

Hippocampal magnetization transfer ratio and not hippocampal atrophy best explains memory dysfunction in patients with multiple sclerosis

Mishkin Derakhshan¹, Gabriel Leonard¹, Daviad Araujo¹, D. Louis Collins¹, Douglas L Arnold¹, and Sridar Narayanan¹
¹Montreal Neurological Institute, McGill University, Montreal, QC, Canada

Background: Postmortem immunohistochemical studies have shown that hippocampal (HC) demyelination is frequent and extensive in patients with multiple sclerosis (MS) and that, while there is a significant decrease in synaptic density, the demyelinated HC have minimal neuronal loss [1, 2]. Recent MRI studies have correlated the number of HC lesions [3], as well as HC atrophy [4], with memory dysfunction (i.e., verbal and visuospatial learning/immediate memory) in patients with MS.

Purpose: To the best of our knowledge, no studies have looked at the relationship between MRI correlates of HC demyelination and memory dysfunction in patients with MS. Thus, we proposed to (i) confirm the postmortem findings of HC demyelination in vivo using measures of magnetization transfer ratio (MTR), a marker of demyelination, and (ii) investigate the relationship of both HC demyelination and neuronal loss (atrophy) with memory dysfunction in patients with MS.

Methods: Subjects: A total of 26 patients with clinically definite MS were recruited from the MS clinic at the Montreal Neurological Hospital. To be included in the study, patients had to display some degree of cognitive disability as indicated by a score of one or greater on the cerebral functional system subscale of Kurtzke's Expanded Disability Status Scale (EDSS). Fifteen healthy volunteers were recruited based on age, gender, and education level to serve as the normal control (NC) group.

Image Acquisition: All participants were scanned on a 3T MAGNETOM Trio TIM scanner (Siemens, Erlangen, Germany). A 3D fast low-angle shot (FLASH) gradient-recalled echo (GRE) sequence [TR=20ms, TE=5ms, FA=27, 256x256 matrix, 256mm field of view, 1mm slice thickness, 1 signal average, voxel size= 1x1x1mm³] provided 1mm isotropic T1-weighted (T1w) anatomical MRI. A pair of 3D FLASH sequences [TR=33ms, TE=3.81ms, FA=10], with geometry matched to that of the high-resolution T1w sequence, was acquired with and without an off-resonance magnetization transfer pulse [Gaussian pulse, offset frequency=1,200Hz, FA=500°, pulse length=9,984µs, bandwidth=192Hz]; MTR maps were created from these images after co-registration as voxel-wise percent difference maps.

Image Processing: Automated HC labels created using the label fusion-based template library scheme [5] were manually corrected as needed by a single trained neuroradiologist (DA). Cortical grey matter (cGM) maps were obtained via SIENAX [7]. All volumes were normalized using the same normalization factor obtained from SIENAX. The mean MTR values inside the HC and cGM labels were calculated.

Neuropsychological testing: All participants completed a detailed neuropsychological assessment within an average of nine days of their MR examination (median = two days). Included in the battery was the Wechsler Memory Scale (third edition; WMS-III), which provides indices of immediate, general/delayed, and working memory. Since HC pathology has a reported effect on immediate memory, we also included visual and auditory immediate subindices that make up the immediate memory index in the analysis.

Statistics: Differences between NC and MS groups for the four MR metrics and five memory indices were calculated with a one-tailed t-test and are accompanied by Cohen's *d* as a measure of effect size. Univariate analysis of both HC volumes and HC MTR values was performed with each of the five memory indices examined. To assess the independent contributions of each, partial *r*² values were obtained for each component using a multivariate analysis. Further stepwise linear regression models were explored in an attempt to identify the best imaging correlate of memory impairment. Bonferroni multiple-comparison correction was applied where appropriate.

Results: Left and right HC volumes and MTR values were combined as no significant differences in lateralization were found (*F*=1.04, *p*=.31, and *F*=2.51, *p*=.12, respectively). With the exception of the auditory immediate subindex, medium to large effect sizes (0.61 < Cohen's *d* < 1.11) were observed for all MR and memory metrics between NC and MS groups. After correction for multiple comparisons, the only differences that remained significant were in the normalized cGM volume (*p*=.03) and the visual immediate memory subindex (*p*<.01).

Univariate correlations revealed a relationship between HC MTR and working memory (*r*²=.22, *p*=.04) as well as between visual immediate memory and both HC MTR (*r*²=.32, *p*<.01) and HC volume (*r*²=.29, *p*<.01). To assess whether the relationships truly reflected localized HC damage and not global damage, cGM MTR and volume were used as covariates. We found that the independent contributions for HC volume disappeared entirely, whereas localized HC MTR still predicted working memory performance (partial *r*²=.23, *p*=.04). Two approaches were used to assess the independent contributions of HC MTR and HC volume to the relationships with the memory findings. In the first analysis, each was assessed independently in a model where it was found that both HC MTR and volume contribute independently to visual immediate memory performance (partial *r*²=.22, *p*=.04, and partial *r*²=.23, *p*=.04, respectively). Though not significant after Bonferroni correction, there was still a trend for the independent contribution of HC MTR to working memory performance (partial *r*²=.19, *p*=.08). The second analysis used stepwise linear regression to predict the best models for each memory index. We found that working memory was best explained with HC MTR and normalized cGM volume; delayed memory with HC MTR; immediate memory with HC MTR and HC volume; auditory immediate memory with HC MTR only; and visual immediate memory with HC MTR and normalized cGM and HC volumes. In all cases, HC MTR was the first parameter to enter the model, thus accounting for the most variance, and always remained in the final model.

Discussion and Conclusions: Our data corroborate the postmortem findings of HC involvement in patients with MS and extend these by showing that the pathology most relevant for negative cognitive effects is driven by demyelination (as measured with MTR) rather than neuronal loss (as measured with HC volume). We found that working memory and, to a lesser extent, visual immediate memory are linked to HC MTR and that whatever contribution HC volume has to memory performance is the result of global atrophy and not HC specific volume loss. We found that visuospatial memory is affected by HC pathology, which supports previous findings [3], but that auditory immediate memory was preserved, in contrast to other studies [4, 8]. These results highlight the importance of including measures related to demyelination as well as atrophy for assessing cognitive dysfunction in patients with MS.

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