

Layer-Specific fMRI of Photic Stimulation in the Rat Retina at 11.7 T

Y.-Y. I. Shih¹, B. H. De La Garza¹, W. J. Lavery¹, E. R. Muir^{1,2}, and T. Q. Duong¹

¹Research Imaging Institute, Ophthalmology/Radiology, University of Texas Health Science Center at San Antonio, San Antonio, TX, United States, ²Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA, United States

INTRODUCTION The retina is about 276 μm thick including the choroid and has highly organized laminar structures. Advances in MRI technologies have enabled: *i*) anatomical laminar resolution of the retina up to $25 \times 25 \mu\text{m}$ (1,2), *ii*) BOLD fMRI laminar resolution associated with physiologic stimulations at $90 \times 90 \mu\text{m}$ (1), and more recently *iii*) quantitative resolution of retinal and choroidal blood flow layers, and the avascular layer in between up to $42 \times 42 \mu\text{m}$ (3,4). These studies revealed not only markedly improved spatial resolutions in the *in vivo* retina but, surprisingly, different layers in the retina have very different basal blood flow, and different BOLD and blood flow responses to physiological stimuli. Moreover, BOLD fMRI of visual stimulation on the cat retina has also been reported with $485 \times 485 \times 2000 \mu\text{m}$ resolution without laminar resolution (5).

In this study, we explored the feasibility of imaging functional laminar resolution of photic stimulation in the rat retina with considerably higher resolution (up to $40 \times 40 \times 600 \mu\text{m}$). High field (11.7T) scanner was employed to improve SNR and spatial resolution, whereas MION was used for blood volume (BV)-weighted fMRI.

METHODS Four rats were anesthetized with α -chloralose (60 mg/kg first dose, maintained with 25 mg/kg/hr, i.v.), mechanically ventilated, paralyzed with pancuronium bromide (3 mg/kg first dose, 1 mg/kg/hr, i.v.) (1,3). Visual stimulus was 8 Hz flickering achromatic light delivered via an optical fiber with a diffuser. MRI acquisitions were typically acquired with five epochs (96 s OFF and 96 s ON for each epoch). MRI studies were performed on an 11.7T/16cm magnet and a 77G/cm B-GA9S gradient insert (Bruker, Billerica, MA). Rats were placed in a head holder consisting of ear and tooth bars. A custom-made small circular surface coil (ID~7 mm) was placed on the left eye. MION was administered (30 mg/kg, iv). BV fMRI were acquired using FLASH with spectral width = 50 kHz, TR = 150 ms, TE = 10 ms, one 1 mm or 0.6 mm slice. For low resolution, matrix = 128×64 (zero-filled to 128×128), FOV = 8.3×8.3 mm. For high resolution, matrix = 192×96 (zero-filled to 256×128), FOV = 7.7×7.7 mm. The nominal in-plane resolution was $65 \times 65 \times 1000$ or $40 \times 40 \times 600 \mu\text{m}$. Two to five repeated measurements were made on each animal. Images were acquired in time series, and corrected for potential motion and drift before additional analysis as needed. Cross-correlation analysis was performed for display only. Quantitative analysis employed linearized profiles of the retina to minimize bias (1). Stimulus-evoked percent changes of the raw fMRI signal, stimulus-evoked changes in ΔR_2^* and percent BV changes were tabulated for the retinal and choroidal vascular layer.

RESULT Fig 1A shows a representative color activation map overlaid on a gradient-echo image from a single animal. Activated pixels are highly localized to the retina. Importantly, layer-specific responses were observed. Fig 1B shows a representative fMRI signal time course from the active pixels within the ROI as shown. fMRI signals decreased with visual stimulation, indicating BV increase. Fig 2A shows the stimulus-evoked percent-change maps on the linearized retina over the area indicated in Fig 1A. Activated pixels are highly localized to the retinal and choroidal vascular layer. Fig 2B shows the activation of the same animal but at higher spatial resolution. Activation in the retinal and choroidal vascular layers can be more clearly identified. Fig 2C shows the group-averaged, stimulus-evoked changes in ΔR_2^* (which reflected changes in BV magnitudes) plotted across the retinal thickness. Lamina-specific activations were reliably detected. The corresponding BV % changes for retinal and choroidal layers were $6.8 \pm 4.2\%$ and $2.8 \pm 0.8\%$, respectively (mean peak values \pm SD, $n = 4$).

