Layer-Specific manganese-enhanced MRI of Diabetic rat retinas associated with light and dark adaptation at 11.7T

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TARGET AUDIENCE: Animal, translational, retinal disease researchers

PURPOSE: Diabetic retinopathy (DR), a leading cause of blindness, affects different cell layers as well as the choroidal and retinal blood supplies bounding the retina. There is evidence that neural retina dysfunction could occur before vascular changes are apparent [1]. The goal of this study was to apply high-resolution manganese-enhanced MRI (MEMRI) to investigate DR in streptozotocin (STZ) induced diabetic rats and compare to non-diabetic controls. We studied light and dark adaptation in the same animals by using two identical radio frequency transceiver coils (one for each eye) to investigate differential manganese uptake activities among different layers of the diabetic rat retina.

METHODS: Male Sprague-Dawley rats (n=3) were injected at 30 days old with 55mg/kg STZ in sodium citrate buffer (0.01M, pH 4.5) via tail vein. Diabetic state confirmed with blood glucose measurement (levels > 250mg/dL). Diabetic rats were imaged at 30 days post STZ injection. Comparison was made with age matched controls (n=4). For light/dark adaptation experiments, rats were anesthetized and one eye was patched (dark adaption). Eye patch was protected with Elizabethan collar. Animals recovered from anesthesia and adapted for 2hrs [3], then re-anesthetized for intravenous $MnCl_2$ administration (88 mg $MnCl_2 \cdot 4H_2O$/kg) over 1 hour and allowed to recover in housing cage under ambient room light (for Mn activity encoding). After 5hrs, animals were anesthetized, intubated, and mechanically ventilated at ~1.5% isoflurane. In dim red light, eye patch was removed, atropine, lubricating eye drops and pancuronium (.5mg/kg, i.v.) was administered.

MRI was performed on an 11.7T/16cm Bruker magnet with 74G/cm B-GA9s with two identical surface coils. MEMRI were acquired using FLASH with TR=150ms, TE=4.625ms, FOV=7.5x7.5mm, slice thickness=0.7mm, 18 repetitions, matrix=384x384 (20x20µm). Time-series data were corrected for motion and drift before offline averaging and profile analysis was used to create average anatomical profiles of the retina for peak intensity comparison [4]. 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.

RESULTS AND DISCUSSION: **Figure 1** shows *in vivo* MEMRI of light- and dark-adapted eyes (one animal), their normalized intensity profiles (one animal), and the group-averaged peak intensities from the control groups. 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]. In the dark adaption, the inner retina peak showed slightly lower intensity, outer retina peak showed higher intensity, and the choroid peak were similar in intensity compared to light adaptation (normal animals). These findings are consistent with the notion that outer retina in the dark has higher calcium activity compared to light, whereas the inner retina has higher calcium activity in light [5].

Figure 2 shows the normalized intensity profiles (one animal) and the group-averaged peak intensities of the DR group. Dark-adapted eye showed overall decreased Mn uptake in inner, outer retina and choroid compared to light adapted eye. The decrease is most apparent in the outer retina when compared to control group. That is the relative outer peak intensities in the dark versus light were opposite those in the control group. This finding suggests reduced Mn uptake in the dark, and thus outer retina dysfunction. Our data are consistent with a previous study that reported dark adaptation in DR rats exhibited a subnormal level of intraretinal manganese uptake although only two layers were detected due to lower spatial resolution [2, 6]. Moreover, our experimental design allowed light and dark adaptation comparison in the same animals.

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. 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. Future studies will investigate earlier DR time points and correlate with other functional measures (such as optomotry).

REFERENCE [1] Jackson et al., Curr Diab Rep 2010, 10:380-384. [2] Berkowitz et al., IOVS 2007, 48:4753-4758. [3] Behn et al., Doc Ophthalmol 2003, 106:153-159. [4] Cheng et al., PNAS 2006, 103:17525. [5] De La Garza et al., IOVS 2012, 53:4352-4358. [6] Berkowitz et al., *Molecular Vision* 2012, 18:372-376.

Figure 1. (A) MEMRI results of light versus dark adapted eyes of representative control animal at 20 x 20µm. Typical vitreous and retina ROI's are shown in white rectangles. ON: optic nerve. **(B)** Normalized intensity profiles showing functional differences in Mn activity between light and dark adapted eyes of control animal. **(C)** group-averaged peak intensity values from control animals.

Figure 2. (A) Normalized intensity profile of representative animal from DR group. (B) group-averaged peak intensity values from DR group.