Early Silent Microstructural Degeneration and Atrophy of the Thalamocortical Network in Multiple Sclerosis

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Abstract: Recent studies on patients with clinically isolated syndrome (CIS) and multiple sclerosis (MS) demonstrated thalamic atrophy. Here we addressed the following question: Is early thalamic atrophy in patients with CIS and relapsing-remitting MS (RRMS) mainly a direct consequence of white matter (WM) lesions—as frequently claimed—or is the atrophy stronger correlated to “silent” (nonlesional) microstructural thalamic alterations? One-hundred and ten patients with RRMS, 12 with CIS, and 30 healthy controls were admitted to 3 T magnetic resonance imaging. Fractional anisotropy (FA) was computed from diffusion tensor imaging (DTI) to assess thalamic and WM microstructure. The relative thalamic volume (RTV) and thalamic FA were significantly reduced in patients with CIS and RRMS relative to healthy controls. Both measures were also correlated. The age, gender, WM lesion load, thalamic FA, and gray matter volume-corrected RTV were reduced even in the absence of thalamic and extensive white matter lesions—also in patients with short disease duration (≤ 24 months). A voxel-based correlation analysis revealed that the RTV reduction had a significant effect on local WM FA—in areas next to the thalamus and basal ganglia. These WM alterations could not be explained by WM lesions, which had a differing spatial distribution. Early thalamic atrophy is mainly driven by silent microstructural thalamic alterations. Lesions do not disclose the early damage of thalamocortical circuits, which seem to be much more affected in CIS and RRMS than expected. Thalamocortical damage can be detected by DTI in normal appearing brain tissue. *Hum Brain Mapp* 37:1866–1879, 2016. © 2016 Wiley Periodicals, Inc.

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INTRODUCTION

Thalamus—the Most Investigated Grey Matter Structure in Multiple Sclerosis

Multiple sclerosis (MS) has traditionally been viewed as an immune-mediated, inflammatory, and demyelinating disease of the human central nervous system predominantly affecting the white matter (WM) [Cifelli et al., 2002; Geurts et al., 2012; Hannoun et al., 2012; Horakova et al., 2012; Hulst and Geurts, 2011]. Although pathological studies dating back to the 19th century have already reported grey matter involvement, it lasted until the 21st century, that grey matter demyelination as well as axonal and neu-

ronal degeneration were increasingly recognized as important pathological hallmarks in this “typical” WM disease [Geurts et al., 2012; Hulst and Geurts, 2011]. Among all grey matter structures, the thalamus as a convergence zone and gateway to the cortex has been studied most extensively [Minagar et al., 2013]. The thalamus with its unique structural division into various nuclei has widespread reciprocal connections to cortical and subcortical regions, making this anatomical structure to a highly sensitive “barometer of diffuse neuropathological damage” [Cifelli et al., 2002] even if these are evolving in remote areas of the brain [Al-Radaideh et al., 2013; Minagar et al., 2013]. Second, the thalamus together with the cerebral cortex forms the thalamocortical loop—one of the most fundamental networks in the brain [McCormick, 1999]. Under physiological conditions, the thalamocortical network generates either rhythmic, high-frequency activity in an awake organism, related to multiple functions such as attention, awareness, perception of sensory input or preparation of movements, or low-frequency rhythms during states of drowsiness or slow-wave sleep [McCormick, 1999]. Alterations in the normal functioning of this highly complex fine-tuned network resulting in inappropriate rhythmic activity were denominated as “thalamocortical dysrhythmia” by Llinás et al. They discussed this “thalamocortical dysrhythmia” as “rosetta stone” of a subset of neurological disorders including movement disorders as “tremorgenic” syndromes, forms of epilepsy, chronic pain, and even neuropsychological dysfunction [Llinas et al., 1999]. Moreover, the thalamus plays a unique role in a wide range of neurological functions including motor, sensory, integrative, higher cortical, and executive functions. Information processing speed, psychomotor speed, visuospatial memory, verbal and visual learning, emotion, mood, motivation, ocular motility, posture, and language modulation are further functions [Batista et al., 2012; Benedict et al., 2013; Houtchens et al., 2007; Minagar et al., 2013; Schoonheim et al., 2012; Tovar-Moll et al., 2009]. Its central and ideal strategic position with fundamental impact on the whole brain, and its significant role in numerous important functions render the thalamus to an interesting grey matter structure in MS, which might help to explain the variety of early appearing permanent but also waxing and waning disease symptoms.

