Supplementary material

Supplementary Table S1:

Clinical disease type	Age	Gender	Total disease duration (months	Cause of death	Deep grey matter Tissue
ACMS 1	35	Male	1,5	Directly MS related	Inactive, Normal appearing
ACMS 2	68	Female	2	Directly MS related	Normal appearing
ACMS 3	69	Female	2	Directly MS related	Normal appearing
ACMS 4	51	Female	5	Directly MS related	Active, Inactive, Normal appearing
ACMS 5	59	Male	5	Directly MS related	Inactive, Normal appearing
ACMS 6	28	Male	2	Directly MS related	Inactive, Normal appearing
RRMS 1	35	Female	108	Unavailable	Inactive, Normal appearing
RRMS 2	39	Female	120	Unavailable	Inactive, Normal appearing
RRMS 3	69	Female	108	Unavailable	Inactive, Normal appearing
RRMS 4	58	Male	120	Unavailable	Inactive, Normal appearing
RRMS 5	57	Female	156	Cardiovascular failure under peripheral septic conditions	Inactive, Normal appearing
RRMS 6	44	Female	262	Pulmonary Embolism	Inactive, Normal appearing
RRMS 7	56	Female	Unavailable	Unavailable	Inactive, Normal appearing
SPMS 1	66	Female	96	Pneumonia / Aspiration Pneumonia	Inactive, Normal appearing
SPMS 2	41	Male	137	Myocardial Infarction	Active, Inactive, Normal appearing
SPMS 3	60	Male	234	Respiratory failure	Inactive, Normal appearing
SPMS 4	53	Female	241	Pneumonia / Aspiration Pneumonia	Active, Inactive, Normal appearing
SPMS 5	56	Female	408	Drowning in bath tube	Inactive, Normal appearing
SPMS 6	46	Female	444	Cardiovascular failure under peripheral septic conditions	Active, Inactive, Normal appearing
SPMS 7	70	Female	120	Unavailable	Inactive, Normal appearing
SPMS 8	31	Female	132	Unavailable	Inactive, Normal appearing
SPMS 9	62	Female	144	Pneumonia / Aspiration Pneumonia	Inactive, Normal appearing
SPMS 10	69	Female	170	Pneumonia / Aspiration Pneumonia	Normal appearing
SPMS 11	45	Female	240	Cardiovascular failure under peripheral septic conditions	Inactive, Normal appearing
SPMS 12	84	Female	264	Pulmonary Embolism	Inactive, Normal appearing
SPMS 13	64	Female	336	Cardiovascular failure under peripheral septic conditions	Inactive, Normal appearing
SPMS 14	56	Male	372	Cardiovascular failure under peripheral septic conditions	Active, Inactive, Normal appearing
SPMS 15	78	Female	372	Pulmonary Embolism	Inactive, Normal appearing
SPMS 16	76	Male	372	Cardiovascular failure under peripheral septic conditions	Inactive, Normal appearing
SPMS 17	69	Female	384	Cardiovascular failure under peripheral septic conditions	Normal appearing
SPMS 18	73	Male	408	Cardiovascular failure under peripheral septic conditions	Inactive, Normal appearing
SPMS 19	81	Female	432	Cardiovascular failure under peripheral septic conditions	Inactive, Normal appearing
SPMS 20	59	Female	492	Cardiovascular failure under peripheral septic conditions	Inactive, Normal appearing
SPMS 21	59	Female	72	Unavailable	Inactive

