

Fatigue in Multiple Sclerosis: the role of thalamus.

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ABSTRACT

Introduction

Fatigue is very common in multiple sclerosis (MS) and is often considered as its most disabling symptom. Over the last twenty years, an increasing number of studies have evaluated the pathogenetic bases of MS-related fatigue. Converging evidence from neurophysiology and neuroimaging research suggests that a dysfunction in a cortico-subcortical pathway, centered on thalamus, is involved in the pathogenesis of fatigue. However, type and significance of such dysfunction remain unknown, and some studies reported an increase in the activity and connectivity within the thalamic network, whereas others suggested its reduction.

Methods and Results

Hereby, we review the results of neuroimaging studies supporting the different hypotheses about the role of thalamic network in the pathophysiology of MS-related fatigue, and discuss limitations and shortcomings of available data, highlighting the key challenges in the field and the directions for future research.

INTRODUCTION

Fatigue, defined as an overwhelming sense of lack of physical and/or mental energy, can affect up to 80% patients with multiple sclerosis (MS) and is often considered as its most disabling symptom.^{1,2}

Fatigue is a poorly defined construct and, hence, difficult to measure and to define in the clinical practice and trials.³ The MS International Federation identified two types of fatigue: i) physical fatigue, characterized by muscle weakness and difficulties to perform daily tasks, and ii) cognitive fatigue, characterized by difficulties in thinking, concentration, and memory.⁴ Fatigue is generally self-reported by patients, using questionnaires such as the Fatigue Severity Scale (FSS),⁵ and the Modified Fatigue Impact Scale (MFIS).⁶ Most frequently-used pharmacological treatments for fatigue are amantadine, 4-aminopyridine, and modafinil. Non-pharmacological interventions include physical (e.g., aerobic exercises, resistance training, yoga, tai-chi), and psychological/cognitive approaches (e.g., cognitive behavioural therapy, education programs, and mindfulness interventions).³ However, evidence supporting the efficacy of these interventions is still preliminary and, sometimes, conflicting. This depends, at least in part, by our poor understanding of the processes underlying fatigue in MS, and by the lack of validated and reproducible biomarkers (e.g., MRI).

Over the last twenty years, an increasing number of studies have evaluated the pathogenetic bases of MS-related fatigue. Converging evidence from neurophysiology^{7,8} and neuroimaging^{9,10} demonstrated that, in patients with MS, fatigue has a central origin and a dysfunction of the circuits between thalamus, basal ganglia and cortex could be its main pathogenic substrate. Initially, many authors focused on areas of possible interest for motor fatigue (cortex and basal ganglia), that were easy to investigate with neuroimaging and neurophysiological tools.^{7,8,11} However, the *a priori* choice of the region of interest (or seed region) has ignored the possible relevance of thalamus. More recently, thanks to the increasing interest in non-motor aspects of fatigue (i.e. cognitive,

psychosocial) and the improved knowledge on the unique role of thalamus as a gateway for cortical areas and a relay between cortical and subcortical structures,¹² thalamus has progressively gained attention in the study of pathophysiology of MS-related fatigue. Overall, a dysfunction in a cortico-subcortical pathway, centered on thalamus, is involved in the pathogenesis of fatigue, but type and significance of such dysfunction remain unknown. Some studies reported an increase in the activity and connectivity of thalamic network,¹³⁻¹⁷ whereas others suggested its reduction.¹⁸⁻²³ Thus, the role of thalamus in the pathogenesis of fatigue remains largely controversial and different hypotheses have been proposed.

In the past, original studies and reviews on fatigue in MS have evaluated different brain regions, whilst, in this review, we have decided to focus on the most promising area in the pathophysiology of MS-related fatigue, the thalamus. In the first part, we will briefly report on normal structure of the thalamus and on its impairment in MS. Then, we will describe the results of neuroimaging studies supporting different hypotheses about the role of thalamic network in the pathophysiology of MS-related fatigue (increased vs decreased activity). We will then unify existing data to discuss potential neuroanatomical mechanisms to explain how damage in (or outside of) the thalamus leads to symptoms of fatigue. Finally, we will discuss limitations and shortcomings of available data, highlighting the key challenges in the field and directions for future clinical trials and observational studies.

THALAMUS: NORMAL STRUCTURE AND FUNCTION

The thalamus is the largest part of the diencephalon and is located between the third ventricle medially, and the internal capsule, laterally. The thalamus is extremely heterogeneous in its anatomical structure, functional connectivity and neuroimaging appearance, and different classifications have been proposed (**Figure 1**).

Anatomically, the thalamus is divided into three regions (anterior, lateral and medial) by the internal medullary lamina. Within each of these regions, there are nuclei with distinct connections (**Figure 1A**). Other nuclei are located within the internal medullary lamina (intralaminar nuclei), and on the lateral aspect of the thalamus (reticular nucleus), out of the external medullary lamina. Finally, in the posterior area of the thalamus, there are the medial geniculate nucleus and the lateral geniculate nucleus.

