Diffusion fMRI detects white-matter dysfunction in mice with acute optic neuritis

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Introduction

Conventional fMRI, based on blood-oxygen level dependent (BOLD) signal change, to identify neuronal activation is largely limited to assess gray matter activation. We have developed a diffusion fMRI method to detect white matter functional activation in normal, healthy mouse optic nerves and observed a 27% decrease in the water apparent diffusion coefficient perpendicular to the axonal fibers ($ADC_\perp$) upon flashing light stimulation. Meanwhile, we excluded the vascular contributions to the $ADC_\perp$ change via measurements in hypercapnic mice [1]. In the current study, we extend this method to non-invasively assess the response of experimental autoimmune encephalomyelitis (EAE) mouse optic nerves to visual stimulation with ongoing optic neuritis. Our findings suggest that acute axonal pathologies present at the onset of optic neuritis led to decreased axonal activation in EAE mice. Thus, diffusion fMRI holds the potential to provide a non-invasive assessment of functional integrity of CNS axons.

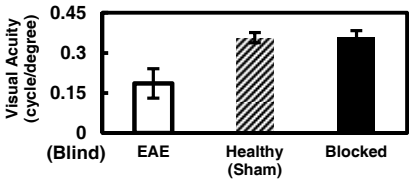

Figure 1 Averaged visual acuity of EAE (n=7), sham (n=7), and contralateral blocked (n=14) eyes (measured before MR experiments) showed the poor vision of EAE eyes and normal vision of sham and blocked eyes. Note: EAE mice in this sample of animals developed unilateral visual impairment.

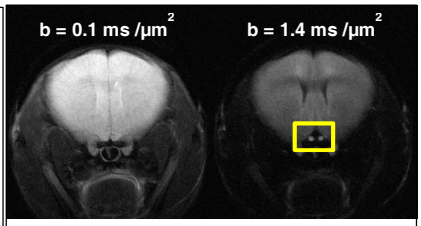

Figure 2 Two diffusion-weighted images (DWIs) with 0.1 and 1.4 ms/μm² b-values were calculated to generate apparent diffusion coefficient ($ADC_\perp$) map. Yellow box indicates the location of optic nerves

Materials and Methods

Animal Model: EAE was induced in seven female ten-week-old C57BL/6 mice with MOG_{35-55} peptide in complete Freund's adjuvant emulsion. The other seven age-matched control mice (sham group) underwent the same procedure without MOG_{35-55} immunization. For EAE mice, daily visual acuity (VA) was measured and MR was performed when VA ≤ 0.25 cycle/degree (Fig. 1). Typically, this occurs by 9-13 days post-immunization. Daily VA measurements assured normal visual function in control mice [2]. *Diffusion-weighted image (DWI) protocol:* Experiments were performed on a 4.7-T Agilent small-animal MR scanner with a multiple-echo spin-echo imaging sequence [3] with the following parameters: TR = 1.5 s, TE = 37.1 ms, inter-echo delay = 23.6 ms, FOV = 20 × 20 mm², matrix size = 256 × 256 (zero-filled to 512 × 512), and thickness = 1.3 mm. The image slice was planned carefully to be orthogonal to the optic nerves, thus minimizing partial volume effects. A pair of one-direction (perpendicular to optic nerve) DWI was acquired with b-value = 0.1 and 1.4 ms/μm², δ = 5 ms, and Δ = 18 ms. Acquisition time was 12.8 minute for each pair of DWI images [1]. *Visual stimulation:* A white LED light was placed 5 cm in front of mouse nose for stimulation of the experimental eye. The other eye was covered with parafilm and blocked using black electrical tape. *Diffusion fMRI strategy:* We acquired one baseline DWI dataset without stimulation. Then, the stimulus-on DWI dataset was acquired during application of flashing light stimulation. Finally, the LED was turned off for last DWI acquisition [1]. *Data analysis:* $ADC_\perp$ maps were generated from two DWIs (Fig. 2). ROI was selected using the same determination as our previous study [1]. *Histology:* mice were perfusion fixed immediately after the *in vivo* MRI for immunohistochemical staining.

