BRAIN UPDATE

Deep grey matter injury in multiple sclerosis: a NAIMS consensus statement

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Although multiple sclerosis has traditionally been considered a white matter disease, extensive research documents the presence and importance of grey matter injury including cortical and deep regions. The deep grey matter exhibits a broad range of pathology and is uniquely suited to study the mechanisms and clinical relevance of tissue injury in multiple sclerosis using magnetic resonance techniques. Deep grey matter injury has been associated with clinical and cognitive disability.

Recently, MRI characterization of deep grey matter properties, such as thalamic volume, have been tested as potential clinical trial end points associated with neurodegenerative aspects of multiple sclerosis. Given this emerging area of interest and its potential clinical trial relevance, the North American Imaging in Multiple Sclerosis (NAIMS) Cooperative held a workshop and reached consensus on imaging topics related to deep grey matter.

Herein, we review current knowledge regarding deep grey matter injury in multiple sclerosis from an imaging perspective, including insights from histopathology, image acquisition and post-processing for deep grey matter. We discuss the clinical relevance of deep grey matter injury and specific regions of interest within the deep grey matter. We highlight unanswered questions and propose future directions, with the aim of focusing research priorities towards better methods, analysis, and interpretation of results.

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Received October 27, 2020. Revised January 28, 2021. Accepted February 1, 2021. Advance access publication 23 March 2021

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Keywords: multiple sclerosis; deep grey matter; thalamus; atrophy; MRI

Abbreviations: DGM = deep grey matter; MR = magnetic resonance

Introduction

Multiple sclerosis has traditionally been viewed as a white matter disease, with focal lesions resulting in neuronal injury and tissue destruction.[1,2] However, detailed neuropathological examination also reveals extensive pathology in cortical and deep grey matter (DGM).[3] While the mechanisms of DGM injury vary across structures, the net effect of this pathology can be examined *in vivo* using MRI. Volume loss/atrophy of the thalamus and other DGM structures has been well-documented in multiple sclerosis using MRI and is clinically relevant.[4–9] The precise mechanisms of DGM loss remain to be fully elucidated and likely represent a complex interplay between the various aspects of pathology in multiple sclerosis.

Conventional MRI can be used to measure DGM volume and lesions. However, DGM lesions are best visualized at 7 T,[10,11] are more difficult to visualize at conventional field strengths, and have even been considered a red flag for a diagnosis of multiple sclerosis.[12] Advanced techniques, such as functional MRI (fMRI), diffusion tensor MRI, relaxometry and magnetic resonance (MR) spectroscopy, can quantify additional changes in DGM structures.[13–15] With recent advances in imaging, there is an opportunity to satisfy an unmet need to fully characterize DGM injury in multiple sclerosis and develop measures for use in clinical care and research. A better understanding of the mechanisms of DGM injury will provide fundamental knowledge about the pathophysiology of multiple sclerosis and may enable more efficient clinical trial design for neuroprotective therapies.

Materials and methods

The North American Imaging in Multiple Sclerosis Cooperative (NAIMS) initially met to discuss DGM injury in San Diego, California in February 2018. Topics included: pathological mechanisms of DGM injury in multiple sclerosis, measurement of DGM volume, clinical relevance and regions of interest, and DGM metrics as clinical trial outcomes. Consensus points were drafted after completion of each topic presentations with input from all attendees. Consensus opinions were presented to the group at the completion of the meeting, and these were further refined by meeting presenters during teleconferences conducted after the meeting. Finally, all authors approved the final version of the manuscript. The goals of this paper are to summarize the proceedings, update and review the current understanding of DGM imaging with a focus on lesions and atrophy based on conventional MRI sequences, and provide recommendations for future research.

Pathological mechanisms of deep grey matter injury in multiple sclerosis

Deep grey matter lesions

Focal DGM lesions have been well-documented in multiple sclerosis and differ quantitatively and qualitatively from white matter lesions.[5] Focal lesions occur in all DGM nuclei but are more common in the caudate, thalamus, and hypothalamus (Table 1).[16] DGM lesions are characterized by demyelination with varying degrees of inflammatory changes.[16] Similar to white matter lesions, DGM lesions can be classified into 'active', 'chronic active', and 'chronic inactive'.[16,33,34] Active DGM lesions show increased perivascular cuffs and lymphocytic infiltrates.[16] Chronic active lesions are characterized by a rim of activated microglia/macrophages, and are common in DGM.[16] When compared to white matter lesions (more inflammation) and cortical lesions (less inflammation), DGM lesions have an intermediate inflammatory pattern.[16]