Functionally, thalamic nuclei can be divided into three basic types: the relay nuclei, the association nuclei and the non-specific nuclei²⁴ (**Figure 1B**). Relay nuclei transmit information from the periphery to the cortex. Depending on the type of relayed information, they can be classified as: a) sensory relay nuclei acting as a gateway for sensory (somatosensory, visual, auditory) inputs to reach different sensory cortical areas;²⁵ b) motor relay nuclei connecting subcortical motor structures, such as cerebellum and basal ganglia, with cortical motor areas such as primary motor and premotor cortex;²⁶ limbic relay nuclei connecting different structures of the limbic system such as mammillary bodies, cingulate cortex, and entorhinal cortex.²⁷ The association nuclei (e.g., pulvinar) receive input from the cerebral cortex and project back to the cerebral cortex and are supposed to regulate the activity of cortical association areas.²⁸ The nonspecific nuclei (e.g., intralaminar and midline thalamic nuclei) send diffuse projections to the cerebral cortex and are probably involved in general functions such as alertness and arousal.²⁹

Recent advances in neuroimaging techniques have enabled to identify, non-invasively, connections between the main thalamic nuclei and the cortex, which have strong correspondence with the anatomical and functional classification of thalamic nuclei derived from animal and human *ex vivo* studies. Using a probabilistic tractography algorithm, Beherens et al.³⁰ classified thalamic grey matter based on its connections with cortex and identified 6 main thalamic regions: frontal, occipital, parietal, temporal, precentral, postcentral regions. This thalamic classification has shown

high reproducibility within and between subjects³¹ and has been confirmed on both functional MRI (fMRI)^{32,33} and DTI.³⁴

THALAMUS IN MS

Evidence of thalamic involvement in MS arises mainly from neuropathological and neuroimaging studies. Neuropathological studies^{35,36} have shown demyelination, inflammation and neurodegeneration (i.e. neuronal loss, neuronal shrinkage and axonal damage) in the thalamus. These findings have been confirmed *in vivo* using different neuroimaging techniques. Volumetric MRI studies showed thalamic atrophy,³⁷⁻³⁹ since the very early stages of the disease, including clinically isolated syndrome (CIS), pediatric-onset MS and radiologically isolated syndrome.⁴⁰ The development of advanced neuroimaging techniques, such as diffusion tensor imaging (DTI), has allowed to detect microstructural alterations in its nuclei and in the white matter tracts towards cortical and subcortical areas, also in absence of obvious thalamic atrophy.⁴¹⁻⁴⁴ Taken together, neuroimaging and neuropathological studies suggest that thalamic damage in MS is common, occurs early and can be related to either a primary damage in thalamus or a distant damage of afferent or efferent thalamic fibers, subsequently affecting the thalamus through mechanisms of Wallerian or trans-neuronal degeneration.²⁴

THALAMUS AND FATIGUE IN MS

The idea that thalamus could be involved in the pathophysiology of fatigue originally arises from studies showing structural and functional changes within the thalamus of fatigued MS patients. Different studies reported on thalamic atrophy along with basal ganglia and fronto-parietal cortex volume loss, in MS patients with fatigue.^{45,46} Moreover, advanced neuroimaging techniques showed indirect signs of demyelination and axonal loss in the thalamus of fatigued MS-patients.^{22,47,48} If a dysfunction in a cortico-subcortical pathway centered on thalamus is nowadays generally accepted as a determinant of fatigue in MS, contrasting results have been reported on type and significance of such dysfunction, with some studies reporting a reduction in the activity and connectivity of

thalamic network,^{18–23} and others an increase.^{13–17} Differences between these findings have produced two main hypotheses about the potential neuroanatomical mechanisms to explain how thalamic impairment leads to fatigue, as discussed below (**Figure 2**).

Reduced thalamic activity produces fatigue

Fatigue can arise from a global reduction in the activity of networks involving thalamus, basal ganglia and cortex. Such reduction can be associated with measurable morphological changes (atrophy) in one or more parts of the network. Of note, functional connectivity may be increased between some areas of the network, as a compensatory strategy, whilst the global activity is globally reduced. This hypothesis is supported by studies showing a sustained reduction in thalamic functionality and connectivity in fatigued MS patients (**Table 1**).

Roelcke et al.²¹ preliminarily described reduced glucose metabolism on 18F-fluorodeoxyglucose PET in the frontal cortex and in the basal ganglia of fatigued patients, when compared with not-fatigued. A specific reduction of thalamic activity in fatigued MS patients was later described in another 18F-fluorodeoxyglucose PET study²², and also on MRI.^{18,20} In particular, Inglese et al.²⁰ found a significant association between fatigue severity and reduction of cerebral blood flow and volume in thalamus and basal ganglia by using dynamic susceptibility contrast enhanced T2*-weighted MRI, as from thalamic dysfunction and damage. Filippi et al.¹⁸ demonstrated that activation of thalamus and sensorimotor cortical areas evoked by a simple motor task on fMRI was reduced in fatigued patients, when compared with not-fatigued. These findings were recently confirmed by Rocca et al.¹⁹ who showed, in a larger cohort, that fatigued patients have reduced activation in thalamus, basal ganglia and fronto-temporal-parietal cortex during a simple motor task, followed by abnormal adaption over time. Similarly, Bonzano et al.⁴⁹ showed that fatigued MS patients presented with worse accuracy on repeated finger motor task and lower BOLD (Blood Oxygenation Level Dependent) signal, when compared with healthy subjects, that did not return to baseline level after rest.

