Diffusion Tensor Imaging of Myelin-Deficient Shiverer Mice Transplanted with Neural Precursor Cells

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Introduction *Ex-vivo* diffusion measurements on myelin-deficient and demyelinated fibers showed that diffusion anisotropy was only marginally reduced relative to normal myelinated axonal fibers. More recently, *in vivo* DTI study on dysmyelinated axons in myelin-deficient *shiverer* (*shi*) mutant mice reported a small reduction in diffusion anisotropy relative to wild type (wt) mice[1], suggesting that most of the DTI contrast arise from restrictions due to axonal membranes. Although CNS axons in *shi* mice apparently remain intact, increased axonal protein content and abnormalities of the axonal cytoskeleton have been reported. These and other potential pleiotropic effects associated with *shi* mutation could however modulate the DTI contrast, and these effects remain to be determined.

In this study, we performed DTI on transplanted mice to address the potential pleiotropic effects associated with *shi* mutation on DTI contrast. We have previously shown that intracerebroventricular transplantation of neural precursor cells in *shi* mice leads to the engraftment and differentiation of transplanted cells that function as oligodendrocytes, producing wt myelin basic protein (MBP) and morphologically normal internodal myelin sheaths[2]. The contribution of myelin *per se* on DTI parameters could thus be directly evaluated.

Methods DTI was performed on wt mice, *shi* mice and *shi* mice transplanted with neural precursor cells (transplant mice) and *shi* mice transplanted with dead precursor cells (transplant control). Homozygous *shi* mutant mice have extensive CNS dysmyelination with morphologically abnormal myelin sheaths. Donor neural precursor cells were harvested from the striatum/subventricular zone, microdissected from wt embryonic mice (expressing green fluorescent protein), on day 16 after overnight mating, and transplanted into the brains of *shi* mice[2]. Similarly, for transplant control mice, dead precursor cells were transplanted into the brains of *shi* mice.

MR imaging was done on 8-12 week old mice, anesthetized at 1% isoflurane, secured in a stereotaxic head restrainer with tooth-, ear- and shoulder-bars. Rectal temperature and respiration rate were monitored and maintained. MRI was performed on a 9.4 T, 89 mm vertical bore magnet with a 100 G/cm gradient insert and a small surface coil. Diffusion-weighted images were acquired in six orthogonal gradient directions[3] at b-value of 1200 s/mm^2 and one direction for low b-value of 5 s/mm^2, TR=2.5 s, TE =14 ms, matrix of 64x64 zero filled to 128x128, FOV = 1.28x1.28 cm, 7 slices of 0.9-mm thickness with interslice gap of 0.1 mm. T_2-weighted images were acquired with similar parameters but a TE of 45 ms. T_2-weighted intensity was normalized to cortical gray matter for comparison across animals. Matlab® programs were used for eigen decomposition and calculation of fractional anisotropy (FA) maps.

After MR imaging, mice were transcardially perfused with paraformaldehyde. Brains were removed and sectioned for immunohistochemistry using a primary antibody directed against MBP (mouse, 1:1,000 = 1 µg/ml) and an anti-mouse Alexa Fluor 594 secondary antibody (Molecular Probes). Slides were examined using fluorescence microscopy, with excitation wavelengths for GFP and Alexa Fluor 594 of 488 and 568 nm, respectively to correlate with MRI.