The most extensively studied DGM structure in multiple sclerosis is the thalamus. Focal thalamic lesions can be identified in over two-thirds of multiple sclerosis cases using microscopy and high-field MRI.[10,11] Pathologically, focal thalamic lesions exhibit

Table 1 Histopathological and MRI findings in DGM and related grey matter regions in multiple sclerosis

DGM Structure	Histopathological findings						MRI findings		
	Lesions	Normal-appearing tissue							
	Focal lesions present[16–18]	Diffuse inflammatory infiltrate[16,17]	Activated microglia[16–18]	Iron deposition[16,*]	Neuronal loss[16,17]	Markers of oxidative stress[16]	Early volume loss[19–21]	DGM lesions MRI-visible[22–24]	Iron accumulation[25–27]
Thalamus[16–20,22]	+ + +	+	+	+	+	+	+ + +	+ + +	+
Caudate[16,17,20,23]	+ + +	+	+	+	+	+	+ +	+	+
Putamen[16,17,19,20,23]	+ +	+	+	+	+	+	+ + +	+	+
Globus pallidus[16,17]	+ +	+	+	+	+	+	NA	NA	+
Substantia nigra[17]	+	+	NA	NA	NA	NA	NA	NA	NA
Amygdala[17]	+	+	NA	NA	NA	NA	NA	NA	NA
Hypothalamus[17,28]	+ + +	+	+	NA	+	NA	NA	NA	NA
Hippocampus[29–32]	+ + +	NA	+	NA	+	NA	+	+ + +	NA

NA = not available.
*Did not reach statistical significance.

neuronal loss,[17] demyelination, and axonal transection.[35,36] MRI and histopathological studies have identified two thalamic lesion types: perivascular lesions, which are typically ovoid in shape, and subependymal lesions, which are thin bands of demyelination that line the third ventricle[18] (Fig. 1). The presence of ovoid, perivascular, thalamic lesions shows conflicting results regarding correlation to cortical lesions.[10,11] The appearance of subependymal thalamic lesions resembles that of subpial cortical lesions, and close proximity to the ventricle suggest a soluble or diffusible factor present in the CSF, perhaps with a role of inflammatory cells.[37,38] The exact reasons for the variability in lesion frequency across DGM structures is unknown, but may relate to myelin content, contact with CSF spaces, and/or vascular distribution.

Non-lesional pathology in deep grey matter

DGM volume loss occurs early in multiple sclerosis and precedes measurable whole brain volume loss.[8,19,39–41] The histopathological changes observed in non-lesional DGM may help explain this early susceptibility. Neuronal density in normal appearing DGM is reduced by as much as 33%[17] compared to controls and could be explained by several mechanisms. Retrograde and anterograde degeneration due to the focal axonal transection that occurs within white matter lesions are probably major mechanisms of DGM neuronal loss.[42–46] Trans-synaptic degeneration, which has been well-documented with hippocampal demyelination but has also been described in the visual system (which runs through the thalamus),[47,48] may also play a role. Finally, deafferentation-induced neuronal loss, as described in the cortex, may also occur in DGM.[49] The cascade of white matter pathology resulting in axonal, neuronal, and synaptic changes suggests that early neurodegeneration in DGM may be a secondary phenomenon; however, a primary mechanism cannot be excluded (Fig. 2).

Iron accumulation is a proposed mechanism for DGM injury. DGM contains high iron content, which increases with age in controls.[50] In multiple sclerosis DGM, iron accumulation has been demonstrated better *in vivo* using iron-sensitive MRI sequences[51] than on pathology.[16] Iron accumulation may lead to oxidative stress, cellular damage, and neuronal injury.[52] Iron release from injured oligodendrocytes results in higher turnover to microglia, which may be heightened in DGM structures and may drive more chronic inflammatory responses.[43] Susceptibility measures from the thalamus are associated with disability[53] and may differentiate relapsing remitting multiple sclerosis from primary progressive multiple sclerosis.[54] Increased iron concentration may be the result of atrophy in the setting of a fixed iron content.[55] In thalamus, some studies have shown increased T_2^*-weighted signal, which may represent loss of iron[27,56] but may also be explained by loss of myelin or inflammation. Overall, the exact role of iron in thalamus is not clear and quantitative susceptibility mapping (QSM) may help resolve these questions. QSM has already been explored in DGM in multiple sclerosis.[43,57,58]

Future directions

Larger and more detailed pathologic studies are needed to elucidate the exact cellular and subcellular substrates of DGM tissue loss in multiple sclerosis. Single cell genomics to further characterize DGM cell types may shed further light on changes secondary to afferent/efferent tracts with lesions or selective vulnerability of various thalamic subregions.

