High field T1 predicts neuronal loss in multiple sclerosis cortical grey matter

K. Schmierer[1], P-W. So[2], S. F. An[3], S. Brandner[3], D. H. Miller[1], T. A. Yousry[4], and H. G. Parkes[1]

[1]Neuroinflammation, Institute of Neurology, UCL, London, England, United Kingdom, [2]Imaging Sciences Department, Imperial College London, Hammersmith Hospital Campus, London, England, United Kingdom, [3]Neurodegenerative Disease, Institute of Neurology, UCL, London, England, United Kingdom, [4]Brain Repair & Rehabilitation, Institute of Neurology, UCL, London, England, United Kingdom

INTRODUCTION: In multiple sclerosis (MS) brain white matter (WM) magnetisation transfer ratio (MTR) and T_1 are associated with myelin content and – to a lesser degree – axonal count (1). The substrate of these MR indices in MS cortical grey matter (CGM) is less clear. In this study we investigated the association between myelin content and neuronal density (ND) with T_1, T_2, and MTR in MS CGM using high-field MRI and quantitative histology.

METHODS: *Post mortem* brain from eight women and four men with MS was used for this study. The patients' age at death was 54 years (SD 10 years), disease duration 23 years (7 years), and expanded disability status scale (EDSS) score 8 (median 8.75). Brain samples had been fixed in 10% formalin for 1090 days (SD 486 days). *Post mortem* interval was 53 hours (35 hours). Coronal brain slices were provided by the UK MS Tissue Bank, Imperial College London, and dissected at the level of the internal capsule. Tissue blocks from these slices were fitted into histology cassettes, inserted into a universal tube, immersed in perfluoropolyether, and placed in a quadrature ^{1}H volume MR coil (24mm diameter) for scanning on a 9.4T Varian Inova system. MR experiments included spin echo acquisitions with TR/TE [ms], 480-4000/16 and 20/24-60 for T_1 and T_2, respectively. Field of view was 30 x 30, matrix size 256 x 192 (2 averages). For MTR maps gradient-echo acquisitions were collected using TR/TE 186/5; FOV 30 x 30; matrix size 256 x 256 (16 averages) with and without RF saturation pulses at 6 and 100kHz offset from water resonance. T_1, T_2 and MTR maps were produced by fitting signal intensities to the appropriate equations on a pixel-by-pixel basis using ImageJ (NIH, Bethesda, USA). After scanning tissue blocks were re-immersed in formalin prior to processing for embedding in paraffin. Sections were (immuno-) stained for myelin-basic protein (myelin) and cresyl-violet (CV). Regions of interest (ROI) were identified on the histological sections alongside the MR images and categorized into healthy looking cortex (HLC) and cortical grey matter demyelination (CGML). CGML were classified according to established criteria into types I (juxtacortical), II (intracortical), III (subpial, not reaching the WM), and IV (subpial, reaching the WM GM border) (2). Myelin content was quantified by measuring transmittance on MBP stained sections (3). Values obtained were expressed as inverse transmittance (1/transmittance, iTrans). Neurons were identified on CV stained sections according to morphological criteria (4), and quantified by unbiased uniform random sampling using the stereology package of Image Pro Plus software. On average ~100 cells were counted for each ROI. The counting frame size was 75µm^2, and neuronal density was expressed as neurons/mm^2. For statistical analysis Student's paired *t*-tests and regression models were applied using SPSS 16.

RESULTS: Nineteen CGML (nine type I, four type II, five type III, one type IV) were analysed. HLC and CGML differed in T_2, MTR and ND whereas only (figure 2), (iii) MTR and myelin content (figure 3), and (iv) T_2 and ND. Marginal significance emerged for an association between iTrans and ND. Duration of fixation was strongly associated with MTR (r= 0.76; p<0.01), T_1 (r= 0.62; p<0.01), ND (r= 0.44; p,0.01) and iTrans (r= -0.55; p,0.01), however not with T_2 (r= 0.03; p=0.88).

