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Myelination and long diffusion times alter diffusion-tensor-imaging contrast in myelin-deficient *shiverer* mice

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Diffusion tensor imaging (DTI) using variable diffusion times (t_{diff}) was performed to investigate wild-type (wt) mice, myelin-deficient *shiverer* (*shi*) mutant mice and *shi* mice transplanted with wt neural precursor cells that differentiate and function as oligodendrocytes. At t_{diff} = 30 ms, the diffusion anisotropy "volume ratio" (VR), diffusion perpendicular to the fibers ($\lambda_\perp$), and mean apparent diffusion coefficient (<*D*>) of the corpus callosum of *shi* mice were significantly higher than those of wt mice by 12 ± 2%, 13 ± 2%, and 10 ± 1%, respectively; fractional anisotropy (FA) and relative anisotropy (RA) were lower by 10 ± 1% and 11 ± 3%, respectively. Diffusion parallel to the fibers ($\lambda_{//}$) was not statistically different between *shi* and wt mice. Normalized T_2-weighted signal intensities showed obvious differences (27 ± 4%) between wt and *shi* mice in the corpus callosum but surprisingly did not detect transplant-derived myelination. In contrast, diffusion anisotropy maps detected transplant-derived myelination in the corpus callosum and its spatial distribution was consistent with the donor-derived myelination determined by immunohistochemical staining. Anisotropy indices (except $\lambda_{//}$) in the corpus callosum showed strong t_{diff} dependence (30–280 ms), and the differences in $\lambda_\perp$ and VR between wt and *shi* mice became significantly larger at longer t_{diff}, indicative of improved DTI sensitivity at long t_{diff}. In contrast, anisotropy indices in the hippocampus showed very weak t_{diff} dependence and were not significantly different between wt and *shi* mice across different t_{diff}. This study provides insights into the biological signal sources and measurement parameters influencing DTI contrast, which could lead to developing more sensitive techniques for detection of demyelinating diseases.

Keywords: DTI; ADC; Diffusion anisotropy; MRI; High fields; Stem cell transplantation; Neural precursor cells; Myelin basic protein; Demyelination; STEAM sequence

Introduction

Diffusion tensor imaging (DTI) (Basser et al., 1994a,b) has gained wide acceptance as a tool for non-invasive imaging of anatomical connectivity (Mori, 1995; Nakada and Matsuzawa, 1995; Makris et al., 1997; Xue et al., 1999) and brain microstructural morphology (Mori et al., 2001a; Mori et al., 2001b; Zhang et al., 2002). DTI has been used for detecting changes in myelination in the developing brain (Wimberger et al., 1995; Prayer et al., 1997; Neil et al., 1998) and in demyelinating diseases (Guo et al., 2001; Larsson et al., 2004), although its underlying contrast mechanism remains incompletely understood. There is some evidence that DTI is more sensitive for detecting demyelinating lesions relative to conventional T_1- and T_2-weighted imaging (Hajnal et al., 1991; Sukama et al., 1991; Wimberger et al., 1995; Prayer et al., 1997; Guo et al., 2001; Larsson et al., 2004). DTI contrast arises from barriers (such as cell membranes of axons and oligodendrocytes) that hinder water diffusion in some orientations more than others, giving rise to anisotropic diffusion. In white matter of the central nervous system (CNS), for example, water diffusion perpendicular to fiber tracts is more restricted than that parallel to the fiber tracts (Le Bihan et al., 1993; Beaulieu and Allen, 1994b).

In principle, DTI contrast could arise from myelin and/or axons. Diffusion measurements on myelin-deficient and demyelinated fibers (Le Bihan et al., 1993; Beaulieu and Allen, 1994b; Ono et al., 1995; Seo et al., 1999; Gulani et al., 2001) showed that diffusion anisotropy was only marginally reduced relative to normal myelinated axonal fibers, suggesting that DTI contrast likely arises from axonal density and anisotropy in axonal structure rather than from myelin content. More recently, in vivo DTI study on dysmyelinated axons in myelin-deficient *shiverer* (*shi*) mutant mice (Song et al., 2002) reported small but significant reduction in diffusion anisotropy relative to wild-type (wt) mice, again suggesting that most of the DTI contrast arises from axons but myelin could contribute to DTI contrast. Although CNS axons in

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shi mice remain apparently intact (Dupouey et al., 1979; Privat et al., 1979; Inoue et al., 1981; Shen et al., 1985), increased axonal protein content and abnormalities of the axonal cytoskeleton have been reported (Brady et al., 1999; Kirkpatrick et al., 2001). These and other potential pleiotropic effects associated with the *shi* mutation, which might modulate the apparent diffusion coefficient (ADC) and DTI contrast, remain to be addressed. Furthermore, the sensitivity of DTI contrast to detect changes in myelin per se has not been unequivocally demonstrated.

Most DTI studies use relatively short diffusion times (t_{diff}), typically ranging from 30 to 60 ms in animal model, in order to minimize signal loss due to T_2 decay. ADC has been shown to be t_{diff}-dependent (Segebarth et al., 1994; Helmer et al., 1995; Pfeuffer et al., 1998). ADC in the whole brain parenchyma had been reported to decrease rapidly from ~2 to ~15 ms and gradually decreases for $t_{diff} > 20$ ms (Segebarth et al., 1994; Helmer et al., 1995; Pfeuffer et al., 1998). The root-mean-squared (rms) displacement for t_{diff} ~20 ms approximates the ensemble-average distant between cell membranes (i.e., on the order of cell size). The effect of t_{diff} on DTI contrast at longer diffusion times (few hundreds of ms), however, remains relatively unexplored. Since diffusion displacement parallel to the fiber tracts in principle is less restricted, while that perpendicular to the fiber is more restricted, we predicted that DTI contrast should improve at longer t_{diff}. Knowledge of the t_{diff}-dependent effects on DTI contrast is important for future experiments aimed at improving sensitivity of fiber tracking.