Recent Findings of Thalamic Damage in MS

By using different segmentation techniques, e.g., voxel-based morphometry, FreeSurfer, or FIRST, a thalamic atrophy was demonstrated in patients with clinically isolated syndrome (CIS) [Audoin et al., 2010; Bergsland et al., 2012;

Abbreviations

ACID	artifact correction in diffusion MRI (software toolbox)
CIS	clinically isolated syndrome
DTI	diffusion tensor imaging
DWI	diffusion-weighted images
EDSS	expanded disability status scale
EPI	echo planar imaging
FA	fractional anisotropy
FLAIR	fluid-attenuated inversion recovery
FOV	field of view
FWE	familywise error
FWHM	full-width at half-maximum (Gaussian kernel size)
GRAPPA	generalized autocalibrating partially parallel acquisition
ICV	intracranial volume
LST	lesion segmentation toolkit
MNI	Montréal Neurological Institute
MPRAGE	magnetization prepared rapid gradient echo
MRI	magnetic resonance imaging
MS	multiple sclerosis
ROI	region of interest
RRMS	relapsing-remitting MS
RTV	relative thalamic volume
TE	echo time
THL	thalamic lesion load
TR	repetition time
TSE	turbo spin-echo
T1w	T1-weighted
T2w	T2-weighted
VBS	voxel-based statistics
WM	white matter
WMLL	WM lesion load
WMLL _{T1}	volume of white matter hypointense lesions in T1w images
WMLL _{FLAIR}	volume of white matter hyperintense lesions in FLAIR images
y	years

Henry et al., 2008; Rocca et al., 2010] and in children and adults with different subtypes of MS [Audoin et al., 2006; Batista et al., 2012; Benedict et al., 2013; Bergsland et al., 2012; Ceccarelli et al., 2008; Hannoun et al., 2012; Houtchens et al., 2007; Mesaros et al., 2008; Mesaros et al., 2011; Ramasamy et al., 2009; Rocca et al., 2010; Schoonheim et al., 2012; Sepulcre et al., 2006; Zivadinov et al., 2012]. Thalamic atrophy was shown to have a prognostic value in predicting conversion from CIS to clinically definitive MS [Calabrese et al., 2011], to proceed gradually in patients with CIS and relapsing-remitting MS (RRMS) compared to secondary progressive MS within one year [Rocca et al., 2010], and to precede cortical atrophy [Bergsland et al., 2012]. MR spectroscopy, histopathological, and immunohistochemical postmortem studies found an extensive neuronal loss and atrophy in the thalamus of patients with different MS subtypes [Cifelli et al., 2002; Geurts et al., 2006; Vercellino et al., 2009; Wylezinska et al., 2003]. Significantly increased iron values were detected in the thalamic pulvinar nucleus of adults with CIS [Al-Radaideh et al., 2013; Hagemeyer et al., 2012] and along with atrophy in patients with RRMS and secondary progressive MS [Hagemeyer et al., 2013; Zivadinov et al., 2012]. It was suggested that iron deposition may precede structure-specific atrophy development [Hagemeyer et al., 2012]. While all these studies have intensively examined alterations of volume, neuronal density, and iron content in the thalamus of patients with MS, measures of more subtle and potentially earlier changes of tissue integrity were neglected [Benedict et al., 2013]. Recently, diffusion tensor imaging (DTI) studies could uncover functionally relevant, microscopic alterations in the thalamus of patients with MS. It was shown that the thalamus of patients with different MS subtypes was not only reduced in volume but also microscopically altered [Benedict et al., 2013; Ciccarelli et al., 2001; Hannoun et al., 2012; Mesaros et al., 2011; Tovar-Moll et al., 2009].

Objectives of This Study

Despite this vast evidence for volumetric and microstructural thalamic alterations in patients with MS, it still remained unclear whether the detected thalamic atrophy was mainly a consequence of WM damage or whether early, WM-independent microstructural pathological processes within the thalamus also played a relevant role. The latter hypothesis was supported by recent findings of our group [Deppe et al., 2013]. In this context, we sought to address the following questions:

I. Can the thalamus already be atrophic in patients with CIS and early RRMS (disease duration ≤ 24 months) who have no thalamic and extensive WM lesions? II. Is early thalamic atrophy accompanied by microstructural thalamic alterations as reflected by reductions of fractional anisotropy (FA)? III. Is the degree of thalamic atrophy correlated with the amount of thalamic FA decrease? IV. Are changes

in the thalamus stronger correlated to microstructural alterations of white matter tracts that traverse typical MS lesion sites or to nonlesional white matter changes next to the thalamus and thalamic white matter tracts? V. Is thalamic atrophy correlated with cortical volume? VI. Is thalamic atrophy related to clinical impairment as measured by the patients' expanded disability status scale (EDSS) or disease duration. VII. How is the thalamus affected in patients with advanced disease and extensive WM lesion load (WMLL)?