Compensatory mechanisms to reduced thalamic activity and fatigue have been hypothesized. Cruz Gomez et al.²³ found that fatigued MS patients presented with decreased levels of RS-MRI functional connectivity between areas related to sensory-motor functions (e.g., thalamus, cerebellum, brainstem, and frontal-parietal cortex), whilst not-fatigued patients displayed increased levels, as from compensatory mechanism reducing fatigue perception. Engstrom et al.¹⁶ found that fatigued MS patients have weaker cortical-to-subcortical connections, but stronger cortical-to-cortical (left posterior parietal cortex and left dorsolateral prefrontal cortex) and subcortical-to-subcortical (right substantia nigra and left thalamus) connections, possibly also acting as a compensatory mechanism.

Fatigue is caused by a compensatory thalamic activity increase

In this hypothesis, fatigue arises from an increase in the activity of the networks involving thalamus, basal ganglia and cortex, as a result of a compensatory mechanism that allows to maintain normal functioning but also produces fatigue. This hypothesis is supported by the results of a number of studies showing an increased activation in thalamus, basal ganglia and fronto-parietal-occipital cortex in fatigued MS patients who performed a cognitive task during fMRI, when compared with controls or with not-fatigued patients (**Table 2**).

Rocca et al.¹³ found that patients experiencing fatigue after Interferon-beta injections have an increased activation of thalamus and frontal cortex compared with patients without fatigue. Zhou et al.¹⁷ found that fatigued patients exhibited changes in the thalamocortical system consisting in structural disconnections at DTI and hyperconnectivity at RS-fMRI, further supporting that fatigue could arise from increased functional connectivity as a compensation to the microstructural damage in the thalamocortical network.

TOWARDS A UNIFYING THEORY

Discrepancies between the above-mentioned studies could be referred to different patterns of (increased or reduced) activation/connectivity, in different parts of the thalamocortical network, at different stages of disease. In early stages, patients could compensate MS-related structural damage by plasticity mechanisms leading to overactivation of the cortical-subcortical network without experiencing any fatigue. Over time, functional reserve reduces, with an additional effort to maintain the overactivation of the network, leading to transient fatigue. Once plasticity is not able to compensate the reduced structural connectivity, functional connectivity drops, and fatigue becomes chronically present. Not least, the structural and functional complexity of the thalamus has not been fully accounted, with most studies considering the thalamus as single region of interest. Preliminary data from volumetric⁴⁵ and functional MRI studies¹⁰ showed that different circuits or different parts of the same circuit within the basal ganglia-thalamus-cortex network could be involved in the pathogenesis of different aspects of fatigue.

To the best of our knowledge, no longitudinal studies have examined patients over time to assess whether fatigue-related brain patterns change over time. Thus, this hypothesis is solely based on the integration of the results from different studies that have investigated patients at different stages of MS. Accordingly, in patients with mild disability and short disease duration, fatigue is associated with microstructural damage in the thalamocortical pathway, compensated by hyperconnectivity at fMRI.¹⁷ On the contrary, in patients with more advanced disease, the structural damage of the thalamocortical system is associated with decreased levels of functional connectivity.²³

In light of the extent of thalamic connections between different cortical and subcortical areas, thalamic pathology in MS could be secondary to a damage of afferent or efferent thalamic fibers.²⁴ According to this hypothesis, fatigue may be related to a non-specific, MS-induced disconnection between different brain regions, with thalamic abnormalities (atrophy, microstructural changes) being an epiphenomenon of MS pathology occurring elsewhere than thalamus. This hypothesis is supported by studies showing that fatigue is associated to a widespread microstructural change in

the white matter,^{50,51} or to a pathology of other structures different from thalamus, such as frontal⁵² and primary somatosensory⁵³ cortex. However, widespread white matter involvement could be representative of more advanced MS, masking the relevance of specific structures (i.e., thalamus) in the pathogenesis of fatigue.

The specific role for thalamus in the pathogenesis of fatigue has been confirmed in both healthy subjects,⁵⁴ and patients with different (and more focal) neurological diseases (e.g., stroke, traumatic brain injury).^{55,56} Indeed, a relatively-small damage to the thalamic network, independently from the extent of diffuse brain involvement, could generate fatigue. Accordingly, in MS, microstructural abnormalities and atrophy in strategic brain regions (e.g., thalamus) have stronger associations with fatigue, than measures of global brain damage, such as atrophy and lesion load.^{9,47} Not least, thalamus can be affected selectively, since the earliest stages of the disease, and independently from the extent of white matter involvement.¹²

CONCLUSIONS, LIMITATIONS AND FUTURE RESEARCH

Neuroimaging studies have demonstrated that the thalamus is involved in the pathogenesis of MS-related fatigue, either as a cause or as a consequence of impaired cortical-subcortical networks. Though a number of brain regions contribute to the pathogenesis of fatigue,^{50,52,53,57,58} thalamus is a strategic area and a relatively-small disruption of its network can generate fatigue, independently from the extent of tissue involvement.^{9,59} In this review, we specifically focused on the results of neuroimaging studies supporting the role of the thalamic network in the pathophysiology of MS-related fatigue.