The general aim of this study was to investigate the potential contribution of myelination and diffusion times to DTI contrast. First, we extended Song et al.'s (2002) study by systematically evaluating the sensitivity of diffusion perpendicular ($\lambda_{\perp}$) and parallel ($\lambda_{//}$) to the fiber tracts, mean apparent diffusion coefficient (<*D*>), fractional anisotropy (FA), relative anisotropy (RA), and volume ratio of the diffusion ellipsoid (VR) to detect anisotropy differences between wt and *shi* mice. Further, we performed the transplantation experiments to ask if and how MR parameters might be altered by the addition of some normal myelin to *shi* mice. We have previously shown that intracerebroventricular transplantation of neural precursor cells in *shi* mice leads to engraftment and differentiation of transplanted cells, including some that function as oligodendrocytes, producing wt myelin basic protein (MBP) and morphologically normal internodal myelin sheaths (Mitome et al., 2001). Such transplantation could potentially reverse the MRI abnormality observed in *shi* mice. DTI of wt, *shi*, and transplanted *shi* mice thus offered a unique opportunity to evaluate the effects of myelination per se on MR parameters. Second, we analyzed the sensitivity of DTI contrast as a function of t_{diff} (30 to 280 ms) in wt and *shi* mice. We modified the Stimulated-Echo-Acquisition-Mode (STEAM) sequence to include diffusion gradients, making it possible to use very long t_{diff} without substantial signal loss due to T_2 decay.

Materials and methods

Phantom experiments

Diffusion-weighted imaging (DWI) was performed using a modified STEAM sequence (Merboldt et al., 1991) with a pair of unipolar diffusion gradients placed during the TE/2 periods. The cross-term interactions of diffusion gradients with each other and with the imaging gradients might change the *b* values for different diffusion-sensitive orientations (Brockstedt et al., 1998; Gullmar et al., 2002), possibly leading to bias in diffusion anisotropy within a single t_{diff} as well as across different t_{diff}. Although these gradient cross-term interactions could be calculated (Brockstedt et al., 1998; Gullmar et al., 2002), these calculations become tedious and inaccurate for STEAM sequence with very long diffusion time because the approximation used in the calculation may be invalid when the cross-terms are large. In this study, an experimental approach was used instead. This was done by experimentally adjusting the diffusion gradients (effective *b* values) such that the diffusion-weighted 1-D signal intensity profiles, and thus the diffusion coefficients, on a uniform water phantom along different diffusion-sensitizing directions were the same using identical imaging parameters as the in vivo part of this study. Phase-encoding gradients were switched off, and six profiles were obtained by sequentially switching on diffusion gradients in each of the six directions (3 axes, +1 and −1 direction for each axis). Since there were no first-order cross-terms in the phase-encoding direction, the profile intensities obtained with diffusion gradients in the phase-encoding directions were equal and these profiles were used as references. The magnitudes of the diffusion gradients in the slice-selection and readout directions were adjusted such that their profile intensities were equal to the reference profile intensities. Cross-terms from higher *k*-space lines were ignored because the positive and negative lobes of the phase-encoding gradients result in cancellation of the cross-term effects and the high *k*-space lines have relatively small "signal power". This correction was done for the larger (~1200 mm^2/s) of the two *b* values; the cross-term effects on the low *b* value (~5 mm^2/s) images were ignored. The advantage of this approach is that it can be readily validated experimentally across a wide range of conditions.

To test the validity of this approach, DTI measurements at different t_{diff} were made on a water phantom and a phantom of *N*-acetyl-aspartate (NAA) dissolved in dimethylsulfoxide (DMSO) at room temperature (20°C); the latter was chosen because it yielded a diffusion coefficient comparable to the ADC in the in vivo brain (i.e., gray matter) at 37°C.

Transplantation methods

Shi mice were used as transplantation hosts. The *shi* mutation is a large deletion in the MBP gene. Homozygous mutant mice fail to produce MBP, which is a major structural component of the myelin sheath, leading to extensive CNS dysmyelination with morphologically abnormal myelin sheaths. The *shi* mutation in our colony is maintained on a B6C3F1 hybrid-based stock, which is >99.9% congenic at other loci. Wide-type mice were obtained from the same hybrid stock.

Donor neural precursor cells were derived from the striatum/subventricular zone microdissected from embryonic transgenic mice on day 16 after overnight mating. These mice express an enhanced form of the jellyfish green fluorescent protein (GFP) under the control of the mouse prion promoter (Borchelt et al., 1996; van den Pol and Ghosh, 1998) and a chicken β-actin-cytomegalovirus immediate early enhancer (Niwa et al., 1991; Ikawa et al., 1995). Cells were isolated and propagated as previously described (Mitome et al., 2001). After 3–8 days in culture, these cells were harvested and transplanted into the brains of *shi* mice.

Neonatal *shi* host mice ranging in age from postnatal days 1 to 4 were cryoanesthetized and injected with 60,000 cells/1 μl into each lateral cerebral ventricle, and 120,000 cells/2 μl into the