METHODS

Subjects and Their Main Demographic Clinical Features

We recruited 110 consecutive patients of our clinic with RRMS and 12 patients with CIS, diagnosed according to the revised McDonald criteria [Polman et al., 2011]. Moreover 30 age- and gender-matched neurologically and psychiatrically healthy controls were included. They were recruited by announcements in local newspapers. Descriptive details of all examined 152 participants are given in Table I. Participants were excluded if they had any pre-existing medical condition known to be associated with brain pathology, were pregnant, previously or currently addicted to substances. Additional exclusion criteria for patients were a relapse or systemic therapy with steroids (intravenous, intrathecal, or oral) within the month before MRI and a history of additional neurological or psychiatric disorders. All patients were neurologically examined. The same specially trained and certified neurologist of our department assessed EDSS scores. Descriptive statistics about the therapies of RRMS patients are given in Table II. All subjects gave their written informed consent to participating in this study. The interdisciplinary ethics committee of the University of Münster and the Physicians' Chamber of Westphalia-Lippe (Ärztekammer Westfalen-Lippe) approved all examinations (2010-378-b-S).

Magnetic Resonance Imaging

Briefly, all participants were scanned using the same 3 T Siemens TIM Trio MRI scanner and a 12-channel (matrix) head coil (Siemens AG, Erlangen, Germany). Employing the same MRI parameters and protocols, we obtained images of the following MRI sequences for all subjects: a native isotropic 3D T1-weighted (T1w) MPRAGE sequence (field of view (FOV) $256 \times 256 \text{ mm}^2$, slice thickness 1.0 mm, matrix 256×256 , no gap, repetition time (TR) 2000 ms, echo time (TE) 2.52 ms, generalized autocalibrating partially parallel acquisition (GRAPPA) factor 2) and an axial diffusion-weighted echo planar imaging (EPI) for DTI (41 slices, FOV $230 \times 230 \text{ mm}^2$, slice thickness 3.6 mm, matrix 128×128 , no gap, slice order interlaced, two averages, TR 7000 ms, TE 104 ms, phase encoding

TABLE I. Descriptive statistics about all examined subgroups

Group	N	Age/years					EDSS		
		Mean	Median	Min	Max	SD	Median	Min	Max
Healthy Control	30	38.1	34.5	23.0	69.0	13.3			
Patients (total)	122	37.0	36.5	18.0	56.0	9.87	2.0	0.0	6.0
CIS	12	43.3	43.0	26.0	56.0	9.1	1.5	0.0	3.0
RRMS (total)	110	36.3	36.0	18.0	56.0	9.8	2.0	0.0	6.0
RRMS Subgroups									
WMLL:low THL:no	46	35.9	36.0	18.0	54.0	10.7	2.0	0.0	6.0
WMLL:low THL:yes	10	35.3	35.0	26.0	46.0	6.7	2.0	0.0	2.5
WMLL:high THL:no	42	36.0	35.0	19.0	56.0	9.7	2.0	0.0	5.5
WMLL:high THL:yes	12	39.8	39.0	25.0	53.0	8.5	2.0	1.0	4.0
Early (Patients with CIS or RRMS and disease duration ≤ 24 months)	32	35.1	34.0	18.0	53.0	10.8	1.5	0.0	4.0

Descriptive statistics about the age-matched examined subgroups of patients with RRMS, CIS, “early” RRMS and CIS (disease duration ≤ 24 months), and the group of healthy controls. Pairwise group comparisons by *t*-tests revealed no indication for any systematic age difference.

N = number; SD = standard deviation; THL:yes = presence of thalamic lesions; THL:no = no thalamic lesions; WMLL = white matter lesion load; WMLL:low = WMLL ≤ 2.5 mL; WMLL:high = WMLL > 2.5 mL.

direction: anterior to posterior, 20 gradient directions with $b = 1000$ s/mm², $5 \times b = 0$ s/mm², 2 averages, GRAPPA factor 2). The following MRI sequences were only applied for patients to assess their lesion load: an axial turbo spin-echo (TSE) fluid attenuated inversion recovery (FLAIR) (44 slices, FOV 250×250 mm², slice thickness 3.0 mm, matrix 256×256 , TR 9200 ms, TE 88 ms, no gap, slice order interlaced, inversion time 2300 ms, flip angle 150°), a sagittal TSE FLAIR (32 slices, FOV 240×240 mm², slice thickness 3.0 mm, matrix 256×256 , no gap, slice order interlaced), a 3D T1w sequence after intravenous Gadolinium-DTPA (diethylene triamine penta-acetic acid) injection (0.1 mmol/kg body weight), an axial T2-weighted (T2w) TSE “short” (46 slices, FOV 250×250 mm², slice thickness