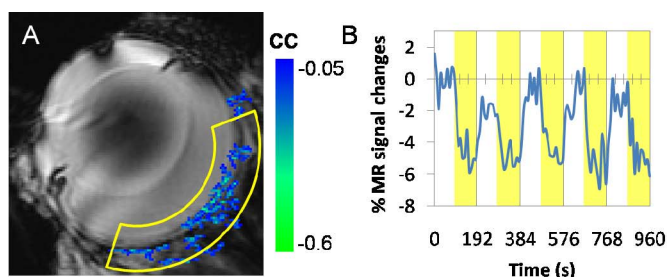


Figure 1. (A) BV fMRI cross correlation map overlaid on gradient-echo image. (B) The time course from active pixels within the ROI.

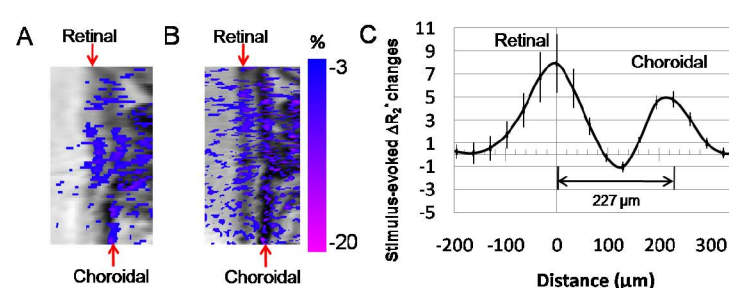


Figure 2. Percent MR signal change maps at (A) $65 \times 65 \mu\text{m}$ and (B) $40 \times 40 \mu\text{m}$ nominal resolution on the linearized retina. (C) ΔR_2^* changes across the retinal thickness from (A).

DISCUSSION This study demonstrates that high-resolution BV fMRI robustly detected visually evoked layer-specific retinal and choroidal response in the rat retina at 11.7T. BV % changes for the retinal layer was slightly higher than the choroidal layer. Retinal blood flow in the optic nerve head has been reported to increase with flicker stimulation as detected by laser Doppler flowmetry. To our knowledge, changes in choroidal blood flow, blood volume or oxygenation have not been reported associated with visual stimulation. The choroid is behind the retina and the retinal pigment epithelium and thus is generally inaccessible by optical techniques. Choroidal vessels are known to be non or less responsive to a range of physiological challenges (such as hypercapnia and hyperoxia) as measured by laser Doppler flowmetry (6,7) and MRI (1). Our findings that choroid responded to diffuse flickers suggest that choroidal vessels could have some local autoregulatory capacity, in contrast to long-held notion that the choroid is not autoregulated. This finding, if confirmed, could have important implications.

CONCLUSION This study reports, for the first time, the functional resolution of lamina-specific retinal and choroidal vascular responses to visual stimulation in the *in vivo* rat retina. This approach has the potential to provide a novel tool to investigate retinal dysfunction and pathophysiology in disease states, such as diabetic retinopathy, glaucoma, and retinal degeneration in animal models. The fMRI approach to study the retina provides unique, clinically relevant data with laminar specificity and has the potential to complement existing retinal optical imaging techniques.

REFERENCE 1) Cheng et al. PNAS 2006, 103, 17525. 2) Nair et al, ISMRM 2008, 3) Li et al. IOVS 2009, 50: 1824. 4) Muir and Duong, ISMRM 2009. 5) Duong et al, IOVS 2002, 43:1176. 6) River et al., Neurosci Let 1991, 128, 291. 7) Riva et al, IOVS 1994, 35:4273. Supported in part by R01 EY014211, R01 EY018855, VA MERIT.