Measurement of deep grey matter injury

MRI acquisition

Recent advances in pulse sequences, increased magnetic field strength, and post-processing algorithms have improved the accuracy of MRI-based DGM volume measurements.[59] Several MRI contrasts can be used to identify DGM structures (Table 2), including T_1-weighted imaging,[60] T_2-weighted imaging,[62] diffusion-weighted imaging,[70] magnetization transfer imaging,[71,73] R_2^* relaxation, and T_2^*-weighted imaging/QSM.[11,56–58] Gadolinium accumulation may preferentially affect DGM structures[74,75] and may affect analysis of pixel intensities on T_1/T_2-weighted images and values of T_2^*, R_2^*, quantitative susceptibility mapping.

MRI analysis

DGM segmentation methods can be either atlas-based, algorithm-based, learning-based, or hybrid.[76] Atlas-based approaches generated using histology or MRI have been the most widely used and include the creation of anatomical labels, which are non-linearly warped to new data.[77] Atlases from several MR contrasts with specific sensitivity to different DGM structures are available. Atlas-based packages using probabilistic algorithms for segmentation of DGM structures are widely used and include FMRIB's

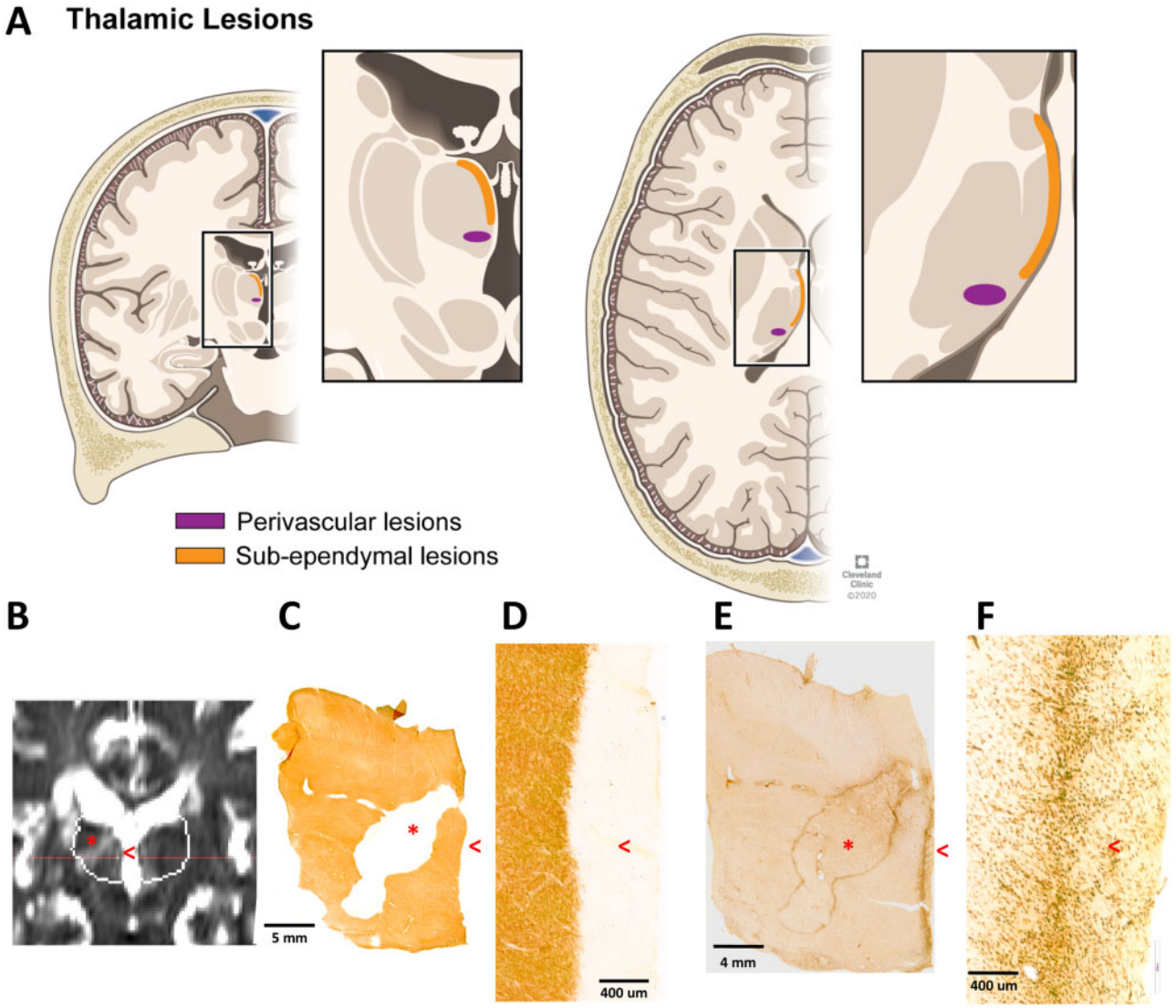

Figure 1 Demonstration of thalamic demyelinating lesion types. (**A**) Thalamic lesions are represented in coronal (*left*) and axial (*right*) planes. Lesions can either border the lateral and third ventricles (termed subependymal lesions, in orange) or have an ovoid appearance around blood vessels (termed perivascular, in purple). (**B**) Thalamic lesions (perivascular labelled with an asterisk and subependymal with a red open arrowhead) on T_2-weighted coronal 3 T MRI from a post-mortem multiple sclerosis case, and matching histological images from the same case (**C**–**F**), highlighting that subependymal lesions are more difficult to visualize. Myelin proteolipid protein immunohistochemistry demonstrates demyelination in perivascular and subependymal lesions (low magnification in **C**; higher magnification of subependymal lesion in **D**). Activated