

Probing axon- and myelin-specific white matter abnormalities in schizophrenia using MRI/MRS

D. Ongur¹, F. Du¹, B. M. Cohen², A. Cooper¹, S. Lukas², and P. F. Renshaw³

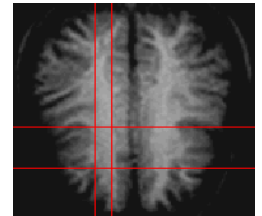
¹Psychotic Disorders Division, McLean Hospital/Harvard Medical School, Belmont, MA, United States, ²McLean Hospital/Harvard Medical School, Belmont, MA, United States, ³Psychiatry, University of Utah, Salt Lake City, UT, United States

Introduction: The pathophysiology of schizophrenia (SZ) is poorly understood but *postmortem* and diffusion tensor imaging (DTI) studies provide converging evidence for white matter (WM) abnormalities. These abnormalities may cause a "dysconnection" syndrome in which coordination of activity between brain regions is disrupted. DTI is a technique based on water molecule diffusion patterns commonly used to quantify WM abnormalities in SZ. However, because water is not restricted to a single compartment in the brain, DTI abnormalities can arise from multiple sources: altered fiber packing orientation/density in the WM, axonal diameter changes, and abnormal myelination. Therefore, DTI cannot identify the exact WM abnormalities in SZ. In this study, we employed two MRI techniques: magnetization transfer ratio (MTR), which is sensitive to myelination changes, and diffusion tensor spectroscopy (DTS), which quantifies diffusion of intracellular metabolites such as N-acetylaspartate (NAA) and is sensitive to axonal changes. We measured myelin- vs. axon-related abnormalities separately in a cohort of patients with schizophrenia and matched healthy controls. We predicted a reduction in MTR in SZ participants, consistent with reduction in WM myelin content. There is bidirectional signaling between myelinating oligodendrocytes and axons in the WM such that axonal diameters are increased when myelin is compromised. Therefore, we also predicted an elevated NAA apparent diffusion coefficient (ADC) in SZ, consistent with greater axonal diameters. Myelin loss would slow down signal transduction, while elevated axon diameter would have the opposite effect. Therefore, we cannot predict whether signal transduction would be impaired in SZ based on this information alone. The balance between amount of myelin and axon diameter is under dynamic control in the nervous system, and there appears to be an optimal balance between the two for maximal signal transduction speed called the g-ratio (defined as axon diameter/(axon diameter+myelin thickness)) (1). The optimal g-ratio in the human CNS is reported around 0.6 and deviations from this value slow conduction speed. We cannot calculate the g-ratio directly *in vivo*, but based on our predictions of MTR reduction (i.e. reduced myelin thickness) and NAA ADC elevation (i.e. larger axon diameter) we predict that the g-ratio in SZ will be higher than in healthy controls, leading to slowed signal conduction.

Because of MRS signal-to-noise considerations, we used a 9cc voxel that is approximately 1000-fold larger than those used in DTI studies. In such a large voxel, WM tracts may arc within the voxel and reduce fractional anisotropy (FA). On the other hand, NAA ADC is determined ultimately by axon diameter in the WM and is unaffected by macroscopic curvature. Therefore, we used ADC as our primary diffusion measure in stead of FA.

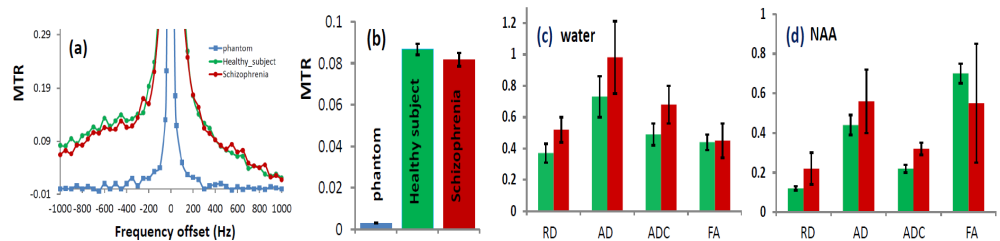
Methods: This study was approved by the McLean Hospital IRB. Healthy controls and age- and sex-matched chronically ill patients with DSM-IV schizophrenia were recruited from the clinical services of the Psychotic Disorders Division; symptom scale and medication information were recorded. Exclusion criteria for all participants: significant medical or neurological conditions, lifetime history of substance dependence or current substance abuse, age <18 or >45, left-handedness, contraindications for MR scan, structural abnormalities as determined by radiologist on routine T1-weighted scanning. On scan day, participants underwent a urine toxicology screen; women of childbearing age also underwent a urine pregnancy test.

