# Extensive axonal damage in the spinal cord of EAE mice detected with in vivo DTI

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## Introduction

Multiple Sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE), an animal model of MS, are characterized by demyelinating lesions in the white matter of the central nervous system (CNS). In addition to demyelination, lesions also contain varying degrees of axonal damage[1]. Identification of lesion pathology has typically only been possible with histological examination. In the current study, we demonstrate that parameters derived from *in vivo* diffusion tensor imaging (DTI) in the spinal cord of EAE mice can detect axonal damage and demyelination. Mice with chronic EAE revealed a significant decrease in relative anisotropy (RA) and axial diffusivity [$\lambda_{\|} = \lambda_1$] compared to control mice, whereas radial diffusivity [$\lambda_{\perp} = (\lambda_1 + \lambda_2)/2$] was not significantly increased. The DTI findings were confirmed with histological staining for β-APP, a marker of axonal damage, and luxol-fast blue, a marker of myelin integrity. This data suggests that while demyelination is confined to focal lesions, extensive axonal damage occurs throughout spinal cord white matter in EAE mice.

## Materials and Methods

*EAE induction*

EAE was induced in ten-week old female C57BL/6 mice through adoptive transfer of ~5 x $10^6$ CD4 T-cells activated against myelin oligodendroglycoprotein (MOG) peptide 35-55. Animals were scored daily for clinical symptoms using a published 0-5 scoring system: 1 = limp tail; 2 = hind limb weakness sufficient to impair righting; 3 = one limb paralyzed; 4 = two limbs paralyzed; 5 = 2 or more limbs paralyzed or moribund.

*Animal Preparation*

Ten-week old normal (n = 5) and EAE (n = 3) female C57BL/6 mice were anesthetized using isoflurane/oxygen mixture (7% for induction and 0.7-1.5% for maintenance). Animals were placed in a custom holder designed to immobilize the spine and monitor respiration. Core body temperature was maintained at 37.0 ± 0.5°C with a circulating warm water pad. Data collection was synchronized with animal respiration to avoid motion artifacts. Mice with EAE were imaged during the chronic stage of clinical disability (clinical score = 2).

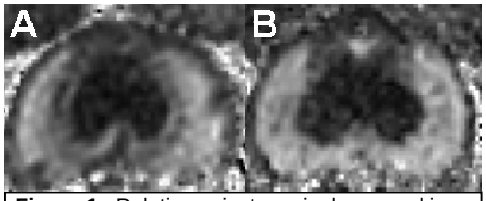


**Figure 1.** Relative anisotropy is decreased in the spinal cord white matter of mice with EAE (A) compared to normal mice (B).

*Diffusion Tensor Imaging*

Diffusion-weighted images were acquired using a spin-echo sequence modified with Stejskal-Tanner diffusion-sensitizing gradients with the following parameters: TR: 0.3s (determined by respiration rate), TE: 37ms, Δ: 20ms, δ: 7ms, FOV: 1x1cm, data matrix 128x128 (zero filled to 256x256), NEX: 4, and b-values = 0 and 0.785 μm$^2$/ms. Images were obtained with diffusion sensitizing gradients applied in six directions: (Gx,Gy,Gz) = (1,1,0), (1,0,1), (0,1,1), (-1,1,0]), (0,-1,1), and (1,0,-1). Each DTI data set consisted of 12 slices that covered vertebral segments L1-L3 and was obtained with an acquisition time of ~2 hours.

*Data Analysis*

The six independent elements of the diffusion tensor were calculated from diffusion-weighted images. The resulting tensor element maps were used to derive the eigenvalues ($\lambda_1$, $\lambda_2$, and $\lambda_3$) and eigenvectors of the diffusion tensor by matrix diagonalization. On a pixel-by-pixel basis, quantitative indices including axial diffusivity ($\lambda_{\|}$), radial diffusivity ($\lambda_{\perp}$), and relative anisotropy (RA) were derived using custom software[2].