microglia/macrophages (MHC class II) immunohistochemistry demonstrating a chronic-active perivascular lesion and a rim at the border of the subependymal lesion (low magnification in **E**, higher magnification in **F**). Panel **A** is reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2020. All Rights Reserved.

Integrated Registration and Segmentation Tool (FIRST),[78] FreeSurfer,[79] and statistical parametric mapping (SPM).[80] These are primarily based on T_1-weighted images, but can be improved by multiple contrasts and MR modalities used simultaneously.[58] Segmentation of lesional multiple sclerosis tissue is an important step to avoid misclassification of gray matter.[81] Integrated probabilistic methods with simultaneous lesion masking are available.[82]

Several comparisons of FSL-FIRST, SPM, and FreeSurfer have been conducted. FSL-FIRST shows the highest correlations with cognitive measures.[83] A recent systematic review compared several automated segmentation techniques[76] in multiple sclerosis and showed that learning-based approaches achieved the highest DGM segmentation accuracy. Multi-atlas approaches and use of additional MRI contrasts (QSM, T_2^*, R_2^*) improved results. These techniques can be used in learning-based approaches for training datasets to establish improved measures of ground truth.

Segmentation of thalamic subregions has been achieved using ultra-high field MRI in combination with multicontrast atlases. Data thus far suggest that certain subregions may show greater volume loss, including the antero-ventral, pulvinar, and habenular regions.[61] Using probabilistic connectivity at 3 T, differences in thalamic subregions have been demonstrated between multiple sclerosis and neuromyelitis optica,[84] with subregion 5 (premotor connection) mean diffusivity being the best discriminator. Selective vulnerability of thalamic subregions may relate to lesion location, susceptibility of specific pathways, and neuronal factors. Lesions in projecting/receiving tracts may be preferentially affected,[46] as well as neurons located closer to the ventricular surface.[85]