SPMS 22	54	Female	168	Unavailable	Inactive, Normal appearing
SPMS 23	43	Male	192	Unavailable	Inactive, Normal appearing
PPMS 1	52	Female	30	Cardiovascular failure under peripheral septic conditions	Inactive, Normal appearing
PPMS 2	55	Female	60	Pulmonary Embolism	Inactive, Normal appearing
PPMS 3	67	Male	87	Cardiovascular failure under peripheral septic conditions	Inactive, Normal appearing
PPMS 4	56	Female	132	Adult respiratory distress syndrome	Inactive, Normal appearing
PPMS 5	34	Female	204	Cardiovascular failure under peripheral septic conditions	Inactive
PPMS 6	38	Female	336	Unavailable	Inactive, Normal appearing
PPMS 7	83	Female	360	Cardiovascular failure under peripheral septic conditions	Normal appearing
PPMS 8	75	Female	372	Pulmonary Embolism	Inactive, Normal appearing
PPMS 9	64	Female	204	Unavailable	Inactive
PPMS 10	73	Female	411	Myocardial infarction	Inactive, Normal appearing
PPMS 11	75	Female	314	Unavailable	Inactive, Normal appearing
Progressive MS 1	66	Female	120	Cardiovascular failure under peripheral septic conditions	Normal appearing
Subclinical MS 1	66	Female	-	Cardiovascular failure under peripheral septic conditions	Normal appearing
Subclinical MS 2	69	Female	-	Cardiovascular failure under peripheral septic conditions	Normal appearing
Benign MS 1	72	Female	-	Systemic malignancy	Inactive, Normal appearing
Control 1	30	Female	-	Cardiovascular failure under peripheral septic conditions	Control tissue
Control 2	36	Female	-	Myocardial Infarction	Control tissue
Control 3	37	Male	-	Pulmonary Embolism	Control tissue
Control 4	39	Female	-	Cardiovascular failure under peripheral septic conditions	Control tissue
Control 5	45	Female	-	Myocardial Infarction	Control tissue
Control 6	46	Male	-	Cardiovascular failure under peripheral septic conditions	Control tissue
Control 7	65	Male	-	Hemoptysis	Control tissue
Control 8	70	Male	-	Renal failure	Control tissue
Control 9	71	Female	-	Pulmonary Embolism	Control tissue
Control 10	71	Female	-	cardiovascular failure under peripheral septic conditions	Control tissue
Control 11	83	Male	-	Systemic malignancy	Control tissue
Control 12	97	Female	-	Pneumonia / Aspiration Pneumonia	Control tissue

Supplementary Table S1: Supplementary Table S1 depicts detailed clinical data of 12 controls and 51 MS patients of whom DGM tissue was available for analysis. Subclinical MS (n = 2) was diagnosed when a routine autopsy revealed multiple sclerosis pathology in patients with no clinical history of neurodegenerative disease. Benign multiple sclerosis (n = 1) was diagnosed when, after 10 years of disease, the EDSS score was below or equal to 3. One MS case was diagnosed with progressive MS but could not be further classified.

Acute MS patients = ACM; Relapsing/remitting multiple sclerosis = RRMS; Secondary progressive multiple sclerosis = SPMS; Primary progressive multiple sclerosis = PPMS

Supplementary Table S2

Patients	**Anatomical structure**				
	Internal capsule	Insula cortex	Putamen	Caudate nucleus	Globus pallidus
n (MS patiens)	25	14	29	20	23
n (MS patients, histo blocks)	30	15	38	25	28
n (Control patiens)	12	7	11	10	9
n (Control patients, histo blocks)	14	7	12	11	9

Supplementary Table S2: Supplementary Table S2 depicts the number of multiple sclerosis (MS), control patients and respective tissue blocks, included in the subanalysis on routine sections for inflammation, iron, oxidative stress and neurodegeneration, separated by anatomical structures.