Future studies will have to clarify the role of thalamus within the pathogenesis of MS-related fatigue, overcoming the limitations of available data by: i) combining different neuroimaging and neurophysiological techniques (e.g. EEG-TMS, non-invasive brain stimulation) in the same sample to obtain multiparametric data; ii) investigating patients at different stages of disease (CIS,

advanced progressive MS); iii) assessing whether thalamic impairment is primary or secondary to the damage of other structures outside of the thalamus (e.g., diffuse white matter damage) with a longitudinal design; iv) considering separately different thalamic nuclei and their connections; v) evaluating possible factors that contribute to fatigue pathogenesis and/or exacerbate its manifestations and perception (demographics, comorbidity, genetics, diet, exercise, depression, cognitive impairment, pain and sleep disorders).⁶⁰⁻⁶³

The management of fatigue requires a multidisciplinary approach including both pharmacological and non-pharmacological interventions, but the evidence supporting their efficacy is still preliminary. Result heterogeneity could be due to methodological differences among studies in terms of sample (e.g., disease duration), and definition of fatigue, with very few studies including neuroimaging protocols. Clinical trials on MS-related fatigue treatments have sometimes requested MRI for baseline patients' stratification.^{64,65} Despite variations in thalamic activity were associated with fatigue development in an open-label study on Interferon-Beta1a-treated patients,¹³ so far, only one clinical trial (evaluating acupuncture and mindfulness for MS fatigue) included functional connectivity on fMRI as a tertiary outcome measure in a subgroup of patients.⁶⁶ Thus, a validated and reproducible set of measures for the thalamic network could be used to i) identifying subgroups of fatigued patients, that share common pathogenetic mechanisms, ii) quantifying objectively the subjective experience of fatigue, and iii) evaluating the efficacy of therapeutic interventions, ultimately leading to tailored pharmacological and non-pharmacological management of fatigue.

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REFERENCES

1. Tur C. Fatigue Management in Multiple Sclerosis. *Curr Treat Options Neurol* 2016; 18: 26.
2. Leocani L, Colombo B, Comi G. Physiopathology of fatigue in Multiple Sclerosis. *Neurol Sci* 2008; 29: 241–243.
3. Khan F, Amatya B, Galea M. Management of fatigue in persons with multiple sclerosis. *Front Neurol* 2014; 5: 177.
4. Multiple Sclerosis International Federation (MSIF). Fatigue and MS. *MS in Focus*, 2011.
5. Krupp LB, LaRocca NG, Muir-Nash J, et al. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. *Arch Neurol* 1989; 46: 1121–3.
6. Fisk JD, Pontefract A, Ritvo PG, et al. The impact of fatigue on patients with multiple sclerosis. *Can J Neurol Sci* 1994; 21: 9–14.
7. Leocani L, Colombo B, Magnani G, et al. Fatigue in multiple sclerosis is associated with abnormal cortical activation to voluntary movement--EEG evidence. *Neuroimage* 2001; 13: 1186–92.
8. Thickbroom GW, Sacco P, Kermode AG, et al. Central motor drive and perception of effort during fatigue in multiple sclerosis. *J Neurol* 2006; 253: 1048–53.
9. Rocca MA, Parisi L, Pagani E, et al. Regional but not global brain damage contributes to fatigue in multiple sclerosis. *Radiology* 2014; 273: 511–20.
10. Hidalgo de la Cruz M, d'Ambrosio A, Valsasina P, et al. Abnormal functional connectivity of thalamic sub-regions contributes to fatigue in multiple sclerosis. *Mult Scler* 2018; 24: 1183–1195.
11. Sheean GL, Murray NM, Rothwell JC, et al. An electrophysiological study of the mechanism of fatigue in multiple sclerosis. *Brain* 1997; 120 (Pt 2): 299–315.
12. Minagar A, Barnett MH, Benedict RHB, et al. The thalamus and multiple sclerosis: modern views on pathologic, imaging, and clinical aspects. *Neurology* 2013; 80: 210–9.
13. Rocca MA, Agosta F, Colombo B, et al. fMRI changes in relapsing-remitting multiple sclerosis patients complaining of fatigue after IFNbeta-1a injection. *Hum Brain Mapp* 2007; 28: 373–82.
14. DeLuca J, Genova HM, Hillary FG, et al. Neural correlates of cognitive fatigue in multiple sclerosis using functional MRI. *J Neurol Sci* 2008; 270: 28–39.
15. Genova HM, Rajagopalan V, Deluca J, et al. Examination of cognitive fatigue in multiple sclerosis using functional magnetic resonance imaging and diffusion tensor imaging. *PLoS One* 2013; 8: e78811.

