Layer-Specific Blood Flow MRI of the Mouse Retina

E. R. Muir[1,2], and T. Q. Duong[2]

[1]Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA, United States, [2]Research Imaging Center, University of Texas Health Science Center, San Antonio, TX, United States

INTRODUCTION The retina is nourished by two separate blood supplies, the *retinal* and *choroidal* vessels. The retinal vessels are located in the inner retina closest to the vitreous. The choroidal vessels are located outside the outer retina, sandwiched between the retinal pigment epithelium and the sclera. The photoreceptor layer in between the two vascular layers does not contain any vessels (7). Importantly, the two blood supplies are substantially different in regards to basal blood flow (BF) and their response to stimulations. Basal choroidal BF is much higher than basal retinal BF and is much less responsive to many factors which regulate BF (1,2). While disruption of retinal vasculature is known to occur in some retinal diseases (3), the lack of non-invasive, depth-resolved imaging techniques has limited layer-specific investigation of the physiologic and functional changes of retinal diseases *in vivo*.

We have previously reported BF MRI of the rat retina at 90x90x1500 µm, but at this resolution the two vascular layers cannot be separated (4). In this study, we developed the continuous arterial spin labeling technique to image BF of the mouse retina at considerably higher resolution (42x42 µm) to resolve the two vascular layers and the avascular photoreceptor layer in between in the retina. We also examined the effects of two common anesthetics, isoflurane or ketamine/xylazine, on BF in the retina.

METHODS Four female C57BL/6 mice at 5-6 weeks (17-22 g) were imaged under 1.1% isoflurane initially and spontaneous breathing conditions. Then ketamine and xylazine (100 mg/kg, 10 mg/kg, ip) were given, isoflurane was discontinued, and imaging was repeated. Respiration rate and rectal temperature were monitored and maintained within normal physiological ranges. MRI was performed on a 7T/30cm Bruker scanner with a 150 G/cm gradient (6 cm ID) using a small surface eye coil with active decoupling (ID = 0.6 cm) and a small circular heart coil (ID = 0.9 cm) for spin labeling placed in the heart position (5). BF images were acquired in coronal orientation using gradient echo EPI with a 6x6 mm FOV, 144x144 matrix (42x42 µm resolution) zero-padded to 256x256, a single 0.4 mm slice, 2 shots, 2.94 s labeling pulse, 3.0 s TR per shot, and 13 ms TE. BF maps (ml/g/min) were calculated as in (6). Automated profile analysis (7) was performed to average BF along the length of the retina.

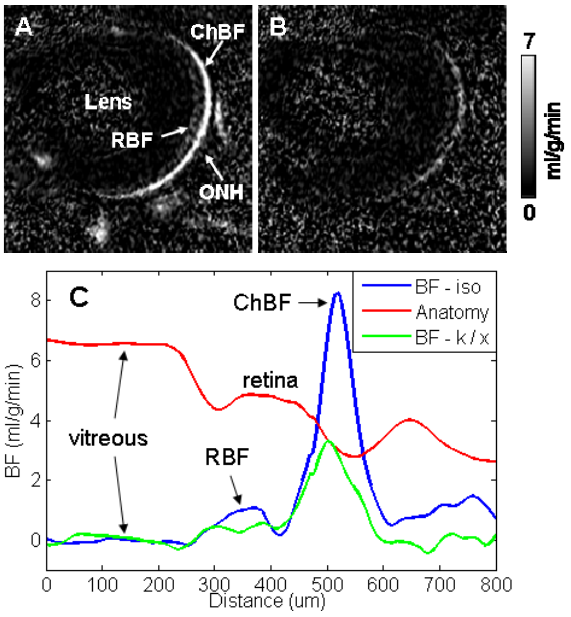
RESULTS BF maps from a single animal are shown in **Figure A** (isoflurane) and **Figure B** (ketamine/xylazine). **Figure C** shows averaged BF profiles across the retinal thickness under isoflurane or ketamine/xylazine from a single animal, with an overlay of the anatomical laminar structures. Two BF layers and the avascular layer in between were distinguished. The peak values of the retinal and choroidal BF averaged along the entire retina are given in **Table 1**. BF of the retinal vasculature was much lower than the choroid under both anesthetics. BF under ketamine/xylazine was 62% lower in the choroid and 34% lower in retinal vessels compared to isoflurane ($p < 0.05$ in both layers).

DISCUSSION This study demonstrated that very high-resolution BF MRI can be measured in the mouse retina. The resolution and sensitivity were sufficient to resolve the choroidal and retinal vascular layers and the avascular photoreceptor layer in between. By comparison, cerebral BF is ~1ml/g/min under isoflurane, which is similar to retinal BF but is markedly lower than choroidal, consistent with previous reports (4,9). Compared to isoflurane, ketamine/xylazine has been reported to reduce cerebral BF by 25-65% depending on the brain region (8), similar to the reductions we obtained in retinal and choroidal BF. Interestingly, choroidal BF was about 7.7 times higher than the retinal BF under isoflurane and 4.4 times higher than the retinal BF under ketamine. These observations suggest that anesthetics could affect choroidal and retinal BF differently and are consistent with the notion that these two vasculatures are regulated differently.

CONCLUSION This study demonstrates a novel approach to image layer-specific quantitative BF of the retina. This approach provides important BF data that is not depth limited, has a large field of view, and has the potential to complement existing retinal imaging techniques. Future studies will focus on developing functional MRI to measure layer specific BF changes in response to physiologic challenges and visual stimulations. These technologies could provide a non-invasive imaging technique that would enable the study of layer-specific physiologic and functional information in longitudinal staging of retinal disease.

REFERENCE 1) Friedman et al, IOVS 1964, 3:539. 2) Xuan et al, J Ocular Pharm Therap 1999, 15:135. 3) Otani et al, J Clin Invest 2004, 114:765. 4) Li et al. NI 2007, 39:1744. 5) Muir et al, MRM 2008, 60:744. 6) Shen et al, JCBFM 2005, 25:1265. 7) Cheng et al., PNAS 2006, 103:17525. 8) Lei et al, Brain Res 2001, 913:174. 9) Alm & Bill, Exp Eye Res 1973, 15:15.

Figures. Blood flow images of the eye under (**A**) isoflurane (**B**) ketamine and xylazine. (**C**) Profiles drawn perpendicular to the retina and averaged over the entire retina. The anatomy profile is the image intensity with arbitrary unit. ChBF: choroidal blood flow, RBF: retinal blood flow, ONH: optic nerve head.



	Choroidal BF	Retinal BF
Isoflurane	7.36 ± 3.18	0.95 ± 0.32
Ketamine/Xylazine	2.78 ± 0.92	0.63 ± 0.18
% Difference	-62*	-34*

Table 1. Blood flow values (ml/g/min) of the choroidal and retinal vasculature under isoflurane or ketamine/ xylazine. Values are given as mean ± SD.
* $p < 0.05$ between anesthetics.