Glutamate reduces in grey matter of MS patients and correlates with cognitive impairment

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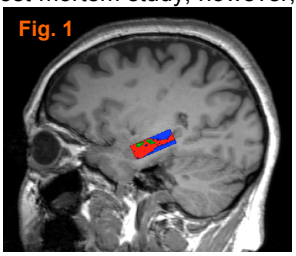
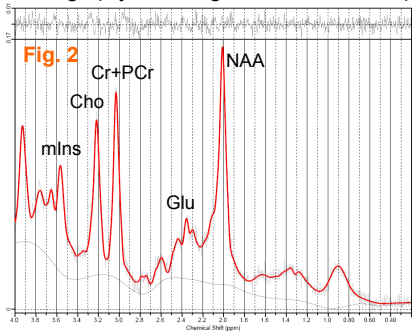
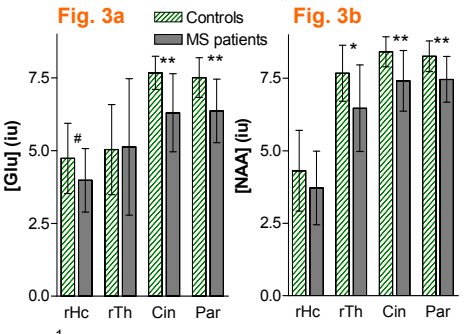
Introduction Glutamate (Glu) is an excitatory neurotransmitter generated in neurons and released into the synaptic space. A previous ^{1}H-MR spectroscopy (MRS) study in multiple sclerosis (MS) has reported elevated levels of Glu ([Glu]) *in vivo* in acute lesions and normal appearing white matter (WM) which may relate to the presence of inflammatory cells and astrocytosis [1]. A 1.5T MRS study did not detect any significant MS-related Glu changes in the cortex, hippocampus or thalamus [2]. A recent a post-mortem study, however, found a reduction in the number of glutamate receptors in demyelinated hippocampi in post-mortem MS brains [3]. We aimed to use short-TE PRESS at 3T to: (i) assess [Glu] in the grey matter (GM) of MS patients and (ii) establish whether [Glu] relates to cognitive dysfunction.

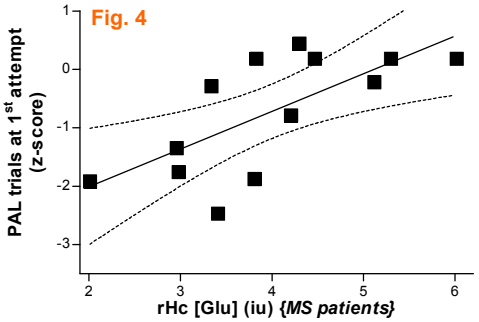


Methods. We used a Siemens 3T Tim Trio (with 32-channel head array coil receiver) to study 18 patients with relapsing-remitting (RR) MS [6 men, mean age 43.5 yrs, median EDSS 2.8] and 17 healthy control subjects [Ctr, 6 men, mean age 39.7] with written informed consent. Imaging included a sagittal 3D MPRAGE with 1x1x1mm^3 resolution. ^{1}H single-voxel MRS used PRESS, with repetition/echo time (TR/TE)=6000/30ms and CHESS water suppression. Given that the T1 of brain water and metabolites is known to vary in MS [1], we employed a relatively long TR to obtain nearly fully-relaxed spectra, with negligible sensitivity to pathological T1 variations. Volumes-of-interest (VOI) were located in right hippocampus (rHc, 7.3ml, 144 averages, avg), right thalamus (rTh, 6.7ml, 144avg) and 2 cortical regions: posterior cingulate cortex (Cin, 12ml, 48avg) and parietal cortex (Par, 15.6ml, 48avg). Concentrations of Glu and other major metabolites (total N-Acetyl-Aspartate, NAA, choline-containing compounds, Cho, creatine-plus-phosphocreatine, Cr and myo-Inositol, mIns) were obtained with LCModel 6.1 [4], scaled using the water signal (from a non-water suppressed spectrum, 2avg) as internal reference for quantification. Concentrations with LCModel standard error estimates (%SD) >20% were excluded from the analysis. The 3D T1-weighted images were lesion-filled [5], then underwent 'unified segmentation' [6] in SPM8 [7] to produce GM, WM and CSF segments; these were used to calculate the fractional tissue content of each VOI (i.e. **Fig. 1**: GM, WM and CSF in rHc VOI). With these, the LCModel output was corrected for the water signal adjusted by brain water content, T1 and T2 and relaxation times (literature values) of GM, WM, CSF. Metabolite concentrations thus corrected are reported in "institutional units" (iu). All subjects underwent a neuropsychological assessment that explored visual memory (Paired associates learning, PAL), verbal memory and speed of information processing (Symbol-digit-modalities test) alongside tests of IQ (WTAR, WASI). We assessed: (i) differences in cognitive performance and metabolite levels between patients and Ctr groups by unpaired t-test; (ii) the predictive value of patients' rHc [Glu] toward performance on visual memory, with linear regression.