3.0 mm, matrix 256×256 , TR 4000 ms, TE 18 ms, no gap, slice order interlaced), an axial T2w TSE “long” (46 slices, FOV 250×250 mm², slice thickness 3.0 mm, matrix 256×256 , TR 3000 ms, TE 72 ms, no gap, slice order interlaced, GRAPPA factor 2), and an axial proton density-weighted TSE (46 slices, FOV 250×250 mm², slice thickness 3.0 mm, matrix 256×256 , TR 3000 ms, TE 17 ms, no gap, slice order interlaced). Foam padding minimized head motion.

Image-Processing Pipeline

We employed an image-processing pipeline that analyzed the 3D T1w, DTI, and FLAIR images completely automatically. This pipeline is exclusively based on open-source tools: FreeSurfer (version 5.3, <http://surfer.nmr.mgh.harvard.edu/>) [Dale et al., 1999; Fischl et al., 2002; Fischl and Dale, 2000; Fischl et al., 1999; Horakova et al., 2012], Lesion Segmentation Toolkit (LST) (version 2.0.4, <http://www.applied-statistics.de/lst.html>) [Schmidt et al., 2012], Artifact correction in diffusion MRI (ACID) toolbox (<http://www.diffusiontools.com/>) [Glauche et al., 2010; Mohammadi et al., 2012], a model-based segmentation tool (FSL-FIRST) (<http://www.fmrib.ox.ac.uk/fsl/first/index.html>) [Patenaude et al., 2011], and a batch processing job control system that integrates the collection of the individual tools into a user friendly, robust web interface-based data analysis system. A schematic overview about the data flow is given in Figure 1. The first DTI processing step (Fig. 1, step 1) corrected all diffusion-weighted images (DWI) effectively for eddy currents and head movements [Mohammadi et al., 2010]. Afterward (Fig. 1, step 2), the multicontrast image registration algorithm of the ACID toolbox registered the DWIs rigorously into the Montréal

TABLE II. Overview over the distribution of therapies of patients with RRMS at the time of MRI examination

Name of active ingredient	Trade name	Number of patients	Number of patients in percent (%)
Interferon beta-1a	Avonex	14	12.73
Interferon beta-1b	Betaferon	3	2.73
Glatirameracetat	Copaxone	7	6.36
Diazoxide versus Placebo ^a		1	0.91
Interferon beta-1b	Extavia	1	0.91
Fingolimod	Gilenya	12	10.91
No therapy		17	15.45
Interferon beta-1a	Rebif	4	3.64
Teriflunomid	Aubagio	3	2.73
Natalizumab	Tysabri	48	43.64

^aThis therapy was taken in the context of a double-blind clinical study.

Figure 1.

Data flow and processing steps integrated into the analysis pipeline (DWI = diffusion-weighted images; FA = fractional anisotropy; FLAIR = fluid attenuated inversion recovery; LST = Lesion Segmentation Toolkit; MNI = Montréal Neurological Institute;

MPRAGE = magnetization prepared rapid gradient echo; ROI = region of interest; T1w = T1-weighted; VBS = voxel-based statistics).

Neurological Institute (MNI) coordinate space (registration type IV, [Mohammadi et al., 2012]). After DWI registration, the DWIs were smoothed (Gaussian kernel FWHM = $2 \times 2 \times 4$ mm) and final diffusion tensors and FA were calculated. The tensor calculation was performed by the robust tensor fitting method as implemented into the ACID toolbox. Because the FA is largely invariant concerning registration based local rotations and translations, no correction of diffusion directions was performed. For quantitative FA analysis (Fig. 1, step 3), the pipeline calculated average FA values within a region of interest (ROI) outlining the thalamus. The reproducibility and validity of this approach has been assessed elsewhere [Deppe et al., 2013]. Individual FA maps of healthy subjects, patients with RRMS and CIS were controlled for spatial congruency with special focus on the thalamus to validate that the ROI actually outlined the thalamus even in patients with high thalamic tissue damage (Fig. 2). We also checked visually all structural MRIs to exclude movement or other artifacts as cause for