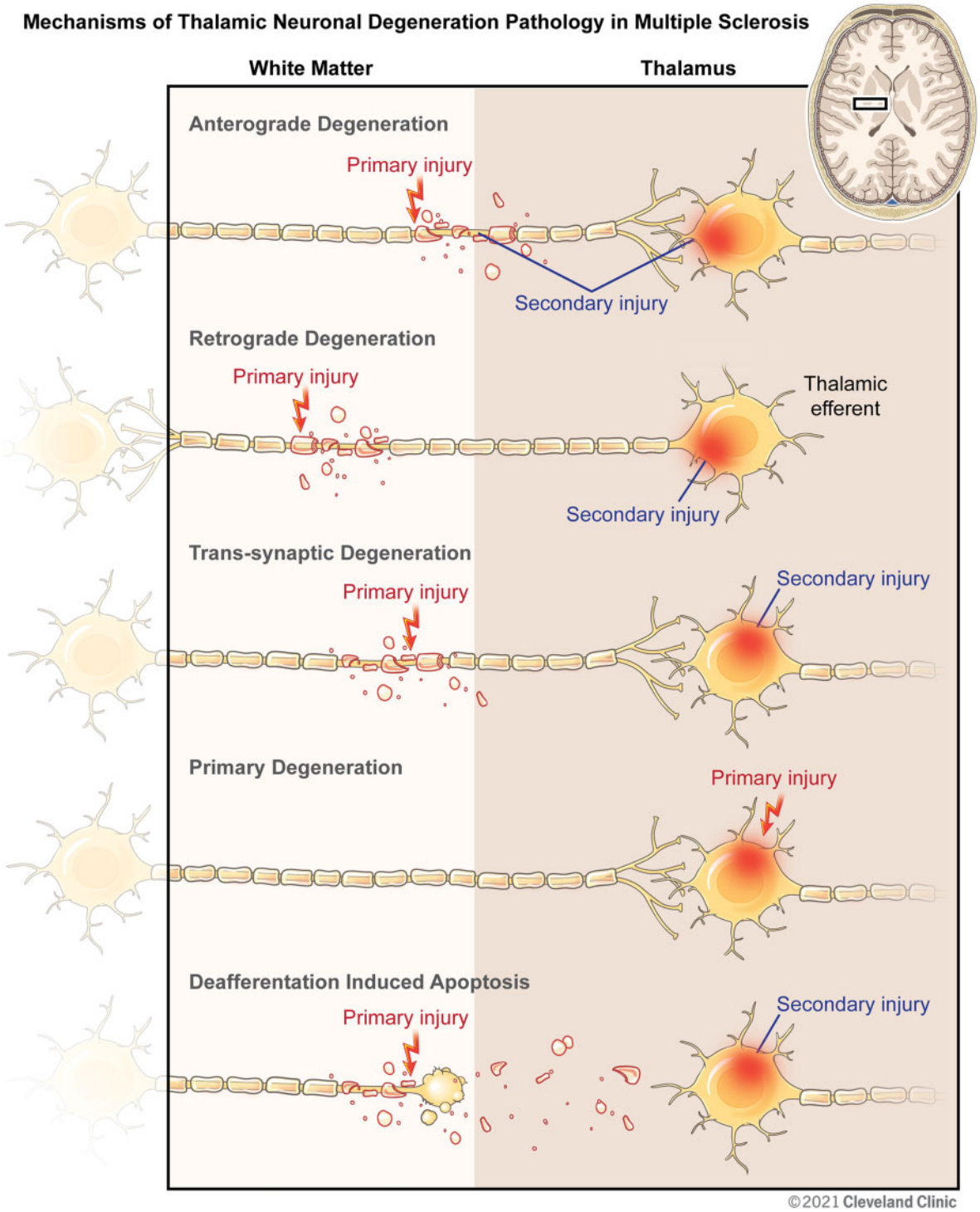

Figure 2 Mechanisms of thalamic neuronal degeneration pathology in multiple sclerosis. The figure shows representations of afferent/efferent neuronal cell bodies and axons in the thalamus and outflow/inflow tracts. Under 'Anterograde Degeneration', a focal white matter lesion in a thalamic afferent with secondary anterograde degeneration in the axon is depicted. Under 'Retrograde Degeneration', a focal white matter lesion in a thalamic efferent is depicted. Under 'Trans-synaptic Degeneration', secondary injury in the neuron as a result of degeneration of the axon with which it forms a synapse is demonstrated. Under 'Primary Neurodegeneration', neuronal injury independent of a direct connection to an axon is illustrated. Under 'Deafferentation Induced Apoptosis' we illustrate loss of neurons resulting as a consequence of axonal transection. Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2020. All Rights Reserved.

Machine learning approaches

Machine learning may offer several distinct advantages over current analysis and processing techniques. Machine learning and deep learning can incorporate several MRI contrasts and measures to more efficiently explore tissue composition, structure, and function. When presented with labelled training data, machine learning algorithms can identify complex patterns from large datasets with a high number of variables, make generalizations from these learned patterns, rank the importance of

Table 2 MRI contrasts in DGM

Contrast	Utility	Acquisition comments
T_1-weighted[10,60,61]	Anatomical identification of boundaries and segmentation for volumetric assessments; potentially useful for lesion identification	High resolution images can be obtained with relatively short acquisition time; substantial experience in multicentre applications; thalamus may be difficult to segment due to high myelin content
T_2-weighted[62] or T_2-FLAIR	Identification of focal demyelinating lesions	Quick acquisition; substantial experience in multicentre applications
T_1 relaxation[63,64]	Intrinsic changes in T_2 signal, some sensitivity to iron Quantitative measure, can differentiate different DGM structures and subregions	Requires multiple acquisitions hence multicentre applications have been limited, but newer single-sequences approaches (e.g. MP2RAGE) may alleviate these drawbacks
T_2 relaxation[64,65]	Quantitative measure, has some sensitivity to iron content in DGM, short component is particularly sensitive to myelin	Time consuming, with limited success in multicentre applications
T_2*[11,56,66,67]	Sensitive to iron and myelin content and blood vessels in DGM, also ideal to identify boundaries of iron-containing deep grey matter structures	High resolution can be attained with relatively quick acquisition times; multicentre application is relatively straightforward; less useful at 1.5 T
Double inversion recovery[68]	Sensitive to detection of DGM lesions	Feasible, and may also allow identification of few DGM lesions but sensitivity unknown
Quantitative susceptibility mapping[69]	Sensitive to iron, myelin and blood vessels; can differentiate diamagnetic and paramagnetic changes	Feasible, but post-processing required; less useful at 1.5 T
Diffusion weighted imaging[70]	Useful sensitive to microstructural	Typically lower resolution than other sequences; acquisition times are long for more complex biophysical models, which are not routinely available on commercial scanners; limited success in multicentre studies
Magnetization transfer imaging[71,72]	Sensitive to deep grey matter demyelination and useful for thalamic segmentation	Feasible clinical acquisition times; needs careful normalization for multicentre application