Results. Patients had comparable IQ to Ctr, but performed significantly worse on visual memory test (PAL errors, p=0.01, PAL trials completed at 1st attempt, p=0.02, PAL total number of trials completed, p=0.005), verbal learning (p=0.002), delayed verbal recall (p=0.04), and processing speed (p<0.001). rHc data for 1 patient and 1 Ctr and Cin data from 1 Ctr was not acquired. LCModel estimated mean (±SD) linewidths to be 10±4Hz, 9±2Hz, 6±3Hz, 5±1Hz and SNR to be 16±6, 18±5, 42±11, 65±10 for rHc, rTha, Cin, Par respectively. A typical rHc spectrum is shown in **Fig. 2**. rHc [Glu] data was excluded for 3 Ctr and 3 patients and rTha [Glu] for 1 patient (due to SD>20%). Patients showed a trend towards lower [Glu] in the rHc (4.0 vs. 4.7 iu, p=0.1) and significant reductions in Cin (6.3 vs. 7.7 iu, p<0.001) and Par (6.4 vs 7.5 iu, p=0.001) than Ctr (**Fig. 3a**; #: p≤0.1, *: p≤0.05, **: p≤0.01). Patients also showed significantly lower NAA in rTha (6.5 vs. 7.7 iu, p<0.01) and cortical regions (**Fig. 3b**), and lower Cho, and Cr in cortical regions, than Ctr (not shown). In MS patients, [Glu] in rHc predicted visual memory function (PAL trials at 1st attempt: R^2 =0.48, p=0.008, (**Fig. 4**; regression line and 95% confidence interval shown); PAL total trials completed: R^2=0.30, p=0.051).





Discussion and Conclusions. The optimal method for brain Glu quantification is still debated, with some groups advocating TE-averaged PRESS [1,8]. We demonstrated that reliable estimation of [Glu] can be achieved at 3T using a widely available short-TE PRESS MRS protocol in cortical and subcortical GM regions. This data suggests that RRMS patients show reduced [Glu] in GM. This reduction is associated with poorer cognitive performance, with reduced [Glu] in the rHc associated with worse visual memory. Reduced levels of Glu, NAA, Cho and Cr in cortical GM of MS patients, together with normal mIns concentrations, are in agreement with post-mortem findings of GM neuronal loss, modest glial proliferation, low degree of inflammation and impaired energy metabolism. MRS of cortical glutamate may provide a surrogate marker for assessing the efficacy of future therapies in reducing memory decline in people with MS.

Acknowledgments: Dr Mark White for setting up the software to calculate the MRS VOI position on 3D-T1-weighted datasets. The Wellcome Trust and the Multiple Sclerosis Society of Great Britain and Northern Ireland for funding. ***References:*** [1] Srinivasan R, *Brain* 2005; 128:1016 [2] Geurts JJ, *MRM* 2006; 55:478 [3] Dutta R, *Annals of Neurology* 201; 69:445. [4] Provencher S, *MRM* 1993; 30:672 [5] Chard DT, *JMRI* 2010; 32:223 [6] Ashburner J, *Neuroimage* 2005; 26:839 [7] www.fil.ion.ucl.ac.uk/spm/software/spm8 [8] Wijtenburg SA, *JMRI* 2011, doi: 10.1002/jmri22638.