abnormal thalamic volumes (Fig. 3). For automated detection of hyperintense lesions in the 2D FLAIR images, the pipeline registered and resliced the FLAIR images into the isotropic 3D T1w images space (Fig. 1, step 4). Binary FLAIR lesion maps and total WM lesion volumes ($WMLL_{FLAIR}$) of each patient were obtained by step 5. Step 6 generated a “lesion probability” map by adding up the 110 binary lesion maps. We used here the term “lesion probability” in conformity to other publications and not in a strict probabilistic sense. In step 7, all T1w images were corrected for contrast and intensity inhomogeneities. Afterward, absolute volume of the left and right thalamus, intracranial volume (ICV), volume of WM hypointensities ($WMLL_{T1}$), grey matter volume, and cortical volume were obtained from FreeSurfer [Deppe et al. 2014]. The amount of automatically detected hypointensities (black holes) in the 3D T1w images in units of mL served as measure for WM lesion load (WMLL) in addition to the $WMLL_{FLAIR}$. From the absolute thalamic volume (unit: mm^3) and the

Figure 2.

Exemplary FA maps of a healthy control subject, a patient with CIS, and a patient with advanced RRMS demonstrating data quality. The thalamic ROI as used for thalamic mean FA calculation is outlined. The three examples demonstrate that thalamic FA alterations occur over the whole thalamus and are not an artifact due to ROI misalignment in atrophic thalami. The color legend represents FA values. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 3.

T1-weighted and FLAIR MRI of a patient (39 y) with RRMS and relatively low white matter lesion load (2.2 mL, n.s.) and normal appearing thalamus. However, quantitative analysis revealed that thalamic volume was significantly reduced (RTV = 0.822%,

$p = 0.014$) and thalamic microstructure was extremely altered (FA = 0.186, $p = 0.0006$, Figure 2). The caudate nucleus (Cd), globus pallidus (Gp), internal capsule (ic), and thalamus (Th) are outlined.

ICV (unit: mm³), we calculated the relative (percentage) thalamic volume as

$$\text{RTV} = \text{absolute thalamic volume} / \text{ICV} \times 100\%. \quad (1)$$

We calculated the thalamic volume by two complementary techniques: (1) FreeSurfer 5.3 performs subcortical and cortical segmentation and assigns a neuroanatomical label to each voxel based on probabilistic information automatically estimated from a large training set of expert measurements. (2) FIRST, a tool from the FSL software suite developed by the Oxford University, Oxford UK, performs subcortical segmentation using Bayesian shape and appearance models. By the application of these two complementary techniques, we achieved quite similar results by Pearson product-moment correlation ($R = 0.81$, $p < 10^{-6}$, Supporting Information, Fig. S1), although FreeSurfer showed systematically smaller volumes. Due to the qualitatively congruent results between FSL-FIRST and FreeSurfer, we decided to present only thalamic volume values as obtained by FreeSurfer.

Lesion Analysis

All images were conventionally inspected for clinically relevant findings by an experienced neuroradiologist who also diagnosed and quantified the thalamic T2w/FLAIR lesion load. The volumes of detected hyperintense WM lesions in FLAIR images ($\text{WMLL}_{\text{FLAIR}}$) and the WMLL_{T1} as calculated by FreeSurfer from the T1w images were compared by a linear regression analysis. Due to the high correlation between both lesion estimates (Supporting Information, Fig. S2), we only used WMLL_{T1} for quantifying lesion volume. WMLL_{T1} and WMLL are therefore used synonymously in the following.

Group Analysis

We subdivided all patients with RRMS into four subgroups according to a “low” or “high” WMLL ($\text{WMLL}:\text{low}$, $\text{WMLL}:\text{high}$) and the presence of thalamic lesions ($\text{THL}:\text{yes}$, $\text{THL}:\text{no}$). As threshold for a “low” WMLL , we used 2.5 mL. This threshold was defined *a priori* and corresponded to the maximum WMLL detected in the group of healthy controls. In addition, we created a subgroup of patients with “early” disease that consisted of patients with RRMS and CIS and a disease duration ≤ 24 months. Resulting subgroup sizes, age, and EDSS statistics are given in Table I. No other inclusion criteria for the subgroups were employed than those mentioned above. It turned out that all subgroups were well age-matched to each other and to the group of healthy controls. Patients with CIS had a slight, but not significant tendency to be older than those of the other groups (Table I). This tendency resulted from the fact that many younger, initially included as CIS patients in the study had a new WM lesion on study MRI and thereby had to be assigned to the

RRMS group (fulfilled criteria of dissemination in time). This is why the group of CIS consisted mostly of patients having a CIS already for a long time, and thereby being slightly older (Table I). The trend to a higher mean age in the CIS group had in particular no impact on the results because we considered the factor Age in our statistics.

