

MEMRI of diabetic retinas during light and dark adaptation at 11.7T

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TARGET AUDIENCE: Animal, translational, retinal disease researchers

PURPOSE: Although diabetic retinopathy (DR) is widely known as a vascular disease, there is substantial evidence that neural retina dysfunction could occur before vascular changes are apparent [1]. The goal of this study was to investigate neural retina dysfunction in DR using high-resolution manganese-enhanced MRI (MEMRI), which measures calcium activities. We studied diabetic rats at 14 and 30 days after streptozocin (STZ) injection under both light and dark adaptation in the same animals.

METHODS: We studied 60 day old age-matched controls (n=7), 14 day STZ treated (n=8), and 30 day STZ treated (n=5) Sprague-Dawley (SD) rats. Diabetes was induced by intravenous injection of STZ 55mg/kg via tail vein and confirmed by blood glucose >250mg/dL. Rats were anesthetized and one eye was patched. Eye patch was protected with Elizabethan collar. Rats recovered from anesthesia and adapted for 2hrs [3], then re-anesthetized for MnCl₂ infusion (88mg MnCl₂·4H₂O/kg, I.V.) over 1hr and allowed to recover in housing cage under ambient room light for Mn activity encoding. After 5hrs, rats were anesthetized, intubated, and ventilated at ~1.5% isoflurane. In dim red light, eye patch was removed.

MEMRI was performed at 11.7T using two identical surface coils, FLASH sequence with TR=150ms, TE=4.625ms, FOV=7.5x7.5mm, THK=0.7mm, NR=18, matrix=384x384 (20x20 μ m). Intensity profiles were normalized with respect to vitreous of each eye, vitreal and retinal ROI's were placed in homologous regions for each eye. Statistical analysis were performed by paired t-test with significance level with P<0.05, Bonferroni corrected.

RESULTS AND DISCUSSION: MEMRI results from a typical control animal with light versus dark-adapted eyes are shown in Figure 1. Normalized intensity profiles show functional differences in Mn activity between light and dark-adapted eyes of same animal. Profiles were normalized with respect to each eye's vitreous. We previously validated that peak #1 was assigned as the inner retina (ganglion and inner nuclear layer) which includes embedded retinal vessels, Peak #2 the avascular outer nuclear layer (outer nuclear layer and photoreceptor segments) and peak #3 the choroid vascular layer [5]. Under dark adaption, the inner retina peak showed slightly lower intensity, the outer retina peak showed higher intensity, and the choroid peak were similar in intensity compared to light adaptation. These findings are consistent with the notion that the outer retina in the dark has higher calcium activity compared to light, whereas the inner retina has higher calcium activity in light [6].

Figure 2 shows group-averaged peak intensity values under light and dark adaption for age-matched controls, 14 days post STZ and 30 day post STZ animals. Age matched control animals had higher intensity in outer retina peaks in the dark relative to the light. Outer retina peak under dark conditions was hyperintense relative to light (P<0.03). The inner retina in dark had lower intensity relative to the light adapted eyes and was hypointense relative to light (P<0.01). Choroid peaks under dark and light adaptation were not significantly different (P>0.05). Animals at 14 day post STZ treatment showed a distinct trend in lessening of intensity differences between light and dark-adapted eyes. At 30 days post STZ injection, outer retina intensity of light was higher than in dark eyes. This was the opposite of what was seen in outer retina in controls. All three layers in the dark had significant decrease in intensity when compared to light.

Figure 3 shows the percent change of light versus dark peak values in control, 14 day, and 30 day post STZ injected rats. The inner retina showed no significant difference (P>0.05) between control and 14 or 30 day post STZ treated animals. The outer retina showed significant difference between control and 14 day post STZ treated animals (P<0.05). Animals at 30 day post STZ treatment were also significantly different when compared to controls (P<0.001). A negative % change in controls represented a dark-adapted outer retinal peak with greater signal intensity versus light adapted peak. At 14 day post STZ this % change value was lessened and at 30 day post STZ, the % change shifted to positive. This reversal in % change reflected the greater calcium activity in dark-adapted eye.

CONCLUSIONS: High-resolution functional MEMRI revealed significant reduced Mn uptake in all three retinal layers (inner, outer, and choroid) in diabetic animals at early DR compared to non-diabetic controls as early as 14 days post STZ treatment. Moreover, differential reduced Mn activity in the dark in the outer layer in the DR compared to control animals, suggesting outer retina dysfunction at early DR.

REFERENCE [1] Jackson et al., Curr Diab Rep 2010, 10:380. [2] Berkowitz et al., IOVS 2007, 48:4753. [3] Behn et al., Doc Ophthalmol 2003, 106:153. [4] Cheng et al., PNAS 2006, 103:17525. [5] De La Garza et al., IOVS 2012, 53:4352. [6] Riva et al., IOVS 1983, 24:737-7.40

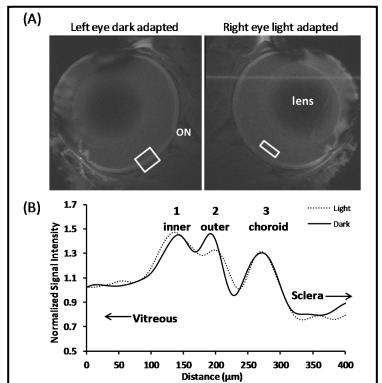


Figure 1. (A) MEMRI results of light versus dark adapted eyes of control animal at 20 x 20 μ m. Typical vitreous and retina ROI's are in white rectangles. ON: optic nerve. (B) Normalized intensity profiles with functional Mn activity differences between light and dark adapted eyes.

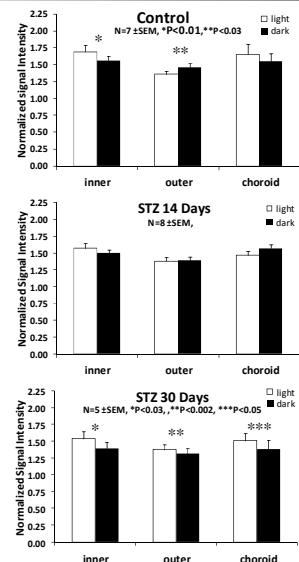


Figure 2. Group averaged peak intensity values for control, 14 day, and 30 day post STZ animals.

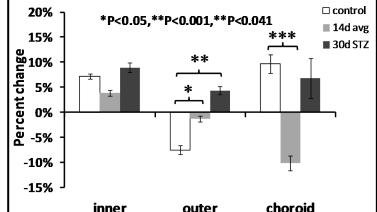


Figure 3. percent change of light versus dark peak values in control, 14 day, and 30 day post STZ injected rats.