Layer-specific fMRI of visual stimulation in the rat retina: responses to different stimulation luminance, frequency, and color

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INTRODUCTION The retina is about 276 μm thick and has highly organized laminar structures [1]. It is nourished by two distinct blood supplies, namely, the retinal and choroidal circulation, which feed the inner and the outer retina, respectively. Retinal neurovascular coupling is studied primarily using flickering light stimulation, which elicits robust increases in retinal and optic nerve head blood flows, but thus far, most studies have failed to detect changes in choroidal blood flow in response to flicker stimulation [2]. One potential concern regarding the choroidal flicker response to date using optical techniques is that in the human studies blood flow measurements have been restricted to the sub-foveal region. Imaging the choroid is further confounded by the light absorbing retinal pigment epithelium. In this study, we report a novel application of very high resolution (≈60x60 μm) fMRI at an 11.7 T scanner to image the retinal and choroidal responses to various visual stimulations.

METHODS Twenty one rats were anesthetized with α-chloralose (60 mg/kg first dose, maintained with 30 mg/kg/hr, i.v.), mechanically ventilated, paralyzed with pancuronium bromide. Four sets of experiments in which visual stimulation parameters were modulated were studied: (i) 10 Hz flickering achromatic light at a luminance level of 374 cd/m^2 with a 50% duty cycle (n = 10); (ii) *Stimulus luminance* of 81, 234, and 374 cd/m^2 achromatic flicker at 10 Hz (n = 9); (iii) *Stimulus flicker frequency* of 1, 10, 30, and 60 Hz achromatic light at a luminance of 373.80 cd/m^2 (n = 7); (iv) *Stimulus wavelength* of red (630 nm), green (525 nm), and blue (472 nm) with 10 Hz flicker and equal quanta of 2.39x10^{13} quanta/s/cm^2 (n = 7). Monocrystalline iron oxide nanoparticle (MION, 30 mg Fe/kg, i.v.) fMRI was performed at an 11.7 T/16cm magnet and a 74G/cm B-GA9S gradient insert. A custom-made small circular surface coil (ID~7 mm) was placed on the left eye. Shimming used FASTMAP on an isotropic voxel of 7x7x7 mm, encompassing the entire eye. fMRI data were acquired using conventional gradient-echo (GE) sequence with spectral width = 14 kHz, TR = 150 ms, TE = 5 ms, FOV = 7.7x7.7 mm, slice thickness = 1 mm, matrix = 128x64 (zero-filled to 128x128), yielding a nominal in-plane resolution = 60×60 μm, and temporal resolution = 9.6 s. Images were corrected for 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 changes in ΔR_2^* were tabulated for the retinal and choroidal vascular layer. Statistical analyses were performed by paired t-tests and ANOVA followed by Bonferroni post-hoc test. Error bars are SEM.

RESULTS & DISCUSSION This study demonstrates a novel high-resolution fMRI approach to resolve layer-specific responses to visual stimulation in the rat retina. The major findings are: (i) In vivo fMRI reveals two distinct laminar signals that correspond to the *retinal* and *choroid* vascular layers bounding the retina, separated by an avascular layer in between. (ii) The stimulus-evoked ΔR_2^* changes were higher in the retinal layer around the optic nerve head. (iii) The retinal ΔR_2^* responses to different graded visual stimuli were similar to those reported previously using different techniques in humans and several other non-rodent species [3], showing that retinal and optic nerve blood flow increases during visual stimulation. Typically the optic nerve response is greater than the retinal response, though both exhibit roughly exponential responses to luminance and bell-shaped responses to frequency and wavelength. Our results showed that the retinal layer ΔR_2^* responses to flickering light of different luminance, frequency and wavelength were greater than in the choroid. The inner layer responses were dependent on luminance, frequency and wavelength revealing characteristic tuning curves of the retinal responses, whereas the choroidal responses were not, suggesting differential neurovascular coupling between the two vascular layers.