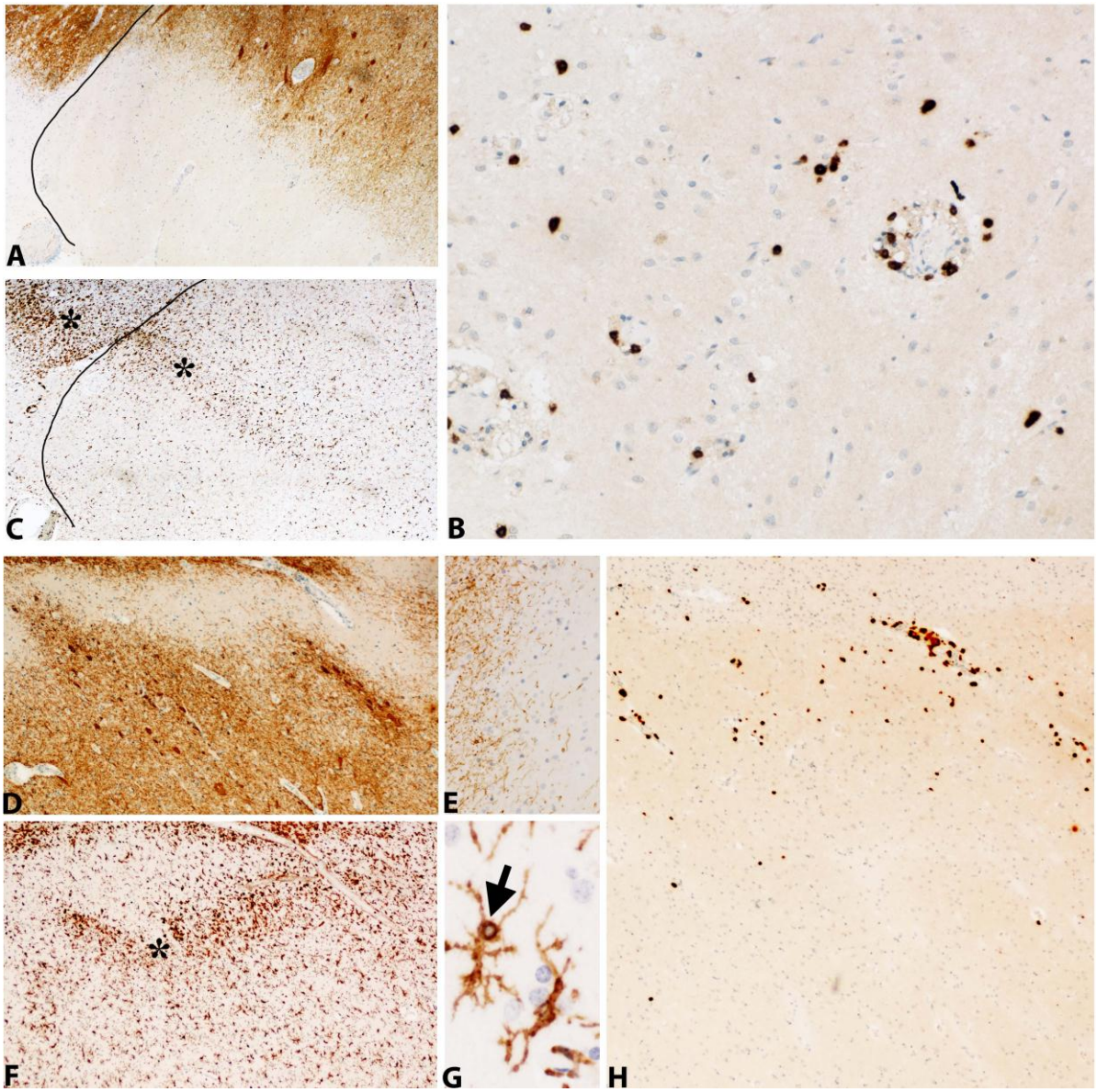
Supplementary Table S3

	Perivascular cuffs		
	none	few 1-10	many >10
Active DGM lesions	1	3	1
	20.0%	60.0%	20.0%
Inactive DGM lesions	32	5	1
	84.2%	13.2%	2.6%
Normal appearing DGM	8	0	0
	100.0%	0.0%	0.0%
Total DGM	41	8	2
	80.4%	15.7%	3.9%

Supplementary Table S3: Evaluation of perivascular cuffs in the DGM of patients with multiple sclerosis

Supplementary Table S3 shows data on the evaluation of the perivascular accumulation of inflammatory cells (cuffs) in the DGM of patients with multiple sclerosis. The values represent the total number and percentage. The data are pooled for total DGM so that one value per patient is given (n = 51). Each patient was categorised as having DGM with active lesions, DGM with inactive lesions, or non-lesioned DGM. The patients with active demyelinating DGM lesions present with significantly more perivascular inflammatory cuffs compared to patients with only inactive DGM lesions or patients with multiple sclerosis without DGM lesions ($p = 0.007$; $p = 0.007$, respectively).

Supplementary Figure S1: CD3 positive parenchymal and perivascular T-cell infiltration is present in active deep grey matter lesions



Supplementary Figure S1 depicts stainings derived from brains of two MS patients. The first subject is a 41-year-old male SPMS patient with 137 months disease duration (A-C). The second subject is a 46-year-old female SPMS patient with 444 months disease duration (D-H).

