Blood-Flow MRI of Retinal Degeneration

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INTRODUCTION Retinitis pigmentosa (RP) is a family of diseases resulting in blindness. The Royal College of Surgeons (RCS) rat (1) is an established animal model of RP with a mutation in the *Mertk* gene. This mutation leads to impaired phagocytosis of the photoreceptor segments by the retinal pigment epithelium, resulting in spontaneous photoreceptor degeneration similar to human patients with RP. While the genetic aspects of the disease are well studied, the lack of non-invasive, depth-resolved imaging techniques has limited investigation of the physiologic and functional changes of retinal degeneration and their temporal progression *in vivo*. Non-invasive imaging technologies that can provide physiologic and functional information would enable longitudinal staging of the disease and testing of therapeutic interventions.

We have previously reported reduced retinal thickness and perturbed BOLD fMRI responses to physiologic challenges in RCS rat retinas (2). In this study, we extend previous findings by studying basal blood flow (BF) and physiologically induced BF changes in the RCS rat retinas and age-matched controls at 90x90x1500-μm. MRI offers some unique information and could complement existing retinal imaging techniques.

METHODS Six male RCS rats at postnatal day 90-100 and age-matched controls were imaged under 1% isoflurane, paralysis and mechanical ventilation. Marked thinning of photoreceptor layers has occurred at this age (1,2). BF was measured at 7T using the arterial spin-labeling technique with a separate neck labeling coil (2), a small eye coil, four-shot EPI with a FOV=11x11mm, matrix 128x128 (90x90μm), slice thickness=1.5mm, TR=3s per shot, and TE=14ms. Each physiologic stimulation trial consisted of 6 mins air and 6 mins of 100% O_2 or 5% CO_2 in air. Automated profile analysis (3) was performed to average BF along the length of the retina. Data and error bars are shown as mean ± SD.

RESULTS **Figure 1** shows the retinal BF image in a normal and RCS rat retina. Among ocular structures, BF was greatest in the retina. There were significant quantitative differences in BF between RCS and normal retinas. **Figure 2A-B** show the BF %-change maps associated with physiologic challenges. Hyperoxia decreased BF whereas hypercapnia increased BF in normal rats, as expected. Group results including RCS rats are summarized in **Table 1**. In contrast to controls, basal BF values among RCS rats differed slightly, possibly due age variances. Nonetheless, RCS BF remained markedly lower than controls. Interestingly, absolute blood flow changes induced by hyperoxia and hypercapnia were not statistically different between RCS and normals. However, due to the diminished basal BF values in RCS rats, these values reach significance when expressed in percentages (**Fig 2C).** BF %-change was -21 ± 16 % in RCS compared to -12 ± 7 % in normals under hyperoxic challenge ($P < 0.05$), and 53 ± 25 % in RCS compared to 14 ± 7 % in normals under hypercapnic challenge ($P < 0.05$).

DISCUSSION Basal BF of the normal retina is significantly higher than cerebral BF (~1mL/g/min (4)) under similar experimental conditions, consistent with reports using the microsphere technique (5). Retinal degeneration in RCS rat retinas markedly decreased BF compared to controls. Changes in absolute blood flow induced by hyperoxia or hypercapnia were similar among RCS and control rats. However, due to the reduced baseline BF of RCS rats the % changes in RCS rats were larger than controls. These results underscore the importance of measuring both absolute quantities and percent changes commonly used in MRI (4). We previously reported an overall retinal thinning and perturbed BOLD fMRI responses to physiologic challenges in RCS rats at postnatal day 120. We were unable to identify publications describing the use of Laser Doppler, autoradiography, or intrinsic optical imaging to determine blood-flow during retinal degeneration as comparators for our data. Thus, our findings, if confirmed, could have important implications.

CONCLUSION This study demonstrates a novel approach to image BF and physiologically invoked BF changes in normal and degenerating retinas. This approach provides important BF data at the tissue level that is not depth limited, has large field of view, and has the potential to complement existing retinal imaging techniques. The retina is supplied by two vascular sources, the *retinal* (at the vitreal border) and *choroidal* (exterior to the retina) vascular layers. At current spatial resolution, the BF and BF %-changes reported are a weighted average of both vascular sources. Future studies will focus on improving resolution to detect lamina-specific BF and to characterize the progression of retinal degeneration at multiple time points to compare the onsets of physiologic fMRI responses perturbation and thickness changes.

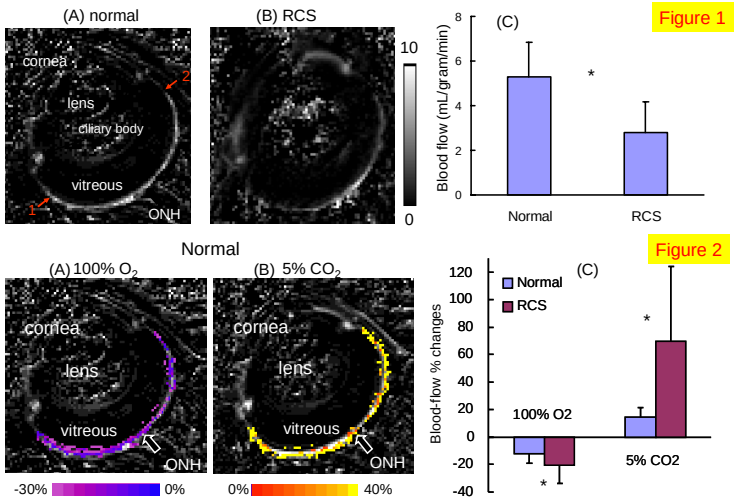

Fig 1. Retinal BF MRI of (A) age-matched control, (B) RCS rat retina, and (C) group-averaged BF of the two groups. *Post-mortem* animals, sacrificed in the scanner, showed no detectable BF contrast in the retina (data not shown).
Fig 2. BF %-change maps associated with (A) 100% O_2 and (B) 5% CO_2 challenges relative to air, (C) group-average BF % changes of the two groups. * $P < 0.05$.

Table 1. Retinal BF (mL/g/min) of normal and RCS rat retina. $P < 0.05$ of air vs. O2 or 5% CO2.

	air	O2	air	CO2
Normal	5.17±0.70	4.51±0.61	5.44±2.04	6.22±2.35
RCS	3.45±0.96	2.77±0.97	1.77±0.84	2.62±1.10

REFERENCE 1) Gal et al, Nat Genet 200, 26:270. 2) Li et al. NI 2007, in press. 3) Cheng et al., PNAS 2006, 103:17525. 4) Sicard & Duong 2005, 25:850. 5) Wang et al., Exp Eye Res 2007, 84:108.