All ¹H MRS and related T₁-weighted brain imaging were acquired by a single tuned TEM-design proton volume coil on a full-body 4T MR scanner (Varian/UnityInova, Palo Alto, CA). A single 9cc voxel (1×3×3cm) in pure WM was placed in the PFC of the right hemisphere (see adjacent Figure). A standard point-resolved spectroscopy (PRESS) sequence was modified with incorporation of diffusion gradients or a saturation pulse train for DTS and MTR measurements, respectively. For DTS, diffusion gradients with 6 directions: [1 1 0] [0 1 1] [0 1 1] [-1 0 1] [0 -1 1] with b value = 1500 s/mm² and one control [0 0 0] (totaling 7 spectra), were applied to calculate diffusion tensors of water and metabolites (TR/TE 3000/135 ms; 8 and 96 repetitions for water and metabolites, respectively). In a separate acquisition within the same scan, water MTR was obtained using a B₁-insensitive selective train to obliterate signal (BISTRO) pulse train saturating NMR signal with specific frequency offsets (2) at the beginning of PRESS sequence (prior to 90 degree pulse). This pulse train is constructed with multiple hyperbolic Sech pulses with varied RF pulse amplitudes. Saturation time (t_{sat}) was controlled by varying the cycling number of the BISTRO pulse train. Other parameters: TR/TE=3000/30ms, repetitions=2 and t_{sat}=2.6 s. Total scanner time including imaging and shimming was around 90 minutes. All MRS processing was carried out blind to diagnosis using Varian software and LCModel (3). DTS constants (axial diffusion-AD, radial diffusion-RD, mean diffusivity-ADC, fractional anisotropy-FA) were calculated using home-grown MATLAB scripts.



Results: This is an ongoing study; thus far data have been analyzed from 4 healthy controls and 4 patients. Phantom studies indicated an MTR very

close to zero, as expected. *In vivo* we observed an approximately 10% reduction of MTR in SZ, consistent with our hypotheses (see (a): profile of the frequency-offset of saturation pulse and (b): average MTR from all frequency offsets excluding data from -300 to +300 Hz around the water frequency to avoid significant RF off-resonance effects). DTS data (see (c) and (d) from healthy participants *in vivo* (green bars in the "Water" and "NAA" panels in Figure) had the expected features: ADC (×10⁻³ mm²/s) of NAA is lower than that of water (because NAA has higher molecular weight and diffuses more slowly), and NAA FA is higher than that of water (because NAA is restricted within axons). SZ data showed elevated water and NAA ADC (red bars in the "Water" and "NAA" panels in Figure) when compared with the control group, also consistent with our hypotheses. Both radial and axial diffusivity were elevated in SZ (RD and AD, respectively). As predicted in this large voxel, water and NAA FA were more variable and not clearly abnormal in the SZ group.



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Discussion: Although many previous studies have reported abnormalities in WM integrity in SZ using DTI, the biological nature of these abnormalities cannot be established using DTI alone. We have developed a combined MRI/MRS approach that examines myelination- vs. axonal-abnormalities separately in the WM in SZ. Here, we present preliminary data using this approach. Our findings are consistent with reduced myelin content in SZ accompanied by an elevation in axon diameter. This suggests that the g-ratio is abnormally high in SZ, and that signal transduction may be slowed. This abnormality is consistent with the "dysconnection" hypothesis of SZ and is likely to have functional consequences, possibly leading to symptom expression in this disorder. One caveat to this discussion is that MTR is not a specific marker of myelination and could be affected by edema or inflammation; however, these changes are not characteristic of schizophrenia. In conclusion, DTI has been a very useful tool for noninvasive assessment of WM integrity in psychiatric disorders but its utility for understanding the biology of WM in health and disease is limited. More detailed examination on the specific biological processes underlying disrupted WM integrity is possible using MRI/MRS as we have observed here. This work may provide deeper insight into the pathophysiology of SZ, and identify novel treatment targets.

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