Differences of absolute thalamic volume and ICV between patients (RRMS and CIS) and healthy controls and of RTV and thalamic FA between all groups were tested by *t*-tests. With a univariate multifactorial general linear model, we investigated the hypothesis of a “silent” (lesion-independent) thalamic degeneration in the group of patients, questioning whether the RTV as the dependent variable was significantly reduced in patients with RRMS and CIS although the (linear) effects of the following covariates and categorical factors had been already considered in the model: WMLL , thalamic FA, age, grey matter volume, gender (F, M), and group (healthy controls, CIS, RRMS-THL:no, RRMS-THL:yes). By regression analyses, we estimated the correlation between absolute thalamic volume and ICV, between RTV, thalamic FA, disease duration, cortical volume, WMLL , and EDSS.

SPM8 Voxel-Based FA Correlation Analysis

A linear regression model was used to estimate the correlation between regional WM FA values as provided by the spatially normalized FA maps and RTV (as covariate) (Fig. 1, step 8). Results were assessed at a threshold of $p < 0.05$, familywise error corrected (FWE) for multiple comparisons and also tested at a $p < 0.001$, uncorrected.

RESULTS

Effect of ICV on absolute thalamic volume: All participants were evenly distributed concerning the ICV. However, patients with RRMS and also those with CIS had on average a 12% lower absolute thalamic volume compared to healthy controls (RRMS: $p < 0.00001$; CIS: $p < 0.02$). Absolute thalamic volume and ICV correlated significantly in all participants (Fig. 4). We carried out all confirmatory statistics by using the RTV as dependent variable to avoid confounding effects of ICV on thalamic volumes [Eq. (1)].

Objectives I and II: The results of pairwise performed *t*-tests about RTV and thalamic FA group mean differences are given in Figure 5. RTV and thalamic FA were significantly reduced in all examined (sub)groups relative to healthy controls. The existence of thalamic lesions showed only a trend, but no *significant* effect on the RTV of patients with RRMS (Fig. 5A). Although four patients with CIS had thalamic lesions and eight were without, there was no indication for a systematic difference of mean RTV (CIS THL:yes: mean RTV = 0.93; CIS THL:no: mean RTV = 0.94, $p = 0.82$, $t = -0.24$) or mean thalamic FA (CIS THL:yes: mean thalamic FA = 0.25; CIS THL:no: mean thalamic FA = 0.26, $p = 0.77$, $t = -0.30$).

Figure 4.

Significant correlation between the absolute thalamic volume and the intracranial volume (ICV) of patients with RRMS, CIS, and healthy controls (CTRL). Patients with RRMS and also those with CIS had on average a 12% lower absolute thalamic volume compared to healthy controls (RRMS: $p < 0.00001$; CIS: $p < 0.02$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Objective III: RTV and thalamic FA were significantly correlated with each other in patients with CIS and in all RRMS subgroups, except for the subgroup “RRMS WMLL:low THL:yes” (Fig. 6).

Figure 6.

Significant linear regressions between thalamic fractional anisotropy (FA) and relative thalamic volume (RTV) for all subgroups of patients except for RRMS WMLL:low THL:yes. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Objective IV: The FLAIR “lesion probability” map showed highest probability for FLAIR lesions within and around the corpus callosum (congruent to Dawson fingers) and around the body and occipital horns of the lateral ventricles (Fig. 7, green areas). The regions of highest

Figure 5.

(A) Mean relative thalamic volume in percent of the intracranial volume (RTV) of all examined (sub)groups. In contrast to the white matter lesion load, the existence of thalamic lesions had no significant (n.s.) effect on the RTV. The significance levels for differences in means of the different samples are marked. **(B)** Mean fractional anisotropy (FA) within the thalamic ROI of all examined

(sub)groups. The existence of thalamic lesions and the white matter lesion load had a significant effect on the thalamic FA of patients with RRMS. The significance levels of the t -tests for the different samples are marked. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 7.

Combination of **(1)** the MNI-152 T1-weighted image for spatial orientation, **(2)** the significant voxels of the SPM8 correlation analysis with dependent variable fractional anisotropy (FA) and independent factor relative thalamic volume (RTV) (red), and **(3)** the white matter lesion distribution in FLAIR images ("lesion probability map") (green). This comparison illustrates that the

microstructural white matter damage (FA reduction) which is correlated with thalamic atrophy occurred mainly next to the thalamus and basal ganglia and showed nearly no overlap with the WM lesion distribution in FLAIR images. Shown t-values survived familywise error correction. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

correlation between local WM FA and RTV were next to the thalamus, basal ganglia, hippocampus, internal capsule, postcentral thalamocortical pathways, midbrain, brainstem, cerebellar WM, and corpus callosum (Fig. 7, red areas).