FIGURE 1

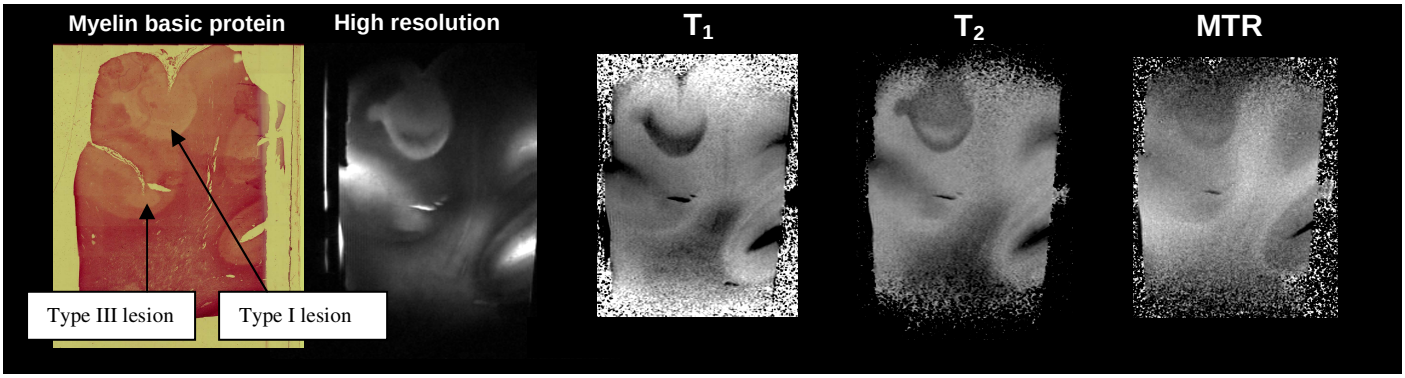


TABLE 1	NAC	(SD)	CGML	(SD)	p
T_1 [ms]	730	246	718	293	0.7
T_2 [ms]	23.8	4.9	27	5.1	<0.01
MTR [pu]	33.9	7.1	27,5	9.5	<0.01
Neurons/mm^2	139	50	115	42	<0.01
iTrans (myelin)	1.41	0.27	1.35	0.24	0.2

TABLE 2	T1	MTR	T2	iTrans (myelin)
MTR	r= 0.84			
	p<0.01			
T_2	r=0.19	r= 0.07		
	p= 0.28	p= 0.77		
iTrans (myelin)	r= 0.31	r= 0.44	r= 0.22	
	p= 0.07	p= 0.04	p= 0.22	
Neurons	r= 0.48	r= 0.3	r= 0.39	r= 0.35
	p<0.01	p= 0.17	p= 0.02	p= 0.04

FIGURE 2 Myelin (iTr) vs MTR

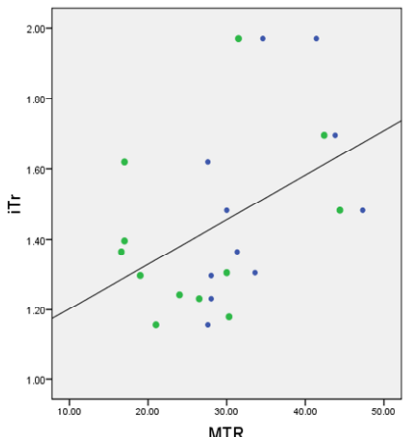
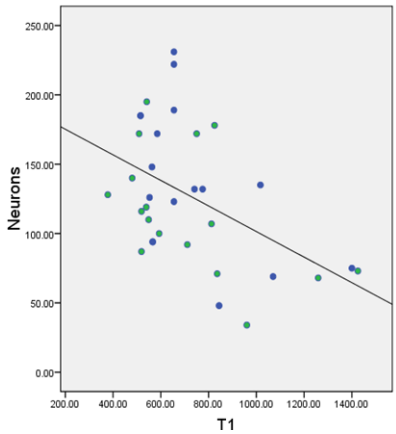


FIGURE 3 Neuronal density vs T_1



CONCLUSION Despite the fact that the cortex contains much less myelin than the white matter, MTR appears mainly dependent on myelin, at least in chronic *pos mortem* MS cortex, whereas T_1 emerged as the strongest predictor of ND. The modest association between ND and myelin content along with a loss of only ~20% o neurons in our sample of chronic MS brains (similar with ref 4) suggests that neurons and/or their environment may be better equipped than axons against th consequences of the damage MS causes. The difference compared to 50% (or more) axonal loos in MS white matter lesions remains striking. Our data further sugges that fixation time is an important confounder that needs to be taken into account in studies using fixed post mortem samples.

REFERENCES (1) Schmierer, et al. J Magn Reson Imaging 2007. (2) Bö, et al. J Neuropathol Exp Neurol 2003. (3) Schmierer, et al. Ann Neurol 2004. (4) Wegner, et al. Neurology 2006.

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