A: Proteolipid protein (PLP) staining of an active deep grey matter lesion in the caudate nucleus.

B: CD3-positive T-cells are found both in the parenchyma and perivascular spaces in the active deep grey matter lesion.

C: p22-phox staining shows increased density of immunoreactive, activated microglia/macrophages at the expanding lesion edge (asterisk). Note the sharp decrease in p22-phox reactivity at the white matter / deep grey matter border, which is indicated by the black line.

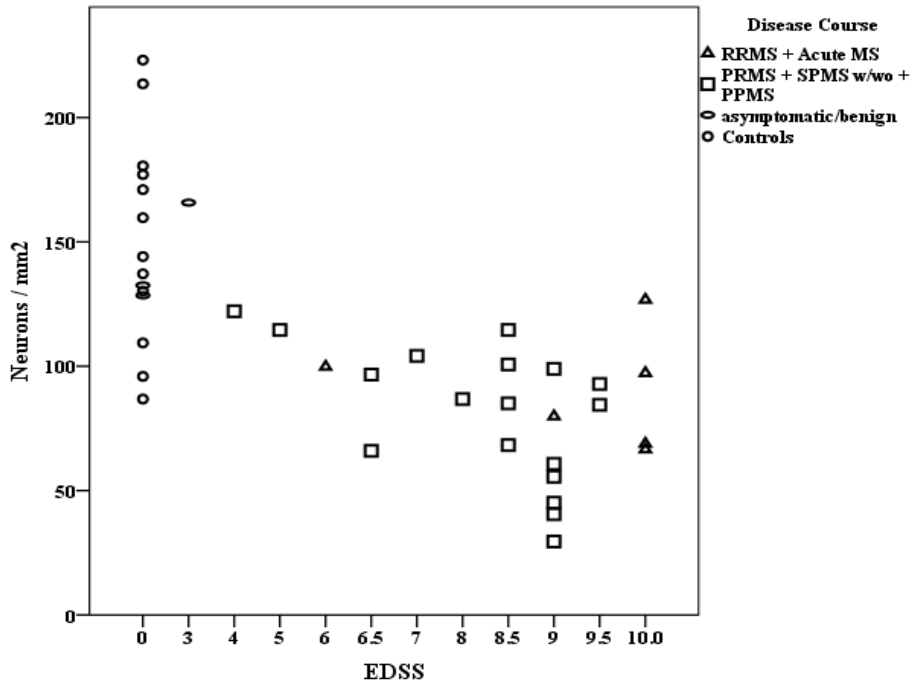
D, E: PLP staining of an active deep grey matter lesion in the caudate nucleus at different magnifications. E: Lesion boarder

F: p22-phox immunoreactivity shows activated microglia/macrophages at the expanding lesion edge (asterisk).

G: Higher magnification of p22-phox immunoreactive activated microglia (arrow) in the DGM lesion.

H: CD-3-positive T-cells are found in perivascular spaces and in the parenchyma.

Supplementary Figure S2: Diffuse DGM neuronal loss is predictive of clinical status



Supplementary Figure S2 shows that the EDSS negatively correlates with neuronal density in the NADGM of patients with multiple sclerosis ($r = -0.5$; $p = 0.004$). The presented multiple sclerosis cases include patients of all disease courses: acute multiple sclerosis, relapsing/remitting multiple sclerosis, progressive-relapsing multiple sclerosis, secondary progressive multiple sclerosis and primary progressive multiple sclerosis as well as benign/subclinical multiple sclerosis. For reasons of graphical visualisation, the neuronal densities of control patients are included in the scatter diagram at an EDSS of zero. It is important to note that these values are not included in the Spearman correlation analysis. In order to obtain these data, neurons identified by the presence of a prominent nucleolus in HE staining were manually counted in 10 visual fields (à 0.0576 mm^2)/normal-appearing putamen and 10 visual fields/normal-appearing caudate nucleus, in both multiple sclerosis and control patients. The mean neuronal density values were calculated per patient. Spearman's correlation analysis was used for the statistical evaluation.