16. Engström M, Flensner G, Landtblom A-M, et al. Thalamo-striato-cortical determinants to fatigue in multiple sclerosis. *Brain Behav* 2013; 3: 715–28.
17. Zhou F, Gong H, Chen Q, et al. Intrinsic Functional Plasticity of the Thalamocortical System in Minimally Disabled Patients with Relapsing-Remitting Multiple Sclerosis. *Front Hum Neurosci* 2016; 10: 2.
18. Filippi M, Rocca MA, Colombo B, et al. Functional Magnetic Resonance Imaging Correlates of Fatigue in Multiple Sclerosis. *Neuroimage* 2002; 15: 559–567.
19. Rocca MA, Meani A, Riccitelli GC, et al. Abnormal adaptation over time of motor network recruitment in multiple sclerosis patients with fatigue. *Mult Scler J* 2016; 22: 1144–1153.
20. Inglese M, Park S-J, Johnson G, et al. Deep gray matter perfusion in multiple sclerosis: dynamic susceptibility contrast perfusion magnetic resonance imaging at 3 T. *Arch Neurol* 2007; 64: 196–202.
21. Roelcke U, Kappos L, Lechner-Scott J, et al. Reduced glucose metabolism in the frontal cortex and basal ganglia of multiple sclerosis patients with fatigue: a 18F-fluorodeoxyglucose positron emission tomography study. *Neurology* 1997; 48: 1566–71.
22. Derache N, Grassiot B, Mézenge F, et al. Fatigue is associated with metabolic and density alterations of cortical and deep gray matter in Relapsing-Remitting-Multiple Sclerosis patients at the earlier stage of the disease: A PET/MR study. *Mult Scler Relat Disord* 2013; 2: 362–9.
23. Cruz Gómez AJ, Ventura Campos N, Belenguer A, et al. Regional brain atrophy and functional connectivity changes related to fatigue in multiple sclerosis. *PLoS One* 2013; 8: e77914.
24. Kipp M, Wagenknecht N, Beyer C, et al. Thalamus pathology in multiple sclerosis: from biology to clinical application. *Cell Mol Life Sci* 2015; 72: 1127–47.
25. Jones EG. The anatomy of sensory relay functions in the thalamus. *Prog Brain Res* 1991; 87: 29–52.
26. Sommer MA. The role of the thalamus in motor control. *Curr Opin Neurobiol* 2003; 13: 663–70.
27. Child ND, Benarroch EE. Anterior nucleus of the thalamus: functional organization and clinical implications. *Neurology* 2013; 81: 1869–76.
28. Shipp S. The functional logic of cortico-pulvinar connections. *Philos Trans R Soc Lond B Biol Sci* 2003; 358: 1605–24.
29. Sherman SM. The thalamus is more than just a relay. *Curr Opin Neurobiol* 2007; 17: 417–422.
30. Behrens TEJ, Johansen-Berg H, Woolrich MW, et al. Non-invasive mapping of connections between human thalamus and cortex using diffusion imaging. *Nat Neurosci* 2003; 6: 750–7.
31. Traynor C, Heckemann RA, Hammers A, et al. Reproducibility of thalamic segmentation based on probabilistic tractography. *Neuroimage* 2010; 52: 69–85.
32. d'Ambrosio A, Hidalgo de la Cruz M, Valsasina P, et al. Structural connectivity-defined thalamic subregions have different functional connectivity abnormalities in multiple sclerosis patients: Implications for clinical correlations. *Hum Brain Mapp* 2017; 38: 6005–6018.
33. Johansen-Berg H, Behrens TEJ, Sillery E, et al. Functional-anatomical validation and individual variation of diffusion tractography-based segmentation of the human thalamus. *Cereb Cortex* 2005; 15: 31–9.
34. Bisecco A, Rocca MA, Pagani E, et al. Connectivity-based parcellation of the thalamus in multiple sclerosis and its implications for cognitive impairment: A multicenter study. *Hum Brain Mapp* 2015; 36: 2809–25.
35. Vercellino M, Plano F, Votta B, et al. Grey matter pathology in multiple sclerosis. *J Neuropathol Exp Neurol* 2005; 64: 1101–7.
36. Cifelli A, Arridge M, Jezzard P, et al. Thalamic neurodegeneration in multiple sclerosis. *Ann Neurol* 2002; 52: 650–3.
37. Houtchens MK, Benedict RHB, Killiany R, et al. Thalamic atrophy and cognition in multiple sclerosis. *Neurology* 2007; 69: 1213–23.