Fig 1

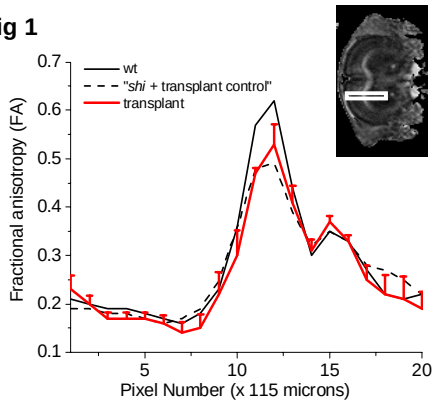
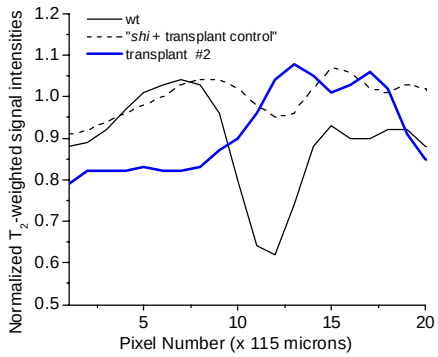
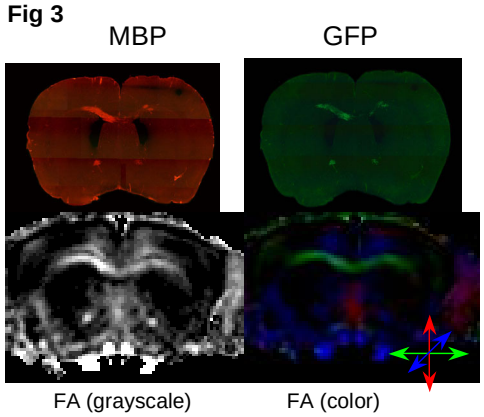


Fig 2



Results There were no statistical differences in DTI parameters between wt and transplant controls and they were grouped together. **Fig. 1** shows a FA profile plot across the corpus callosum (inset) for the wt, "*shi* + transplant control" and transplanted mice. Profiles of FA maps from wt and "*shi* + transplant control" groups were similar in hippocampal gray matter but differed significantly in the corpus callosum. **Fig. 2** shows normalized T_2-weighted profile plot of a successful transplant mouse (#2) as determined by GFP and MBP immunohistochemistry. **Fig. 3** shows another transplanted mouse (#5) with grossly asymmetric hemispheric GFP cellular distribution and wt MBP immunoreactivity in the corpus callosum (top row). FA image in grayscale appeared brighter in the corpus callosum of the hemisphere with relatively more successful transplantation. FA color tensor map did not show any grossly irregular patterns in deposition of donor-derived myelin.

Fig 3



Discussion The major findings of this study were: 1) There were marked differences in T_2 and diffusion anisotropy between wt and shi mice. 2) Partial renormalization of DTI parameters was observed with transplantation, although the sample size of the transplant mice was small due to the challenging transplantation experiments. 3) The spatial distribution of increased anisotropy showed good correspondence to the distribution of donor-derived myelination in individual mouse brains as indicated by immunohistology and GFP distribution.

One surprising finding is that, although T_2-weighted images showed dramatic differences between wt and *shi* mice in the corpus callosum, they were not sensitive to transplant-derived myelination. A possible explanation is that normal and densely packed myelin appears to be necessary for T_2 contrast but not necessary for anisotropic water diffusion in white matter. Although further validation is needed, our initial results are consistent with other studies involving human Krabbe's disease and stem-cell transplantation[4], which found RA to be more sensitive than T_2-weighted images in detecting dysmyelination. Similarly, Larsson et al.[5] found that DTI is relatively more sensitive than T_1- or T_2-weighted images in delineating myelin-related lesions. The combined use of T_2- (or T_1-) weighted imaging and DTI is likely necessary for proper delineation and for monitoring the progression of white-matter diseases and therapeutic interventions.

Conclusion T_2-weighted images detected obvious differences between wt and *shi* mice in the corpus callosum but did not detect donor-derived myelination in *shi* mice transplanted with neural precursor cells. By contrast, FA showed relatively small differences between wt and *shi* mice in the corpus callosum but detected changes due to donor-derived myelination in the transplant group.

References: 1) Song et al., Neuroimage 2002 17:1429. 2) Mitome et al., Brain 2001 124:2147. 3) Basser and Pierpaoli, MRM 1998 39:928 4) Guo et al., Radiology 2001 218:809. 5) Larsson et al. Dement Geriatr Cogn Disord 2004 17:316.