Layer-Specific Blood-Flow and BOLD fMRI of the Mouse Retina Associated with Hypoxic Challenge

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INTRODUCTION: The retina is nourished by two separate vasculatures: the retinal and choroidal vessels. The retinal vessels are localized within the inner retina next to the vitreous, and the choroidal vessels are behind the retinal pigment epithelium. The photoreceptors and segments between these two vascular layers are avascular (1). Oxygen tension in the photoreceptor layers is in fact near 0 mmHg under normal conditions (2). As such, the photoreceptors are more susceptible to hypoxic injury. The ability to image retinal and choroidal blood flow (BF) and oxygenation associated with hypoxic challenge could have important applications in retinal diseases such as retinal ischemia, diabetic retinopathy and glaucoma.

Optical imaging techniques, widely used to study the retina, cannot unambiguously resolve choroid BF (ChBF) and retinal BF (RBF) or oxygenation. In contrast, MRI has recently been shown to be capable of measuring quantitative RBF and ChBF in mice at 42x42x400 μm (3). In this study, BF MRI was further developed to include inversion-recovery suppression of the vitreous to improve sensitivity in detecting layer-specific BF in the retina. We applied this new approach to investigate lamina-specific (retinal and choroidal) BF and BOLD MRI responses to mild hypoxic challenge.

METHODS: Female C57BL/6 mice (17-25 g) were imaged under 1.2% isoflurane and spontaneous breathing conditions. Respiration rate and rectal temperature were monitored and maintained within normal ranges. Mild hypoxic challenge involved 4.5 min of 30% O_2 in N_2 as baseline and 4.5 min of hypoxia (10% O_2 in N_2). MRI was performed on a 7T/30cm Bruker scanner with a 100 G/cm gradient and a small surface eye coil with active decoupling (ID=0.6 cm) and a circular coil (ID=0.9 cm) for arterial spin labeling placed at the heart (4). Combined BF and BOLD MRI were acquired with inversion-recovery to suppress the strong vitreous signal and reduce noise and motion artifacts in BF images (5). Images were acquired in coronal orientation with gradient-echo inversion-recovery EPI with 6x6 mm FOV, 2.1s labeling pulse, 4.0s TR per segment, and 2.12s TI. Basal BF images (n=7) had 144x144 matrix zero-padded to 256x256, single 0.4 mm slice, 2 segments, and 11.7ms TE. fMRI of hypoxia (n=7) used 128x128 or 112x112 matrix, single 0.6 mm slice, 1 segment, 12.7 or 14.9ms TE, and non-labeled images used for BOLD. BF images in ml/g/min were calculated using a formula modified from (6). Automated profile analysis (1) was used to align the retina and to average BF along the retinal length.

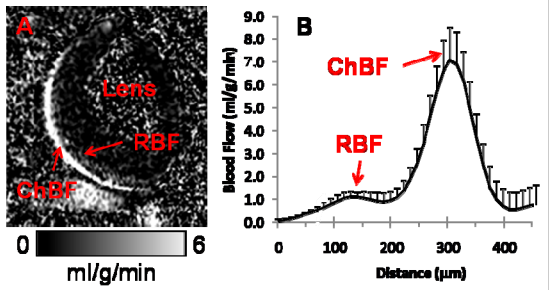

Figure 1. (A) Basal BF from a single mouse. Scale bar = 0-6 ml/g/min. (B) Group average BF profile (n=7, SD).

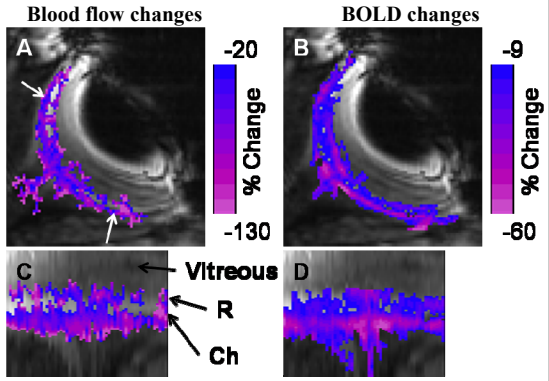

Figure 2. (A) BF and (B) BOLD % change maps due to hypoxia overlaid on the EPI image. The white arrows delineate the region selected for profile analysis. (C) and (D) show corresponding images of the linearized retina. R – retinal vascular layer, Ch – choroidal vascular layer.

