The contribution of myelin to the T2 of Corpus Collosum in Shiverer mouse

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INTRODUCTION: Injury to brain white matter (WM) often involves cytoskeletal and/or myelin structures, but the relative specificity of MRI transverse relaxation (T2) to these specific structures is limited. We employed two types of mice, Shiverer mice (Molineaux et al., 1986), exhibiting markedly reduced CNS myelin sheath formation, and HM-DKO mice (Yuan at al., 2003), exhibiting markedly reduced neurofilament content of CNS axons to examine the effect of these changes upon the T2. Immunocytochemical (ICC) and electron microscopic (EM) techniques were used to characterize the morphology behind the T2 relaxation rate within the corpus collasum (CC).

METHODS: MRI Four month old mice (10 SH mice, 10 controls (WT-SH), 6 HM-DKO mice 6 controls (WT-HM-DKO) were examined at 7 Tesla (Varian Inc.). Maps of T2 were calculated from phase cycled multislice spin echo data (200 um slices, 0.2x0.2x0.2 mm^3), processed in MEXD and fitted using a two parameter, single compartment fit (M0 and T2). Registration of images employed the ART software package. ANOVA followed by t-tests were used as indicated. ICC: NF subunits and alpha-internexin were studied using anti-alpha-internexin, SMI33 for NF-H and NF–M, anti-NF-L b-cyto-13 for NF and SMI 99 for myelin basic protein (MBP). Slides were stained with crestyl violet for light microscopic visualization. Western blot and intensities were calculated by subtracting the background optical density (OD) from the measured OD of each immunolabled protein (SMI 33, NR-4, SMI-31, SMI-99 and alpha internexin) from excised and prepared corpus callosa. EM: Myelin sheath thickness and axon caliber were measured BIOQUANT software in uniform identical regions of the CC (C2, C3 and C4) studied using EM.

RESULTS: **MRI** *Volumetric analysis* revealed reductions in whole brain (WB, $p<0.01$) and CC volume(33% reduction, $p<0.01$) in HM-DKO mice, and in CC (17% reduction, $p<0.05$) when compared to WT controls (Figure 1). *T2 relaxation rate* increased in nearly all brain regions in SH mice compared to their control WT mice (Figure 3), reaching significance is several regions, most notably CC and TM (Figure 2, $p< 0.01$). In HM-DKO mice only a modest increases in T2 in the CC (7.8% increase, $p<0.05$) was observed. **IC Results** : HM-DKO mice exhibited a nearly complete loss of NF's and a significant ($p < 0.05$) increase (40%) in MBP within the CC although no change in myelin thickness or axonal volume or numbers were observed. In SH mice, NF's and alpha-internexin were unchanged, while MBP and axonal myelin was nearly absent, while mean axonal volume and axon diameters increased. **EM Results**: Dramatic differences were visible in the ultrastructure of the CC. In SH mice myelinated axons all but disappeared while unmyelinated axons increased and occupied most of the CC volume. In SH mice, nearly 99% of axons were unmyelinated, and mean axonal diameter increased in both myelinated ($p< 0.001$) or unmyelinated axons ($P<0.001$) while in HM-DKO animals, mean axonal diameter of myelinated axons decreased ($p< 0.025$). While the relationship between axonal diameter are myelin wrap thickness was eliminated in SH mice due to the absence of MBP, loss of NF's from the cytoplasm of axons in HM-DKO mice had no effect upon the relationship.

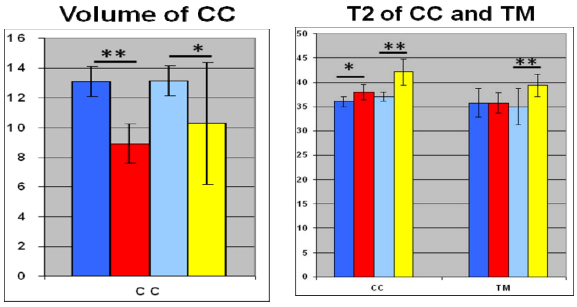

Figure 1 (left). MRI measurements in HM-DKO confirmed that volume decreased in CC ($p< 0.01$) but not elsewhere. CC volume also decreased in SH mice ($p<0.05$). **Figure 2** (right). T2 increased significantly in the CC of both SH and HM-DKO mice compared to their WT littermates.

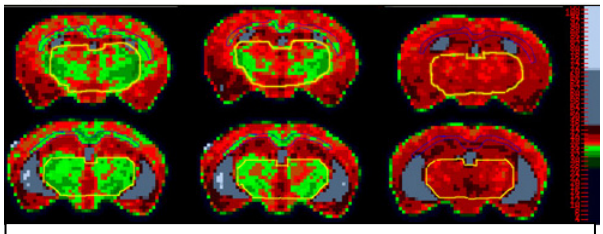

FIGURE 3. Representative Maps of T2 from WT (left), HM-DKO (middle) and SH (Right) mice. Note the substantially higher T2 across white matter and cortical regions in the SH mice.

DISCUSSION: In SH mice, T2 increased in the CC because myelin bound water was nearly absent. Thus, the intra-axonal compartment (cytoskeletal water) dominated the T2 as extra-cellular space was less than 1%. White matter within the SH mouse can therefore be represented as a single T2 compartment, rather than 3 compartments (myelin trapped, axoplasmic and extracellular water) [Lancaster et al., 2003] . In contrast, in MH-DKO mice, elimination of neurofilaments from the cytoplasm of axons only slightly elevated the T2 in CC, suggesting a minimal effect of NF's upon cytoplasm T2. These results suggest that Transgenic or knock-out models of white matter disruption, combined with multicompartment measurement of tissue relaxation (T2) can be used to improve the specificity of MRI-based tissue relaxation (T2) measurements, thereby facilitating improved specificity of the T2 assessment of white matter pathology in vivo.

References:

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