Regions of interest (ROIs) were defined for dorsal, ventral, left-lateral, and right-lateral white matter. A non-paired t-test was performed on DTI parameters between the normal and EAE mice from the L2 level of the cords. Significance was assessed at $p < 0.05$.

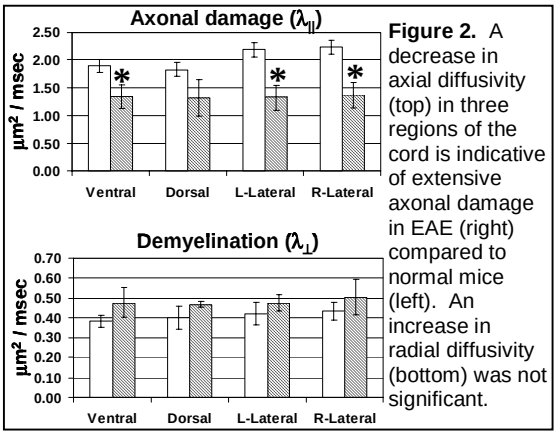


**Figure 2.** A decrease in axial diffusivity (top) in three regions of the cord is indicative of extensive axonal damage in EAE (right) compared to normal mice (left). An increase in radial diffusivity (bottom) was not significant.

*Histological Analysis*

Mice were perfused with 4% paraformaldehyde in phosphate buffered saline at the conclusion of DTI measurements *in vivo*. Axonal injury was examined on fixed spinal cords incubated with β-amyloid precursor protein (β-APP). Myelin integrity was examined on fixed sections stained with luxol-fast blue.

## Results and Discussion

A decrease in the RA of spinal cord white matter is evident in a mouse with EAE compared to a normal mouse (Fig 1). No apparent changes in the spinal nerve roots are observed (Fig. 1A). The DTI parameters from the predefined ROIs from three EAE mice at the chronic stage of clinical disability (CS=2) were examined and compared with those obtained from a cohort of 5 normal mice (Fig. 2). The group averaged axial diffusivity is significantly decreased in EAE mice in all white matter regions of cord, except the dorsal white matter. In contrast, there is no statistically significant increase in the group averaged radial diffusivity in any region.

To verify that axial and radial diffusivities reflect axon and myelin degeneration, spinal cords were obtained from representative EAE and normal mice. Both β-APP and LFB stains were performed at segment L2 following DTI measurements (Fig. 3). Intense staining of β-APP in all regions of the EAE cord indicates extensive axonal damage compared to the normal cord (Figs. 3A and B). In contrast, LFB staining in the same level of the EAE cord is more inhomogeneous than that of the normal cord (Figs. 3C and D).

The present findings indicate that the spinal cord white matter in mice with EAE exhibits widespread axonal damage and regional demyelination. The results show that parameters derived from DTI, $\lambda_{\|}$ and $\lambda_{\perp}$, can be used to assess the extent of axonal damage and demyelination, respectively, and that these measurements are in good agreement with the pathology.

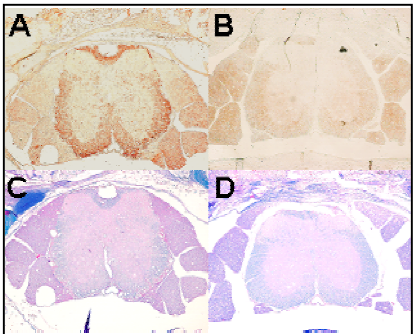


**Figure 3.** β-APP staining demonstrates extensive axonal damage in spinal cord white matter in EAE (A) vs. normal (B) mice. Luxol-fast blue staining for myelin in EAE (C) vs. normal (D) white matter shows regional demyelination.

## References

1. Kornek *et al.*, *Am. J. Pathol.* 157:267-76 (2000).
2. Song *et al.*, *Neuroimage*, 20:1714-1722 (2003).