variables, and then use this information to make predictions on new data.

From an image-processing standpoint, deep learning may improve the accuracy of image segmentation in multiple sclerosis,[86,87] and importantly, can reduce image heterogeneity across studies. Deep learning methods can normalize image intensity, resolution, and contrast across time points and across subjects.[88] Supervised machine learning methods using random forest classification of volume, thickness, surface area of cortical grey matter regions, and volumes of DGM nuclei, distinguished relapsing remitting multiple sclerosis from neuromyelitis optica with an accuracy of 74%.[89] Further development of supervised machine learning and unsupervised deep learning approaches may improve analysis of DGM structures to identify optimal regions of interest.

Future directions

Significant progress has been made in DGM segmentation. Use of multimodal approaches, multi-atlas approaches, subregional approaches, and machine learning may significantly improve DGM segmentation and its utility. Pulse sequences that generate quantitative data should take into consideration the local contrast generated for each specific structure and the resolution needed. Creation of data repositories and imaging toolboxes as shared resources will advance discovery. To enhance real-world utility, segmentation would ideally use pulse sequences that are widely available or postprocessing algorithms that are robust to different input sequences. Finally, the long-term goal of these measures is clinical translation, which requires multicentre validation and determination of clinical meaningfulness.

Clinical relevance of deep grey matter injury

Thalamus

Thalamic volume loss is observed on MRI in the earliest identifiable phases of multiple sclerosis, including pediatric multiple sclerosis,[90,91] clinically isolated syndrome (CIS),[40,41] and radiologically isolated syndrome,[19] whereas whole brain volume and total grey matter volume may still be preserved. Thalamic volume declines consistently as a function of disease[7] and, correlates with clinical end points, including cognition,[6,7,92,93] and provides feasible sample sizes as a primary end point for clinical trials.[7] Thalamic volume was shown to be modifiable in several recent randomized, placebo-controlled trials (see below). Given its high sensitivity in early disease and correlation with cognition, thalamic volume may be particularly useful in studies targeting cognition, or younger patients early in the disease.

Impact of normal ageing on deep grey matter measurements

Limited data exist regarding the impact of normal ageing on grey matter structures in multiple sclerosis. Whole and regional brain volumes can follow a linear or non-linear trajectory of decline,[94–96] suggesting that the contribution of normal ageing to brain atrophy in multiple sclerosis may not be constant.

In a recent analysis, normal ageing was shown to contribute to whole brain and thalamic atrophy, whereas it did not in the putamen and caudate.[20] Most atrophy observed in whole brain and thalamus in early adulthood was multiple sclerosis-related, and by age 60, most of the atrophy was primarily attributable to normal ageing. The lack of ageing effect in the caudate and putamen

suggests DGM atrophy may differ across structures, an area of high interest for future studies.

Deep grey matter as an outcome in clinical trials

While the effects of disease-modifying therapies on whole brain volume in multiple sclerosis[97] have been extensively described, effects of disease-modifying therapies on DGM structures are only beginning to be investigated. Thalamic volume loss was reduced by ozanimod and is the first example where thalamic volume was reported in the primary analysis of a phase 3 study in multiple sclerosis.[98,99] Thalamic volume loss was slower in laquinomod,[100] fingolimod,[101] and ibudilast[102] treated patients compared to placebo. In a multivariate retrospective analysis of a small, non-randomized cohort, natalizumab and rituximab were associated with a slower rate of thalamic and putaminal atrophy compared to interferon-beta and glatiramer acetate, whereas whole-brain volume decline did not differ between the two groups.[103] Exploring the differential effect of treatments on DGM may provide insight into specific pathways of injury related to these structures[101]; however, the feasibility of advanced MRI sequences must be considered for trial application (Table 2). It is likely that DGM MRI end points will be incorporated in future clinical trials. Tailoring the MRI end point to the patient population or clinical end point being studied could reduce sample sizes and improve trial efficiency.

