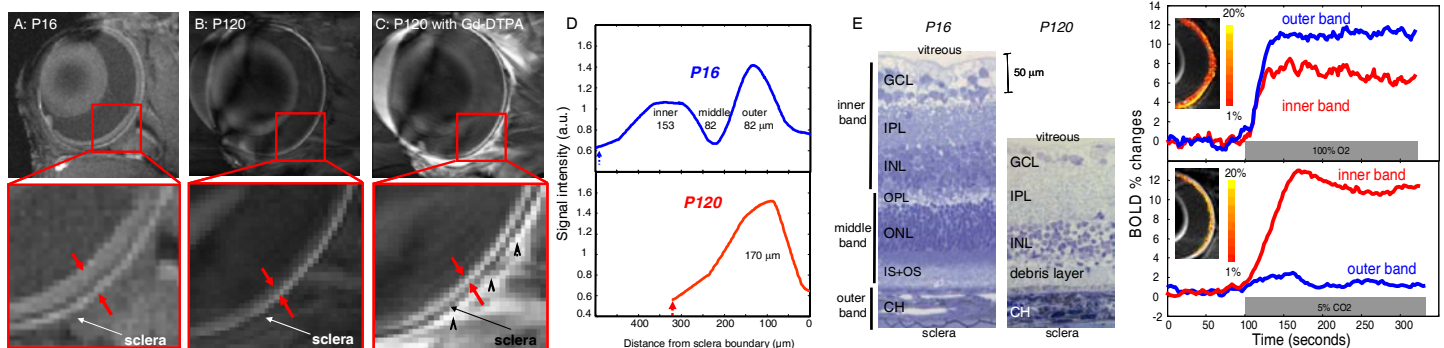
Figure 1. Anatomical images at 60x60 µm resolution of (**A**) P16 RCS retina before photoreceptor degeneration (control), and degenerated P120 RCS retina (**B**) before and (**C**) after iv administration of Gd-DTPA. Arrowheads in C indicate signal enhancement outside the retina. Intensity profiles (**D**) and histological sections (**E**) show thinning of the P120 compared to the P16 RCS retina.
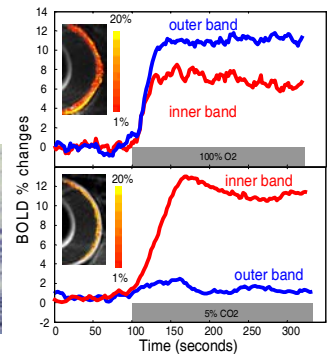
Figure 2. BOLD fMRI responses to (**A**) hyperoxia (100% O_2) and (**B**) hypercapnia (5% CO_2) from a normal rat retina at 90x90 µm.