White-Matter Diffusion fMRI in the Healthy Mouse Optic Nerve

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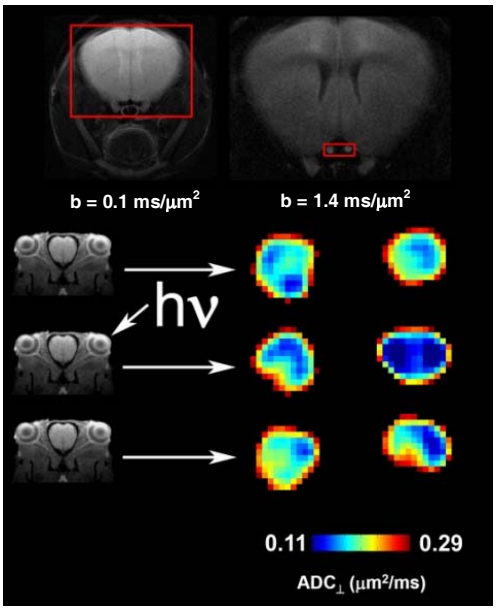
Introduction. Functional MRI measurements are, with few exceptions, limited to investigations of gray matter wherein Blood Oxygen Level Dependent (BOLD) effects are revealed via hemodynamic coupling with cortical activation.[1,2] Diffusion fMRI has been suggested as an alternate approach to directly detect neuronal activation in the gray matter of the human visual cortex.[2,3] In the present work we describe diffusion fMRI, as recently implemented, to detect effects produced by axonal activation in the optic nerve of normal, healthy mice with visual stimulation.[4]

Methods. All protocols were approved by the Washington University Animal Studies Committee. Female C57BL/6 mice (age 10-12 wks) were used in the study. Normal visual acuity was ensured via assessment with a Virtual Optokinetic System (Cerebral Mechanics, Inc.).[5] Medetomidine was employed for anesthesia[6] and animals were held immobilized in a stereotactic headholder. Visual stimulus was delivered via a high-intensity white LED placed 5 cm in front of the animal's nose (part of a home-built MR-compatible visual stimulation apparatus). A near-optimal flash frequency of 1.4 Hz was used with a flash duration of 150 ms.[7] In each animal, one eye was covered with black electrical tape and served as a control. Diffusion MRI measurements were acquired via a multi-echo, spin-echo sequence[8] at 4.7 T using an Agilent DirectDrive small- animal MR system. DWI acquisition parameters included TR = 1.5 s, three echoes co-added for increased S/N with TE_1 = 37.1 ms and $TE_{2,3}$ = 23.6 ms, δ = 5 ms, Δ = 18 ms and a 20 × 20 mm^2 (256 × 256) field-of-view. The slice orientation was planned to be perpendicular (as nearly as possible) to the long axis of the axonal fibers to minimize partial volume effects. Maps of $ADC_\perp$ and $ADC_\parallel$ were generated from two separate diffusion-weighted images (low and high b values). In addition to experiments carried out in room-air-breathing animals, hypercapnic animals (breathing a 5% CO_2/95% O_2 mixture) were studied in order to evaluate the possibility of vascular contribution to the observed diffusion fMRI response.[3,9]

Results. The color-coded maps in Figure 1 Illustrate the effect of the applied visual stimulus on $ADC_\perp$ in mouse optic nerve. Averaged across five air-breathing mice, a statistically significant ~27% decrease in $ADC_\perp$ is observed in the optic nerve of the stimulated eye (Figure 2), from 0.18 ± 0.02 μm^2/ms at baseline to 0.13 ± 0.01 μm^2/ms with stimulation. This $ADC_\perp$ change is completely reversible after removing the stimulus. In the contralateral, non-stimulated optic nerve $ADC_\perp$ is unchanged. Experiments carried out in hypercapnic animals indicate that any vascular contribution to the observed effect is minimal (data not shown). $ADC_\parallel$ was independent of visual stimulus application.



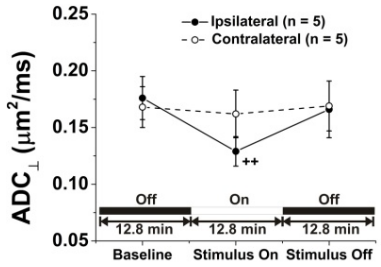


Figure 1. (Left) The images in the top row show views with image slice perpendicular to the axis of the optic nerves at low (b = 0.1 ms/μm^2) or high (b = 1.4 ms/μm^2) diffusion weighting applied in the phase-encode direction. The optic nerves are visible within the red box on the b = 1.4 ms/μm^2 image. The lower set of three color-coded maps show the effect of visual stimulus on the optic nerve $ADC_\perp$ when applied to the indicated eye (top = baseline; middle = stimulus on, hν = light; bottom = stimulus off). **Figure 2. (Above)** Application of visual stimulus produces a statistically-significant decrease in $ADC_\perp$ for the optic nerve of the stimulated eye.

Discussion. Decreased ADC is a hallmark of repeated, synchronous neuronal discharge in the CNS.[10-13] Electrical stimulation applied to *ex vivo* rat optic nerve preparation resulted in frequency- and duration-dependent shrinkage of the extracellular space (ECS) with higher stimulus frequency and longer stimulus duration producing larger reductions in ECS volume.[14] Age-dependent myelination of the rat optic nerve is correlated with the extent of activity-induced ECS shrinkage in these *ex vivo* preparations. It should be noted that the extracellular volume fraction emerges as the dominant biophysical parameter determining white-matter diffusion characteristics in mathematical models.[15]

As outlined in a recent review by Rash,[15] evidence from multiple investigators indicates that the panglial syncytium (a network of gap-junction interconnected astrocytes, ependymocytes, and oligodendrocytes, including direct involvement of myelin-bounded spaces) provides a critical mechanism for dynamically siphoning K^+ and osmotically associated water away from repetitively-discharging axonal fibers, allowing rapid membrane repolarization. In myelinated axons, the voltage-gated potassium channels (opened with propogation of the action potential) are predominantly localized to the intermodal axonal membrane underneath the internodal and juxtaparanodal myelin and form the tributaries to the panglial syncytium.

Conclusions. The optic nerve can be regarded as a prototypical white-matter structure, hence diffusion fMRI may be extended to investigations of white-matter function throughout the CNS and may provide interesting information about activity in distributed neural networks that is not directly accessible by BOLD studies of gray matter. Monitoring white-matter function in diseases progression/therapeutic response that directly target white matter also suggest themselves.

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