Incorporating deep grey matter measurements into clinical practice

Incorporating brain volumetrics, including DGM, into clinical practice has proven challenging for several reasons, which have been reviewed.[104] Developing image-processing algorithms that are robust to image heterogeneity would address many barriers. A gold standard for DGM measurements is needed, and the variability in each structure needs to be known and accounted for. Differences in acquisition, gradient distortion, intrascanner variability, movement, and scanner upgrades have been cited as sources of variability for brain volume.[105] Whether DGM measures are susceptible to these same confounders as well as the effects of pseudoatrophy (initial apparent brain volume loss associated with anti-inflammatory effect),[106,107] in measures of total versus individual DGM structures, are outstanding questions. Statistical techniques to translate DGM measurements into clinically relevant outcomes at the individual level are needed, and will need to account for age, treatment status, and measurement error at a minimum.

Hippocampus

Although not considered a DGM region, many studies examining DGM include the hippocampus in the analysis and classification of DGM structures,[108] and also because imaging methods sensitive to DGM damage are used to assess the hippocampus. The hippocampus is composed of several subregions, each of which has a distinct function and susceptibility to pathology.[109] It plays an important role in memory, regulation of mood, and emotional response.[110] Histopathological studies show extensive involvement of the hippocampus in multiple sclerosis, including widespread demyelination, neuronal loss, and synaptic loss.[29–32] Using MRI, total hippocampal volume loss has been associated with cognitive impairment in multiple sclerosis.[111] Atrophy in the CA1 subregion is associated with deficits in verbal memory in early relapsing remitting multiple sclerosis,[21] and atrophy in the CA3 subregion is associated with depression.[112] CA4 subregion/dentate gyrus may be the earliest subfield with volume loss compared to healthy controls, and that CA4/dentate volume predicts atrophy in the CA1 subregion 1 year later,[113] suggesting a 'dynamic spread' of pathology.

Total hippocampal volume, or CA1 and/or CA3 subregional volumes, could be useful to study cognitive dysfunction and depression in multiple sclerosis, which lack sensitive and specific MRI correlates. Moreover, because of the potential for structural and functional plasticity within the hippocampal network,[114] the hippocampus may be an interesting target for cognitive rehabilitation and neuroprotection interventions. The effects of exercise[115] and cognitive rehabilitation[116] on the hippocampus have been studied in several populations, including multiple sclerosis. Exercise interventions preserve or even increase hippocampal volume or activity, potentially leading to improvements in memory.[116,117]

Because of low MR contrast with surrounding tissues, the hippocampus remains a technically challenging region to segment, particularly at the subregion level. Multiple automated segmentation algorithms exist, with variable levels of agreement with manual segmentation.[118] Areas of interest for future hippocampal work in multiple sclerosis include improving longitudinal volume measurements, validating automated subregion techniques, pursuing hippocampal connectivity studies, and exploring the potential to preserve or reverse hippocampal damage, memory loss, and mood impairments using rehabilitative strategies.

Deep grey matter atrophy to understand disease biology better

Most of the current literature in multiple sclerosis has examined whole-brain and DGM volumes as predictors of clinical worsening. However, brain atrophy represents irreversible tissue loss, which is precisely what disease-modifying therapies aim to prevent. Future studies may use atrophy as the outcome rather than the predictor, to study biologic mechanisms of volume loss. The DGM is particularly well-suited for such studies because of the breadth of multiple sclerosis pathology present, mixed white and GM structures, early disease impact, and the highly connected nature of several regions. Different structures may provide insights into specific pathogenic mechanisms. For example, the mechanism of thalamic atrophy likely differs from that of hippocampal or basal ganglia atrophy, with different contributions from white matter lesions, intrinsic DGM lesions, iron deposition, oxidative stress, primary degeneration, etc., within each structure.[10,11,18,45,46] A long-term goal is to understand the contribution of mechanisms of injury within each structure, leading to more specific targets using imaging endpoints for specific biological pathways.

Conclusions

DGM injury in multiple sclerosis is common and results from a combination of focal lesions and changes in normal-appearing DGM, as well as upstream/downstream injury secondary to distant lesions. Significant improvement in DGM segmentation has resulted in ample evidence demonstrating the clinical relevance of DGM in multiple sclerosis. Improvements in acquisition, segmentation (including subregions), and analysis methods promise to refine DGM measures further (DGM lesional volume, thalamic volume, caudate volume, hippocampal volume) and enable future use in clinical trials, potentially even as primary outcome for phase 2 studies focusing on neuroprotection. DGM clinical diagnostic tools and biomarkers of therapeutic response will be

accelerated by shared resources including pulse sequences, imaging datasets, and analysis tools.