Objective V: RTV correlated also with cortical volume, but only in RRMS patients and not in the healthy control group (RRMS: left hemisphere $R = 0.33$, right hemisphere $R = 0.34$, $p < 0.001$, healthy controls: left and right hemisphere: $p > 0.35$, respectively).

Objective VI: The RTV of the 122 patients correlated to the EDSS ($R = 0.20$, $p = 0.022$) and highly significantly to the disease duration ($R = 0.46$, $p < 10^{-6}$, Fig. 8). The WMLL of the 122 patients also correlated to the EDSS ($R = 0.20$, $p = 0.028$) and highly significantly to the disease duration ($R = 0.49$, $p < 10^{-6}$).

Objective VII: Thalamic atrophy and FA reduction were most pronounced in patients with a high WMLL (> 2.5 mL) (Fig. 5). However, the relationship between RTV and WMLL was not linear. Instead we found a logarithmic dependency between thalamic atrophy and WMLL in the 122 patients ($\text{RTV} [\%] = 1.0028 - 0.1693 \log_{10}(\text{WMLL}$

[mL]), Formula 2). The higher the lesion load, the smaller is the relative impact on thalamic volume (Fig. 8).

Control of potential confounders: The results of the univariate general linear model (Table III) confirmed that age, gender, and grey matter volume had no significant confounding effect on the RTV of the examined subjects. We further found no indication for a Group by Gender effect. Thalamic FA had the highest effect on the RTV followed by WMLL. Group membership (healthy control, "RRMS THL:no", "RRMS THL:yes", CIS) produced an additional significant effect on the RTV (Table III). A *post hoc* analysis considering the RTV corrected for thalamic FA, WMLL, Age, and grey matter volume revealed that the group of healthy controls still showed a significantly ($p < 0.05$) higher RTV compared to the group of CIS and RRMS (Fig. 9). In other words, although all RTVs of all groups (healthy control, "RRMS THL:no", "RRMS THL:yes", CIS) were in the *post hoc* analysis adjusted to the same thalamic FA, WMLL, Age, and grey matter volume patients with CIS and RRMS showed still a significantly reduced RTV compared to healthy controls. The reference values used in this analysis (Age = 37.24 years; thalamic FA = 0.254,

Figure 8.

(A) Logarithmic dependency between the relative thalamic volume in percent of the intracranial volume (RTV) and the white matter lesion load (WMML) in mL of all examined (sub)groups and healthy controls. The higher the WMML, the smaller is the relative impact of WMML on thalamic volume. Patients with a WMML ≤ 2.5 mL, a threshold determined by “healthy” controls, showed significantly reduced RTV compared to the group of healthy controls. (B) Significant correlation between the relative thalamic volume (RTV) and the disease duration in months in patients of all subgroups. Even patients with very early RRMS

and CIS (disease duration ≤ 24 months) have already a reduced RTV compared to healthy controls. The continuous line represents the regression function of CIS and RRMS patients’ uncorrected RTV as function of disease duration ($RTV = 0.982 - 0.000532 \times \text{“Duration in months”}$). The subgroup of patients with early RRMS or CIS (subgroup “early”) is delineated. Mean RTV of healthy controls: 1.0395 (95% confidence interval: [1.00087, 1.070]). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 9.

Age, fractional anisotropy (FA), grey matter volume, and white matter lesion load (WMML)-corrected least square means of relative thalamic volume as resulted from the general linear model ($F(3,140) = 2.78, p < 0.05$), computed for covariates at following means: age = 37.24 years; thalamic FA = 0.254, grey matter volume = 0.682 l; WMML = 3.316 mL. Vertical bars denote 0.95 confidence intervals.

grey matter volume = 0.682 l; WMML = 3.32 mL) represented the means over all participants, respectively. The group “RRMS THL:no” and “RRMS THL:yes” did not differ in the corrected RTV, which means that the presence of thalamic lesions had no effect on the corrected RTV.