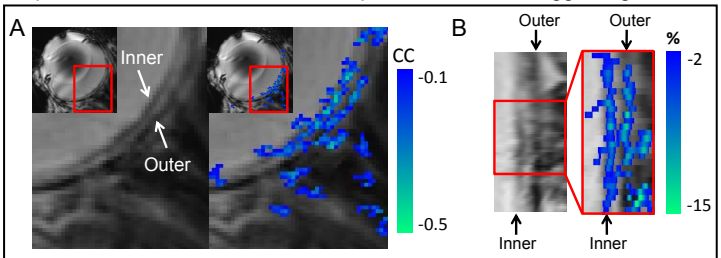

Fig 1. Layer-specific MION fMRI of achromatic 10 Hz flicker stimulation. **(A)** Cross-correlation maps overlaid on gradient-echo MRI with 30 mg/kg MION). Two arrows in the expanded view indicate the inner and outer bands corresponding to the two vascular layers bounding the retina. **(B)** Linearized images showed two well-resolved bands activated by visual stimulation. Negative % changes indicate vasodilation.

CONCLUSION To our knowledge this is the first report of layer-specific fMRI of visual stimulation in the *in vivo* retina, providing important insights into the unique stimulus-evoked changes in *retinal* and *choroid* vascular layer. Because MRI gives simultaneous, global information about the ocular circulations without depth limitation and can be done repeatedly, this approach could have applications in early detection and longitudinal monitoring of retinal diseases, such as retinal ischemia, glaucoma, diabetic retinopathy, and retinitis pigmentosa, where retinal and choroidal hemodynamics may be perturbed differently at different disease stages [4]. This approach may also have long-term clinical applications because similar iron oxide contrast agents are approved for clinical use.

REFERENCES [1] Cheng et al., PNAS 2006, 103:17525. [2] Garhofer et al., Curr Eye Res 2006, 24:109. [3] Riva et al., Progress Retinal Eye Res 2005, 24:183. [4] Pournaras et al., Prog Retin Eye Res 2008, 27:284.

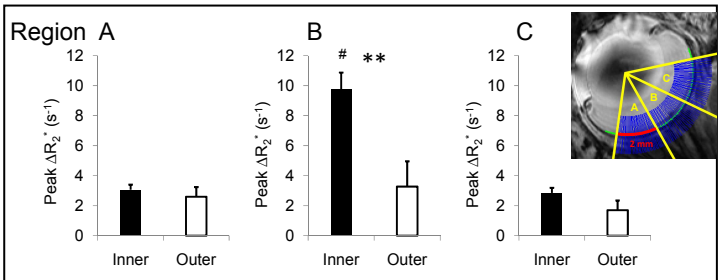

Fig 2. Regional differences in magnitude and percent changes of layer-specific MION fMRI (n = 10). Visual stimuli were achromatic 10 Hz flickers at 374 cd/m^2. **p < 0.01 indicates statistical difference between inner and outer peak. #p < 0.01 indicates statistical difference from region A and C.

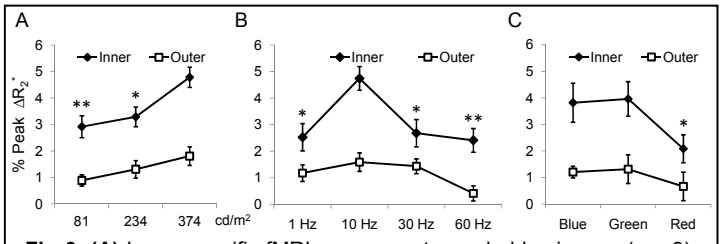

Fig 3. (A) Layer-specific fMRI responses to graded luminance (n = 9). *(p < 0.05) and **(p < 0.01) indicates statistical difference from 374 cd/m^2 for the inner peak. **(B)** Responses to graded flicker frequency (n = 7). * and ** indicates statistical difference from 10 Hz for the inner peak. **(C)** Responses to equal quanta red, green, and blue color (n = 7). * indicates statistical difference from green and blue for the inner peak. No difference was found at the outer peak for all stimulation conditions.