38. Rocca MA, Mesaros S, Pagani E, et al. Thalamic damage and long-term progression of disability in multiple sclerosis. *Radiology* 2010; 257: 463–9.
39. Nygaard GO, Celius EG, de Rodez Benavent SA, et al. A Longitudinal Study of Disability, Cognition and Gray Matter Atrophy in Early Multiple Sclerosis Patients According to Evidence of Disease Activity. *PLoS One* 2015; 10: e0135974.
40. Azevedo CJ, Overton E, Khadka S, et al. Early CNS neurodegeneration in radiologically isolated syndrome. *Neurol Neuroimmunol neuroinflammation* 2015; 2: e102.
41. Davies GR, Ramió-Torrentà L, Hadjiprocopis A, et al. Evidence for grey matter MTR abnormality in minimally disabled patients with early relapsing-remitting multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2004; 75: 998–1002.
42. Mesaros S, Rocca MA, Pagani E, et al. Thalamic damage predicts the evolution of primary-progressive multiple sclerosis at 5 years. *AJNR Am J Neuroradiol* 2011; 32: 1016–20.
43. Inglese M, Liu S, Babb JS, et al. Three-dimensional proton spectroscopy of deep gray matter nuclei in relapsing-remitting MS. *Neurology* 2004; 63: 170–2.
44. Wylezinska M, Cifelli A, Jezzard P, et al. Thalamic neurodegeneration in relapsing-remitting multiple sclerosis. *Neurology* 2003; 60: 1949–54.
45. Calabrese M, Rinaldi F, Grossi P, et al. Basal ganglia and frontal/parietal cortical atrophy is associated with fatigue in relapsing-remitting multiple sclerosis. *Mult Scler* 2010; 16: 1220–8.
46. Bernitsas E, Yarraguntla K, Bao F, et al. Structural and Neuronal Integrity Measures of Fatigue Severity in Multiple Sclerosis. *Brain Sci* 2017; 7: 102.
47. Niepel G, Tench CR, Morgan PS, et al. Deep gray matter and fatigue in MS: a T1 relaxation time study. *J Neurol* 2006; 253: 896–902.
48. Wilting J, Rolfsnes HO, Zimmermann H, et al. Structural correlates for fatigue in early relapsing remitting multiple sclerosis. *Eur Radiol* 2016; 26: 515–23.
49. Bonzano L, Pardini M, Roccatagliata L, et al. How people with multiple sclerosis cope with a sustained finger motor task: A behavioural and fMRI study. *Behav Brain Res* 2017; 325: 63–71.
50. Novo AM, Batista S, Alves C, et al. The neural basis of fatigue in multiple sclerosis: A multimodal MRI approach. *Neurol Clin Pract* 2018; 8: 492–500.
51. Bisecco A, Caiazzo G, d'Ambrosio A, et al. Fatigue in multiple sclerosis: The contribution of occult white matter damage. *Mult Scler* 2016; 22: 1676–1684.
52. Sepulcre J, Masdeu JC, Goñi J, et al. Fatigue in multiple sclerosis is associated with the disruption of frontal and parietal pathways. *Mult Scler* 2009; 15: 337–44.
53. Riccitelli G, Rocca MA, Forn C, et al. Voxelwise assessment of the regional distribution of damage in the brains of patients with multiple sclerosis and fatigue. *AJNR Am J Neuroradiol* 2011; 32: 874–9.
54. Jiang Z, Wang X-F, Yue GH. Strengthened Corticosubcortical Functional Connectivity during Muscle Fatigue. *Neural Plast* 2016; 2016: 1726848.
55. Mutai H, Furukawa T, Hourri A, et al. Factors associated with multidimensional aspect of post-stroke fatigue in acute stroke period. *Asian J Psychiatr* 2017; 26: 1–5.
56. Clark AL, Sorg SF, Holiday K, et al. Fatigue Is Associated With Global and Regional Thalamic Morphometry in Veterans With a History of Mild Traumatic Brain Injury. *J Head Trauma Rehabil* 2018; 33: 382–392.
57. Biberacher V, Schmidt P, Selter RC, et al. Fatigue in multiple sclerosis: Associations with clinical, MRI and CSF parameters. *Mult Scler* 2018; 24: 1115–1125.
58. Bakshi R, Miletich RS, Henschel K, et al. Fatigue in multiple sclerosis: cross-sectional correlation with brain MRI findings in 71 patients. *Neurology* 1999; 53: 1151–3.
59. Eshaghi A, Marinescu R V, Young AL, et al. Progression of regional grey matter atrophy in multiple sclerosis. *Brain* 2018; 141: 1665–1677.
60. Comi G, Leocani L. Assessment, pathophysiology and treatment of fatigue in multiple sclerosis. *Expert Rev Neurother* 2002; 2: 867–76.
61. Pommerich UM, Brincks J, Christensen ME. Is there an effect of dietary intake on MS-

- related fatigue? - A systematic literature review. *Mult Scler Relat Disord* 2018; 25: 282–291.
62. Amatya B, Khan F, Galea M. Rehabilitation for people with multiple sclerosis: an overview of Cochrane Reviews. *Cochrane database Syst Rev* 2019; 1: CD012732.
 63. Heesen C, Nawrath L, Reich C, et al. Fatigue in multiple sclerosis: an example of cytokine mediated sickness behaviour? *J Neurol Neurosurg Psychiatry* 2006; 77: 34–9.
 64. Testosterone for Fatigue in Men With MS. ClinicalTrials.gov Identifier: NCT03000127., <https://clinicaltrials.gov/ct2/show/NCT03000127>.
 65. Evaluation of Natalizumab for thE Relief of MS Associated FatiGue (ENER-G). ClinicalTrials.gov Identifier: NCT00464074, <https://clinicaltrials.gov/ct2/show/NCT00464074>.
 66. Acupuncture or MBSR for Patients With Fatigue and MS (MS-Fatigue). ClinicalTrials.gov Identifier: NCT01864707., <https://clinicaltrials.gov/ct2/show/NCT01864707>.

Table 1. Imaging studies showing reduced thalamic volume, microstructure and activity in MS patients with fatigue.

Table shows studies showing reduced thalamic volume (on structural imaging), microstructure (on DTI) and activity in relation to MS fatigue (on functional, metabolic and perfusion imaging). Imaging technique, population characteristics, fatigue assessment, main findings and limitations are reported.

| Imaging Technique | Population | Fatigue scale (assessor) | Main findings in fatigued patients (compared with HC and/or not-fatigued patients) | Limitations | Reference |
|---------------------------------------------------------------|--------------------------|---------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------|----------------------------------------|
| Structural imaging | | | | | |
| 1.5T MRI | RRMS (n=60) HC (n=18) | FSS (self-reported) | ↑↑↑ atrophy in areas related to the sensory-motor network. | Significant difference in EDSS between fatigued and not fatigued patients. | Cruz-Gomez et al, Plos One, 2013 |
| Microstructural imaging | | | | | |
| 3T MRI (DTI) | RRMS (n=79) HC (n=40) | FSMC (self-reported) | ↓↓↓ FA in thalamus and basal ganglia ↑↑↑ MD in thalamus and basal ganglia (in absence of differences in thalamic volume) | HC not well matched. | Wilting J et al, Eur Radiol 2016 |
| Functional imaging | | | | | |
| 1.5T fMRI (motor task with dominant hand) | RRMS (n=29) HC (n=15) | FSS (self-reported) | ↓↓↓ activation in contralateral thalamus and intraparietal sulcus, and in ipsilateral thalamus and rolandic operculum. | Small sample size | Filippi M et al, Neuroimage 2002 |
| 3T fMRI (motor task with dominant hand) | RRMS (n=79) HC (n=26) | MFIS (self-reported) | ↓↓↓ activation in the fronto-temporal-parietal regions, basal ganglia, thalamus and supplementary motor area. ↑↑↑ activation in the ipsilateral middle frontal gyrus. | Analysis restricted to the motor network | Rocca MA et al, Mult Scler. 2016 |
| 3T rs-fMRI | RRMS (n=22) HC (n=12) | FSMC (self-reported) | Immediate ↑↑↑ FC between frontal cortex and caudate Delayed ↓↓↓ FC within thalamus | Small sample size Patients not well matched. | Pravata' et al, Mult Scler 2016 |
| 1.5T fMRI (demanding motor task with dominant hand) | RRMS (n=14) | MFIS (self-reported) | ↓↓↓ activation in contralateral thalamus, basal ganglia and amigdala. | Absence of a control group. Small sample size | Bonzano L et al, Behav Brain Res. 2017 |
| 1.5T rs-fMRI | RRMS (n=60) | FSS | ↓↓↓ FC between the supplementary motor | Significant difference in | Cruz-Gomez et |