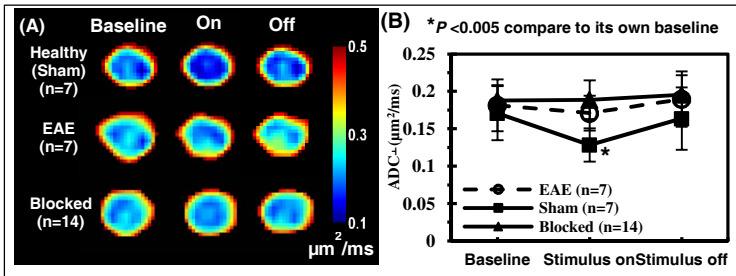

Figure 3 Averaged $ADC_\perp$ maps of sham, EAE, and contralateral blocked eyes at baseline, with and without stimulation (A). Comparing to its own baseline map, sham group showed obvious $ADC_\perp$ decrease. Statistical $ADC_\perp$ average plot (B) was in agreement with the pattern of averaged $ADC_\perp$ maps (A). A significant 25% $ADC_\perp$ decrease was observed in sham group. EAE group only showed a slightly 5% decrease. Regarding blocked eyes, there was no difference between the scans of baseline and stimulation.

Results

Color-coded group-averaged $ADC_\perp$ maps for the different experimental groups are shown in Figure 3A. Group-averaged $ADC_\perp$ at baseline, with and without visual stimulus for EAE, healthy, and blocked optic nerves are depicted in Figure 3B. In healthy optic nerves, application of visual stimulus led to a significant $ADC_\perp$ decrease (~25%) while in EAE optic nerves only a slight $ADC_\perp$ decrease (~5%) was observed. ADC changes were reversible with cessation of stimulus. Blocked eyes showed no difference between baseline and stimulation scans. From Figure 3A it can also be appreciated that optic nerves in EAE mice are slightly swollen. Representative SMI31 (intact axons), MBP (myelin sheaths), and SMI32 (injured axons) staining images of optic nerves were showed that EAE optic nerve developed axonal beading, distorted myelin sheaths, and injured axons (Fig. 4).

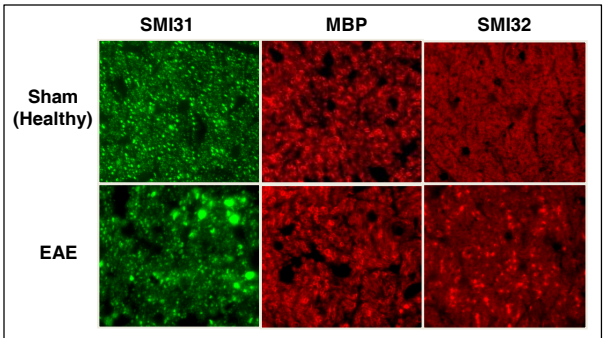

Figure 4 Representative 60× SMI31 (intact axon), MBP (myelin sheath), and SMI32 (injured axon) staining images from control and EAE mouse. EAE mouse optic nerve showed obviously axon beading (SMI31), distorted myelin sheath (MBP), and positive SMI32 staining.

Conclusion

Our results demonstrate that diffusion fMRI can non-invasively differentiate between patterns of *in vivo* axonal activation in healthy and diseased optic nerve. Histology of retinal samples is ongoing, however, since measurements are made early in the EAE time course, it is likely that the pathology is localized to the optic nerve-- prior to any retinal ganglion cell loss [4]. More broadly, with other stimulus paradigms, this technique may enable global detection of white-matter dysfunction throughout the CNS.

Reference

[1] Spees et al., Neuroimage (in press); [2] Chiang et al., Proc. Intl. Soc. Magn. Reson. Med. 20 (2012), 3058; [3] Tu et al., Proc. Intl. Soc. Magn. Reson. Med. 18 (2010), 4001; [4] Shindler et al., Exp. Eye Res. 87: 208-213.