Table 1. Basal BF and BF and BOLD responses to hypoxia. Values are mean ± SD (n = 7).

	Retinal layer	Choroid layer
Basal BF (ml/g/min)	1.2 ± 0.2 [a]	7.1 ± 1.4 [a]
BF change (ml/g/min)	-0.53 ± 0.31 [b]	-2.4 ± 1.3 [b]
BF % change	-47 ± 18	-49 ± 16
BOLD % change	-13 ± 3.8 [c]	-25 ± 8.8 [c]

P < 0.005 for baseline versus hypoxia for BF and BOLD.
[a,b,c] P <0.005 between retinal and choroid layers.

RESULTS: Figure 1 shows a basal BF image at 42x42x400 μm from a single animal and the group-averaged basal BF profile across the retina. RBF, ChBF, and the avascular layer in between were well resolved. Basal ChBF was about 6x higher than RBF. **Figure 2** shows BF and BOLD % change maps associated with mild hypoxia from a single animal. Hypoxia decreased BF and BOLD signals, with distinct responses in the retinal and choroid layer. Group-averaged data are summarized in **Table 1**. In magnitude, ChBF decreased 4.5x more than RBF, but % changes were the same in both. Choroid BOLD % changes were larger than the retinal vessels.

DISCUSSION: Basal ChBF is 6x higher than RBF. Cerebral BF is ~1 ml/g/min under isoflurane, similar to RBF but lower than ChBF, consistent with previous reports (7). During mild hypoxia, RBF and ChBF were reduced because hypoxia also induces hyperventilation and thus hypocapnia, causing vasoconstriction that overcomes any vasodilation due to mild hypoxia. This finding is consistent with that of the brain under similar experimental conditions (8).

BF response: The RBF responses in magnitude and % changes were similar to the brain, in which hypoxia is reported to reduce BF by 48% (8). ChBF magnitude decrease was larger than RBF, but the ChBF % decrease was similar due to its high basal value. The ChBF magnitude change was also larger than cerebral BF while % changes were similar. These findings caution interpretation of % fMRI changes in diseased states where basal BF is significantly altered.

BOLD response: Hypoxia-induced BOLD decrease was larger in the choroid than retinal layer. In the brain, hypoxia is reported to reduce BOLD by 9% (8), similar to the retinal response. The large choroid BOLD change compared to the retinal layer and the brain could be a result of high choroid blood volume and large absolute ChBF decrease.

Together, these findings demonstrated differential responses to hypoxia between the retinal and choroidal vasculatures. This is consistent with a previous MRI study which showed layer-specific BOLD responses to hyperoxia and hypercapnia in the rat retina at 90x90x1000 μm (1). This is also consistent with laser Doppler flowmetry studies of hyperoxia and hypercapnia in the optic nerve head (RBF index) where retinal vessels dominate and in the fovea (ChBF index) where retinal vessels are absent (9).

In conclusion, this study demonstrates for the first time the ability to non-invasively resolve quantitative, layer-specific retinal and choroidal basal BF, BF changes, and BOLD changes associated with hypoxia, making it possible to study neurovascular coupling specific to the retinal and choroid vessels.

Reference: 1) Cheng et al, PNAS 2006, 103:17525. 2) Yu & Cringle, Exp Eye Res 2005, 80:745. 3) Muir et al, ISMRM 2009, 1536. 4) Muir et al, MRM 2008, 60:744. 5) Shen et al, ISMRM 2009, 1528. 6) Shen et al, JCBFM 2005, 25:1265. 7) Bill, Circulation in the eye. Editors: Renkin & Michel, Handbook of physiology 1984. 8) Duong, Brain Res 2007, 1135:186. 9) Riva et al, J Appl Physiol 1986, 61:592. Supported in part by R01 EY014211, R01 EY018855, VA MERIT.