| | | | | | |
|-------------------------------------------|------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------|------------------------------------------------|
| | HC (n=18) | (self-reported) | area and associative somatosensory cortex. | EDSS between fatigued and not fatigued patients. | al, Plos One, 2013 |
| 1.5T fMRI (working memory task) | RRMS (n=11) SPMS (n=3) PPMS (n=1) HC (n=11) | Self-reported fatigue and heat sensitivity (self-reported) | ↓↓↓ activation in thalamus, basal ganglia and frontal cortex. ↑↑↑ activation in the parietal cortex. | Lack of standardized fatigue assessment Possible cognitive impairment | Engstrom et al, Brain Behav. 2013 |
| 1.5T rs-fMRI | | | ↑↑↑ FC within the cerebral cortex and within subcortical regions, ↓↓↓ FC between cerebral cortex and striatum. | | |
| Metabolic imaging | | | | | |
| [¹⁸F]-FDG-PET | RRMS (n=17) | MFIS (self-reported) | ↓↓↓ [¹⁸ F]-FDG uptake in the GM of frontal and temporal regions, and in bilateral thalamus and basal ganglia. | Measurements limited to the GM. Absence of a control group. Small sample size. | Derache N et al, Mult Scler Relat Disord. 2013 |
| Perfusion imaging | | | | | |
| 3T MRI (perfusion measures) | RRMS (n=11) PPMS (n=11) HC (n=11) | Multidimensional Fatigue Inventory (psychologist) | ↓↓↓ cerebral blood flow and cerebral blood volume in deep GM. | Measurements limited to deep GM. Small sample size. Concomitant immunomodulatory treatments. | Inglese M et al, Arch Neurol. 2007 |

MS: multiple sclerosis; MRI: magnetic resonance imaging; DTI: diffusion tensor imaging; fMRI: functional MRI; RRMS: relapsing-remitting MS; HC: healthy control; FSS: Fatigue Severity Scale; MFIS: Modified Fatigue Impact Scale; RS: resting state; GM: grey matter; PPMS: primary progressive MS; SPMS: secondary progressive MS; FC: functional connectivity.

Table 2. Imaging studies showing increased thalamic activity in MS patients with fatigue.

Table shows studies showing increased thalamic activity in relation to MS fatigue (on functional imaging). Imaging technique, population characteristics, fatigue assessment, main findings and limitations are reported.

| Imaging Technique | Population | Fatigue scale (assessor) | Main findings in fatigued patients (compared with HC and/or not-fatigued patients) | Limitations | Reference |
|----------------------------------------------------|-----------------------------------------------------|----------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|-------------------------------------|
| Functional imaging | | | | | |
| 3T fMRI (cognitive task) | RRMS (n=12) PPMS (n=3) HC (n=15) | mSDMT score (psychologist) | ↑↑↑ activation in thalamus, basal ganglia and fronto-parietal-occipital cortex. | Neuropsychological fatigue, without self-reported fatigue. Small sample size. | DeLuca J et al, J Neurol Sci. 2008 |
| 3T fMRI (task-switching task) | RRMS (n=9) PPMS (n=1) SPMS (n=1) HC (n=11) | Self-reported cognitive fatigue (self-reported) | ↑↑↑ activation in prefrontal cortex, left postcentral gyrus, precuneus, precentral gyrus, inferior temporal gyrus, and declive of the cerebellum. ↓↓↓ activation in the left superior frontal gyrus, right cuneus and bilateral temporal regions. (in presence of microstructural impairment on DTI, with ↓↓↓ FA in the anterior internal capsule) | Neuropsychological fatigue, without self-reported fatigue. HC not well matched. Small sample size. | Genova et al, PLoS One. 2013 |
| 1.5 fMRI (motor task with dominant hand) | RRMS (n=22) | FSS (self-reported) | ↑↑↑ activations of thalamus and frontal cortex | Reversible Interferon-induced fatigue. Absence of a control group. | Rocca et al, Hum Brain Mapp. 2007 |
| 3T rs-fMRI | RRMS (n=20) HC (n=20) | MFIS (self-reported) | ↑↑↑ FC in thalamocortical pathways (in presence of microstructural impairment on DTI, with ↑↑↑ MD and RD in the thalamocortical somatosensory WM tract, and ↑↑↑ AD in the thalamocortical prefrontal WM tract) | Poor standardization of MRI methods | Zhou et al, Front Hum Neurosci 2016 |

