Diffusion Tensor Imaging and T2 Relaxography in Myelin Deficient Mice

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INTRODUCTION- Myelin abnormalities are present in a number of neurological diseases and may contribute to the pathophysiology of schizophrenia and Alzheimer's disease. Mouse models, such as the myelin basic protein (MBP) knockout *shiverer*, have been studied with MRI and neurohistology and represent an opportunity to better understand the impact of myelin–related defects on brain structure. Song et al. (Neuroimage, 17: 1429-36, 2002) used diffusion tensor imaging (DTI) in *shiverer in vivo* and showed that the MBP null mutation is associated with decreased diffusion anisotropy attributable to increased radial diffusivity. Here we extend these findings to *shiverer* fixed brain and report associations between MBP, DTI and T_2 relaxation measures.

METHODS- All mice used were 3-4 months of age. Following deep anesthesia with isofluorane, *shiverer* mice and their age-matched littermates (Jackson Labs) were perfused with 4% formalin-PBS. The top part of the skull was removed so that the head could be positioned and immobilized in a 15 ml centrifuge tube, filled with 4% formalin, such that the tube could be easily positioned at the coil's center. Postfixation continued for a minimum of 3 months. All the data were taken on a 7.0 T MRRS (Guildford, UK, formerly SMIS) 40 cm bore system. The imaging protocol and hardware details have been described in detail in Guilfoyle et al. NMR Biomed (2003) 16: 77-81. A 3-plane localizer was used initially to help standardize slice selection across samples and reliability was achieved. Briefly, coronal DTI scans (beginning at the callosal genu) were acquired using a distortion-free spin echo sequence (TR/TE 1000/40 ms, 128 x 128 matrix, b=900s/mm^2, 12 slices, 0.3mm slice thickness, 0mm gap). In-plane resolution was 85µm. Diffusion was measured along six equally spaced nonlinear directions and also with no diffusion gradients (b=0 images). A multi-echo spin echo sequence was used for the T_2 measurements with a total of six separate echoes (in msec: 15, 20, 25, 30, 35, 55, 75) for the T_2 fit. Matrix size was 128 x 96 (zero-filled to 128 x 128) and TR was 2 s. The FOV, slice thickness and slice selection of the T_2 measurements were the same as used in the DTI experiments. ROIs were created on b=0 images as they retained sufficient white matter contrast to define regional boundaries (though this was clearly diminished in *shiverer*). ROIs for white matter regions- corpus callosum (CC), anterior commissure (AC), internal capsule (IC), optic tract (OT) and fornix (FX)- and cerebral cortex (as a negative gray matter control, CTX) were then transferred to DTI-derived maps of fractional anisotropy (FA), trace diffusivity, axial (DAx) and radial [$(\lambda_2+\lambda_3)/2$] diffusivity (DRa) bilaterally. Measures were averaged across both sides. Group differences were analyzed using students T-test (2-tailed) and Pearson correlations to evaluate the relationships between DTI metrics and T_2.

Table1 DTI Measures and T2 Time in *shiverer* and control Mice

REGION	CONTROL	SHIVERER
Fractional Anisotropy (mean ± sd)		
AC*	.72 ± .05	.65 ± .01
CC**	.69 ± .03	.63 ± .01
IC*	.64 ± .02	.51 ± .09
OT***	.86 ± .05	.67 ± .04
FX**	.57 ± .05	.48 ± .02
CTX^{NS}	.33 ± .05	.35 ± .02
Axial Diffusivity (mean ± sd)		
AC^{NS}	.84 ± .05	.88 ± .02
CC^{NS}	.76 ± .08	.85 ± .03
IC^{NS}	.73 ± .07	.77 ± .03
OT^{NS}	.99 ± .09	.89 ± .05
FX^{NS}	.66 ± .05	.69 ± .03
CTX^{NS}	.75 ± .05	.72 ± .06
Radial Diffusivity (mean ± sd)		
AC^{NS}	.23 ± .03	.26 ± .01
CC***	.22 ± .01	.29 ± .01
IC**	.24 ± .01	.32 ± .05
OT**	.19 ± .02	.25 ± .03
FX*	.27 ± .04	.32 ± .01
CTX^{NS}	.33 ± .05	.35 ± .02
T2 time in msec (mean ± sd)		
AC^{NS}	38.9 ± 5.8	39.4 ± 1.3
CC^{NS}	38.9 ± 3.7	40.5 ± 2.0
IC^{NS}	35.5 ± 3.1	38.3 ± 0.6
OT^{NS}	42.6 ± 5.6	40.1 ± 1.4
FX^{NS}	37.8 ± 2.4	40.5 ± 2.3
CTX^{NS}	40.6 ± 0.9	34.5 ± 10.6

* p ≤ .10
**p ≤ .05
***p ≤ .01

RESULTS- This study is ongoing. Preliminary analyses for 3 pairs (1 *shiverer* and 1 control matched for age and gender) show remarkably similar results to the *in vivo* work of Song et al. (2002). These are illustrated in Table 1. FA measures were significantly lower ($p \le .05$) in 3 of 5 white matter regions and marginally lower ($.05 \le p \le .1$) in the other two. Radial diffusivity measures were significantly higher ($p \le .05$) in 3 of the 5 white matter regions and marginally higher in 1 of the remaining 2. Mean axial diffusivity did not differ across groups, and no cortical DTI measures were statistically differentiated by group. There were nonsignificant trends toward higher mean T_2 times in *shiverer* mice in 5 of the 6 white matter ROIs. (The mean for the OT in the control set was driven by a signal high value for one control mouse.). However, in the two largest white matter ROIs (CC and IC) T_2 time was significantly and positively correlated with DRa in control mice (CC: p=.004, IC: p=.01) and were correlated in IC in *shiverer* (p=.04).

CONCLUSIONS- These data replicate those of Song et al. and show a relationship between developmental myelin disruption and radial diffusivity. They also extend these data by showing that diffusion tensor morphology is preserved in perfusion-fixed specimens. The data are consistent with the possibility that myelin bound water contributes to a short-TE component of the T_2 relaxation behavior in white matter, though the study is currently underpowered to demonstrate group differences in overall T_2 time in the ROIs examined. *Shiverer* may prove to be a useful model for studying myelin-based white matter abnormalities in human disease processes.