Deep Gray Matter T2 Hypointensity Correlates with Disability in a Murine Model of MS

I. Pirko[1], A. J. Johnson[2], A. K. Lohrey[2], J. Ying[3], D. Lindquist[4], and R. S. Dunn[4]

[1]Department of Neurology, University of Cincinnati, Cincinnati, OH, United States, [2]Department of Neurology, University of Cincinnati, United States, [3]Department of Biostatistics and Epidemiology, University of Cincinnati, United States, [4]Imaging Research Center, Cincinnati Childrens Hospital Medical Center, Cincinnati, OH, United States

Objective: To determine if the development of disability in a TMEV induced murine MS model is accompanied by deep gray matter T2 hypointensity.

Background: The hallmark of MS is the white matter lesion, which represents an area of inflammatory demyelination. In addition to white matter lesions, gray matter is also known to be affected in MS, including lesional[1] and non-lesional pathology[2]. Most non-lesional features of MS were discovered using advanced MRI methods. Many serve as important paraclinical markers, having strong associations with clinical outcome measures[3]. T2 hypointensity (T2H) in deep gray nuclei[4,5] have been linked with ambulatory, cognitive dysfunction and brain atrophy[6]. These areas are thought to represent iron deposition, although histology correlation data is limited[7]. Animal models of MS-related T2H would allow for mechanistic investigations of its pathogenesis. However, the presence of T2H has never been demonstrated in MS models, although iron deposition has been reported[8]. In this study, we demonstrate that T2H of the thalamus is present in a Theiler's Murine Encephalitis Virus (TMEV) induced murine model of MS, and it correlates well with rotarod detectable disability.

Design and Methods: The experiments were approved by the Institutional Animal Care and Use Committee. 8 TMEV infected and 6 age matched control SJL/J mice were studied. TMEV infection in susceptible strains is followed by a demyelinating disease, and serves as an MS model[9]. ***MRI scans*** were performed at 1, 4, 6 and 12 months using a Bruker Biospec 300 MHz horizontal bore system (Bruker Biospin, Billercia, MA) equipped with custom-built coils. A respiratory gated T2 weighted volume acquisition RARE sequence was used (TR: 1500ms, TE: 70ms, RARE factor: 16, FOV: 3. 20x1. 92x1. 92cm, matrix: 256x128x128). ***Disability*** was monitored using the rotarod assay (Columbus Instruments, Columbus, OH): mice are trained to march on a constantly accelerating rotating rod. Eventually, the animals are no longer able to keep up with the rod and fall. The time spent on the rod serves as a quantitative disability measure. ***MRI post-processing:*** 3D images were co-registered to a base image using the 3D Voxel Registration module in Analyze 8. 1 (Mayo Clinic BIR, Rochester, MN[10]). The ROI Tool was used to extract a specific coronal slice. To measure intensity, a standard ROI was placed in the medio-dorsal thalamus (Fig. 1). Intensity from an identical sized ROI placed in CSF was also taken for each subject for normalization. The adjusted intensity (medio-dorsal nucleus/CSF intensity ratio) was used as the MRI outcome measure (referred to as MEAS/CS). ***Statistical analysis:*** Mixed effect models were used to assess associations of numerical measures to time and treatment, using a random effect (individual mice) to account for within subject correlation caused by repeated observations. *Post hoc* comparisons of means longitudinally between months in each group and cross sectionally between groups at each month were performed under the mixed effect model framework and adjusted for type 1 error using Tukey's method in SAS 9.1 (SAS, Cary, NC).

Results Mice in the experimental group showed progressive decline of thalamic T2H after month 1, while controls showed no significant change over the time. The rotarod score was decreased in cases after month 4 ($p<0.05$) consistent with the animals' worsening disability (Table 1 and Fig.2). The intensity measurements showed a strong association with the rotarod score among infected mice, indicating a link between T2H and disability (Fig. 2).

Discussion: Our results clearly establish that deep gray matter T2H is present in this MS model, and that T2H shows a strong correlation with disability. The model will allow us to determine the main contributors to T2H formation. We will investigate the temporal characteristics of T2H in all deep gray nuclei affected in human MS (caudate, putamen, red and dentate nuclei [5,11]). We will also investigate additional TMEV susceptible mouse strains for inter-strain differences, which would enable us to define genetic determinants of T2H. Since our model is viral induced, we will also determine the contributions of viral load. While early stage TMEV involves neuronal infection (also in the deep gray matter), in the late demyelinating stage (incl. the studied time points) neurons are no longer infected. A limitation of the current study is that spin echo-based sequences were used, whereas gradient echo is more sensitive to susceptibility effects. In future extensions, both sequences will be utilized and compared. We propose that our newly established T2 hypointensity model will serve as a fertile ground for future research into the role and significance of this novel MS-related MRI finding.

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	Thalamic intensity			Rotarod score		
	E†	C†	p (E-C)‡	E†	C†	p (E-C)‡
1	0.32 ± 0.01	0.32 ± 0.02	0.956	99.05 ± 9.95	101.23 ± 14.10	0.901
4	0.25 ± 0.01 ##	0.32 ± 0.01	<0.001	31.90 ± 9.95 ##	113.46 ± 11.51	<0.001
6	0.23 ± 0.01 ##	0.30 ± 0.01	<0.001	13.71 ± 10.61 ##	151.23 ± 12.59 #&	<0.001
12	0.20 ± 0.01 ##&	0.27 ± 0.02	<0.001	2.96 ± 9.92 ##&	126.29 ± 14.04	<0.001

Table 1. Summary of rotarod and MRI findings by month. †: mean ± standard error (or SE); E = experimental C = control group. ‡: p<0. 05; "#" and "##" indicate the mean of month 4, or 6 or 12 is different from that of month 1, with p<0. 05 and 0. 01; and a "&" indicates the mean of month 6 or 12 is different from that of month 4, with p<0. 05.

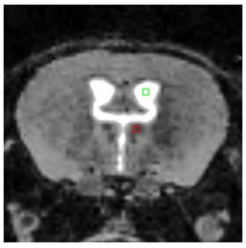

Fig. 1. Measurement technique. Representative coronal slice extracted from co-registered 3D datasets. Green frame: ROI for CSF intensity measurement; red frame: ROI for medio-dorsal thalamus intensity measurement in Analyze.

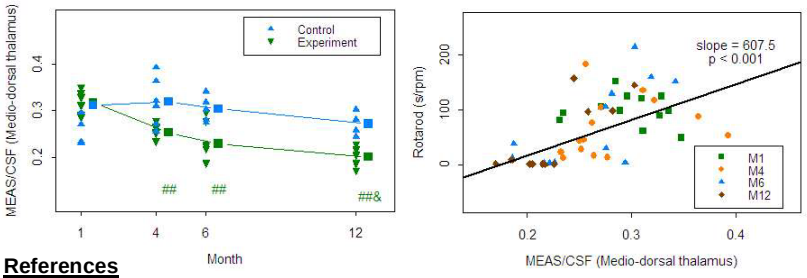

Fig.2. Results. **Left panel:** CSF normalized thalamic intensity ratios show a steady decline in cases (green) but not in controls (blue). Triangles represent actual measurements, squares are mean values.
Right panel: the slope of 607.5 and p<0.001 suggest a strong association between rotarod score and hypointensity.

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