RRMS (n=20)
HC (n=20)MFIS

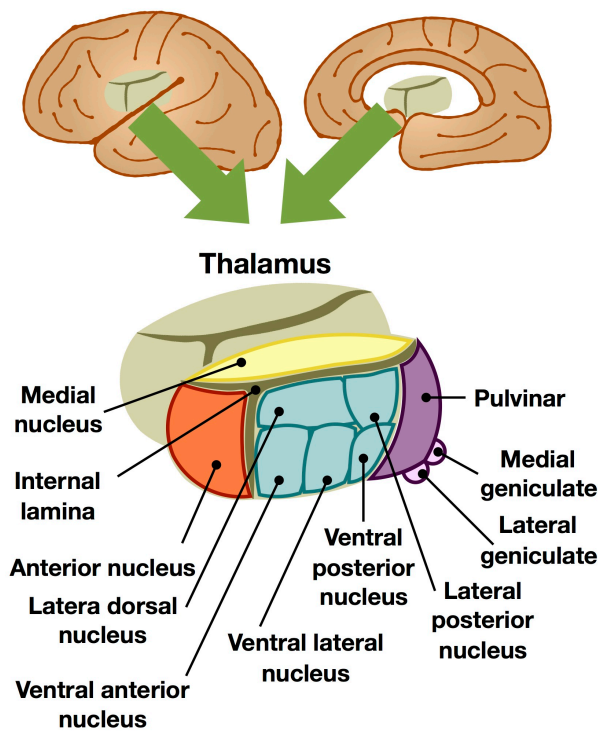
(self-reported)MS: multiple sclerosis; MRI: magnetic resonance imaging; fMRI: functional MRI; RRMS: relapsing-remitting MS; HC: healthy control; FSS: Fatigue Severity Scale; MFIS: Modified Fatigue Impact Scale; RS: resting state; GM: grey matter; WM: white matter; PPMS: primary progressive MS; SPMS: secondary progressive MS; FC: functional connectivity; FA: fractional anisotropy; MD: mean diffusivity; RD: radial diffusivity; AD: axial diffusivity; FSMC: Fatigue Scale for Motor and Cognitive Functions; DTI: diffusion tensor imaging.

Figure 1. Anatomical and functional classification of the thalamus.

Anatomically (A), the thalamus is divided by the internal medullary lamina (green) into anterior nucleus (red), lateral region (containing lateral dorsal, ventral anterior, ventral lateral, ventral posterior and lateral posterior nuclei, in blue), medial region (yellow), and posterior region (including pulvinar, medial geniculate and lateral geniculate nuclei, in violet).

Functionally (B), thalamic nuclei can be divided into relay nuclei, association nuclei and non-specific nuclei. Sensory relay nuclei send somatosensory (red), visual (pink), and auditory (green) inputs to different sensory cortical areas. Motor relay nuclei connect subcortical motor structures with cortical motor areas (blue). Limbic relay nuclei connect different structures of the limbic system (violet). The association nuclei receive input from the cerebral cortex and project back to the cerebral cortex (orange). The nonspecific nuclei send diffuse projections to the cerebral cortex.

A. Anatomical classification



B. Functional classification

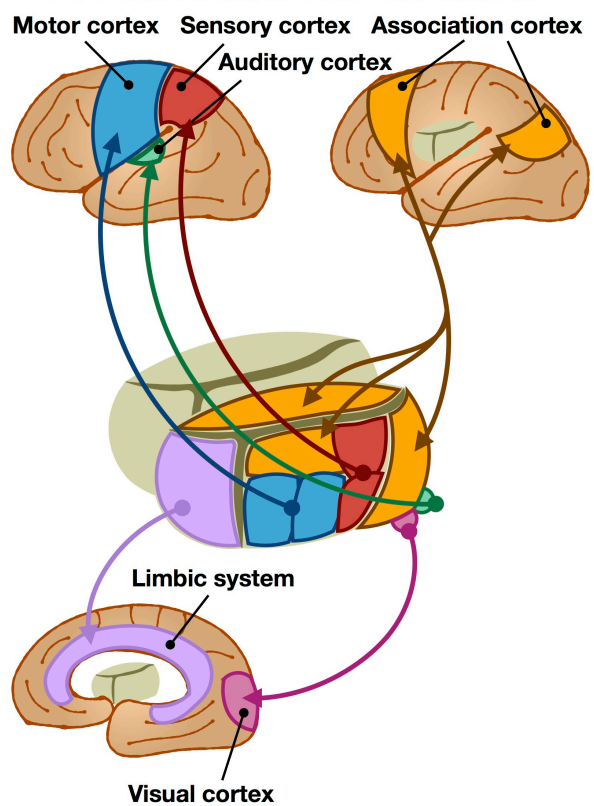


Figure 2. Normal thalamic function and possible changes in fatigued patients.

In healthy subjects, thalamus acts as a relay between different cortical and subcortical areas (A). In fatigued MS patients, fatigue can arise from an increase in the activity of the network, as a result of a compensatory mechanism that allows to maintain normal functioning but also produces fatigue (B). Alternatively, fatigue can arise from a global reduction in the activity of the network involving thalamus, basal ganglia and cortex (C).

