

## Effect of Metabolic Rate on Postocclusion Reactive Hyperemia in Rat Retina

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**Target Audience:** Basic and translational researchers of the retina.

**Background:** Blood flow and metabolism are intricately coupled under normal physiological conditions. A common method used to probe such coupling and the hemodynamic reserve is to induce a transient occlusion and observe the reactive hyperemia during reperfusion under different metabolic demands, i.e., when metabolic activity is higher, the metabolic byproducts accumulated during the occlusion result in a larger reactive hyperemia. Indeed, reactive hyperemia has been found to be modulated by increased metabolic demand in dog intestine [1], dog stomach [2], human heart [3] and calf muscle [4]. Reactive hyperemia and its modulation by varying metabolic demand, however, have not been studied in the retina. The goal of this study was to investigate reactive hyperemia and hemodynamic reserve in the rat retina by measuring blood flow and oxygen tension (i.e., BOLD fMRI) while the level of metabolic demand was modulated by light conditions (i.e. dark, constant light and flicker).

**Method:** Long-Evans rats were used for BOLD fMRI (N=9) and laser speckle imaging (LSI, N=11). The rats were anesthetized with urethane (~1 g/kg), mechanically ventilated and paralyzed. Their femoral arterial blood pressure (BP) was continuously monitored. The left common carotid artery was exposed. A custom-made reversible occlusive device was wrapped around the artery to reversibly occlude the artery from outside the scanner. 2~3 trials were collected for each condition with 60-sec occlusions in each rat. A 15-min break was given between consecutive trials.

MRI was performed on an 11.7 T Bruker Biospec. A surface coil (ID = 1 cm) was used to image the left eye. BOLD fMRI of a single axial slice bisecting the optic nerve head was acquired using 4-segment EPI with TR/TE = 1500/12 ms, resolution = 51×51×800  $\mu$ m. The retinal BOLD signals were extracted as described elsewhere [5]. The extracted BOLD time courses were detrended and normalized to their baseline period. A custom-made LSI system was used to measure the retinal BF (RBF) [6]. ROIs were manually drawn to avoid visible retinal vessels in the LSI images. The mean value of the pixels in the ROIs of each image was reported.

To perform further statistical analyses, we calculated the accumulated RBF and RBOLD during the hyperemia period, which are the area under the RBF and RBOLD time course curves during the hyperemia period.

**Results:** The mean normalized RBF and retinal BOLD (RBOLD) time courses under the constant light, flicker and dark conditions showed dramatic drops in blood flow and oxygen tension followed by an overshoot (the red rectangle shades), indicative of reactive hyperemia (Figure 1A & B).

During the occlusion, the mean RBF changes were not different among the 3 conditions, whereas the RBOLD decreased the most under the flicker and the least under dark (Figure 1C & D). These data suggest that the metabolic rate in the inner retina was the highest in the flicker light, followed by the constant light and then the dark condition.

During the reactive hyperemia, the mean accumulated RBF and RBOLD changes among the 3 conditions were not significantly different from each other (Figure 1E & F), suggesting that the level of metabolism in the inner retina did not affect the hyperemia. Experiments were also repeated with 15-sec occlusion, and they gave similar results (data not shown).

**Discussion & Conclusions:** The accumulated RBF and RBOLD data during the hyperemia period were not modulated by constant light, flicker and dark conditions, which are known to change retinal metabolism. This is in contrast to other tissues (dog intestine [1], dog stomach [2], human heart [3] and calf muscle [4]), where reactive hyperemia is modulated by metabolism. There are also differences in the hyperemic response magnitude in the retina compared to other tissues. In the human heart and calf muscle, the maximum BF during the reactive hyperemia is 4~5 times of the baseline, and in the intestine, it was almost doubled. By contrast, the maximum RBF during the reactive hyperemia was ~25% above the baseline.

These differences suggest that there is less BF reserve in the retinal circulation for a higher metabolic demand than in other tissue. This conclusion is supported by a pO<sub>2</sub> study in the retina [8], which found inner retinal pO<sub>2</sub> decreases during the flicker and constant light stimulation, suggesting that RBF does not compensate fully for the increased metabolic demand by visual stimuli. Hence, an increase in metabolism by visual stimuli does not increase the reactive hyperemia.

In summary, the results indicate that the changes in metabolic rate by visual stimuli do not affect the reactive hyperemia because of the small BF reserve in the retinal circulation, suggesting that metabolic autoregulation in the retina is less able to compensate for increased metabolism. To our knowledge, this is the first study of the effect of changing metabolism on the reactive hyperemia in the retina, providing novel insights into retinal metabolic autoregulation.

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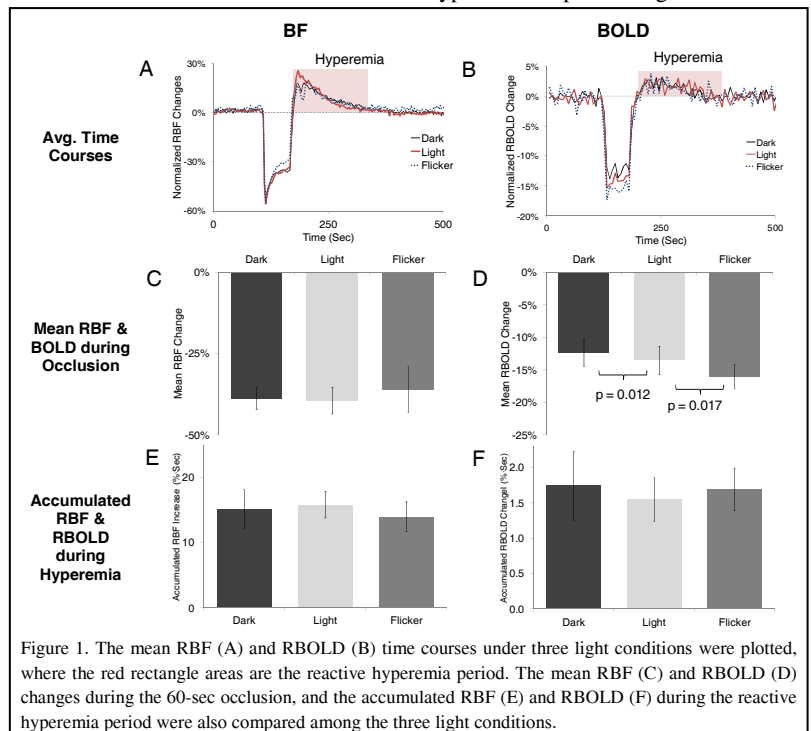


Figure 1. The mean RBF (A) and RBOLD (B) time courses under three light conditions were plotted, where the red rectangle areas are the reactive hyperemia period. The mean RBF (C) and RBOLD (D) changes during the 60-sec occlusion, and the accumulated RBF (E) and RBOLD (F) during the reactive hyperemia period were also compared among the three light conditions.