Acknowledgements

The authors acknowledge Jennifer Brydges and Emily Viles from AMPed for meeting organization and the American Committee for Research and Treatment in MS for use of space for the consensus meeting. The authors also acknowledge Amanda Mendelsohn for assistance with creation and editing of figures.

Funding

No direct funding supported this work. The authors thank the Race to Erase MS Foundation for support of NAIMS and meeting logistics.

Competing interests

D.O. reports research support from the National Institutes of Health, National Multiple Sclerosis Society, Patient Centered Outcomes Research Institute, Race to Erase MS Foundation, Genentech, Genzyme, and Novartis. Consulting fees from Biogen Idec, Genentech/Roche, Genzyme, Novartis, and Merck. K.R.M. reports research support from the National MS Society and National Institute of Health. D.L.A. reports personal compensation for serving as a Consultant for Alexion, Biogen, Celgene, Frequency Therapeutics, GENeuro, Genentech, Merck/EMD Serono, Novartis, Roche, and Sanofi. D.L.A. has an ownership interest in NeuroRx. M.G.D reports research support from the National Institutes of Health, National Multiple Sclerosis Society, Novartis, Merck, and Bristol Myers Squibb. Consulting fees from Novartis, Bristol Myers Squibb, and Keystone Heart. S.A.G reports research support from the National Institutes of Health, Genentech, Genzyme, and Mallinckrodt. Consulting fees from Biogen Idec. D.N.G. reports no competing interests related to this work; research support from the National Institutes of Health grants R01EB023281, R01NS105820, and R01NS112161. D.M.H. reports research support from the National Institutes of Health, National Multiple Sclerosis Society, Genentech, and EMD-Serono. Consulting fees from Biogen, Genentech, EMD-Serono, and Sanofi-Genzyme. Royalties and writing fees from UpToDate Inc. and the American College of Physicians. R.G.H. reports consulting fees from Roche/Genentech, Atara, Sanofi/Genzyme, Celgene/Bristol Myers Squibb, MEDDAY, QIA Consulting and grants from Roche/Genentech, MEDDAY, and Atara. D.K.B.L. has received research funding from the Canadian Institute of Health Research and Multiple Sclerosis Society of Canada. He is Emeritus Director of the UBC MS/MRI Research Group which has been contracted to perform central analysis of MRI scans for therapeutic trials with Roche and Sanofi-Genzyme. The UBC MS/MRI Research Group has also received grant support for investigator-initiated studies from Sanofi-Genzyme, Novartis and Roche. He has served on the PML-MS Steering Committee for Biogen. He has given lectures, supported by non-restricted education grants from Academy of Health Care Learning, Biogen, Consortium of MS Centers and Sanofi-Genzyme. C.M. has received funding from Sanofi-Genzyme, EMD Serono, Genentech, the MS National Society, the National Institute of Health and the Department of Defense outside the scope of this work. W.M. reports current research funding from the Multiple Sclerosis Society of Canada. At the time of this conference he was a member of the Medical Advisory Committee of the Multiple Sclerosis Society of Canada. In the past, he has received a grant in aid of research from Berlex Canada, has been a consultant for Schering, and received an honorarium from Teva for teaching. S.N. reports research funding from the Canadian Institutes of Health Research, the Myelin Repair Foundation and Immunotec; consulting fees from Genentech; part-time employment with NeuroRx Research. J.O. reports esearch support from the MS Society of Canada, Brain Canada, National MS Society, Biogen-Idec, Roche, EMD-Serono. Consulting fees from Biogen-Idec, EMD-Serono, Sanofi-Genzyme, Roche, Novartis, BMS, and Alexion. D.P. has received consulting fees from Biogen, Roche, Sanofi Genzyme, Novartis, and EMD Serono. A.R. has received research funding from the Canadian Institutes of Health Research and the National Multiple Sclerosis Society. W.D.R. reports research support from the National Institutes of Health, Conrad N. Hilton Foundation, Paul G. Allen Family Foundation, Race to Erase MS Foundation, and Revalesio, Inc. N.L.S. reports research support from the National MS Society, Patient Centered Outcomes Research Institute, and the National Institute of Health. R.T. reports grants from Multiple Sclerosis Society of Canada, Natural Sciences and Engineering Research Council of Canada, Mitacs, Praxis Spinal Cord Institute, and UBC Data Science Institute. D.S.R is supported by the Intramural Research Program of NINDS, NIH. C.J.A. has received consulting fees from Guerbet LLC, Genentech, Biogen Idec, Novartis, Sanofi Genzyme, EMD Serono, and Alexion Pharmaceuticals, outside the submitted work. All other authors report no competing interests.

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