DISCUSSION

We found evidence for all seven objectives as formulated in the introduction. The RTV was significantly reduced even in patients with very early RRMS and CIS (disease duration ≤ 24 months) who showed no indication for thalamic degeneration by visually inspecting conventional MRIs (Objective I, Fig. 8). This early thalamic atrophy was accompanied by microstructural thalamic alterations as reflected by significantly reduced thalamic FA (Objective II). Even if thalamic FA, alterations can only infer and not directly measure microstructural degeneration, a potential interpretation of these findings is that “silent” (not visible lesion-generating) destructive processes already alter the thalamic microstructure and cause measurable atrophy. This hypothesis is further supported by three new findings of our study: (1) RTV and thalamic FA were significantly correlated, even in the subgroup

TABLE III. Results of the general linear model

Dependent variable: RTV					
Factors	SS	DF	ms	F	p
Thalamic FA	0.2631	1	0.2631	60.83	0.000000 **
White matter lesion load	0.0560	1	0.0560	12.95	0.000443 **
Group (healthy control, CIS, RRMS THL:no, RRMS THL:yes)	0.0360	3	0.0120	2.77	0.044 *
Gender (F,M)	0.0056	1	0.0056	1.29	0.258 \$
Gender (2) × Group (4)	0.0138	3	0.0046	1.06	0.367 \$
Age	0.0021	1	0.0021	0.47	0.491 \$
Grey matter volume	0.0010	1	0.0010	0.22	0.638

Results of the general linear model with dependent variable RTV (considered as significant: * $p < 0.05$, ** $p < 0.001$; \$no differences expected; SS = sums of deviation squares; DF = degrees of freedom; ms = mean square; F = Fisher's F value; p = probability of type I error). Group sizes are given in Table I.

“RRMS WMLL:low THL:no” (*Objective III*, Fig. 6). In other words, patients with RRMS, low WMLL, and no thalamic lesions showed already a reduced average thalamic volume, while the individual reduction was proportional to the individual microstructural damage as assessed by DTI. (2) Even when the RTV was corrected for many volume influencing factors, including age, thalamic FA, grey matter volume, and WMLL, patients with RRMS and CIS presented still significantly lower RTV than healthy controls (Fig. 9). This means that although RTV was associated with WMLL and disease duration (Fig. 8), neither WMLL alone nor disease duration could completely explain the RTV reduction (general linear model, Table III). Instead, the RTV was already significantly reduced at the very beginning of the disease (disease duration ≤ 24 months) and in patients with minor WMLL (Fig. 8). (3) The epicenters of predominant correlations between WM FA and RTV were not in areas susceptible for FLAIR/T2w WM lesions (Fig. 7). This finding clearly contradicts the common opinion that retrograde neuroaxonal degeneration or anterograde transsynaptic changes secondary to axonal transection of fibers passing through areas of WM lesions (Wallerian degeneration) are the most likely pathological substrates of thalamic volume loss [Hannoun et al., 2012].

While recent studies have previously shown a negative correlation between thalamic volume and disease duration [Batista et al., 2012; Bergsland et al., 2012; Houtchens et al., 2007; Wylezinska et al., 2003], we could furthermore demonstrate that even patients with CIS and early RRMS (median EDSS = 1.5) already at the manifestation of their first symptoms have an average RTV reduction of about 10% relative to healthy controls (Fig. 8). The relevance of this initial RTV loss becomes clear if one considers that the interindividual variability of RTV in the healthy control group was only 7.9% (coefficient of variation) and that the initial RTV decrease of the patients roughly corresponds to the estimated average RTV loss per decade of disease duration (Fig. 8B).

By employing a multifactorial data analysis, including patients with CIS, and early RRMS (disease duration ≤ 24

months), and minor WMLL, and by defining appropriate and well-matched subgroups, our study is the first one demonstrating systematically a thalamic atrophy caused by “silent” (not visible lesion-generating) neurodegenerative processes within the thalamus, even in patients without THL, low EDSS (EDSS ≤ 2), low WMLL, and short disease duration. In patients with higher WMLL (> 2.5 mL), the additional effect on the thalamic volume might be caused by “extrathalamic” WM degeneration. The SPM8 voxel-based FA correlation analysis revealed that thalamic atrophy could cause damage of the thalamo-cortical network (cortical volume reduction and WM FA reductions next to the thalamus and basal ganglia, in the hippocampus, internal capsule, postcentral thalamocortical pathways), which cannot be explained by WM lesions.

From a theoretical point of view, an actual *neurodegenerative*, tissue-destroying process in the thalamus should lead in the long term to a *reduced* FA and increased mean diffusivity as demonstrated by many studies [Benedict et al., 2013; Ciccarelli et al., 2001; Mesaros et al., 2011]. However, in the very early phase of MS, acute (nonlesional) *inflammatory processes* in the thalamus can cause temporary edema that will lead to a specific increase of longitudinal diffusivity and thus to a FA *increase* [Deppe et al., 2013]. In our study, we found on average a systematically reduced FA, consistent with the majority of current studies. However, some studies reported also increased thalamic FA [Hannoun et al., 2012; Tovar-Moll et al., 2009] in patients with MS. There might be (at least) two explanations for these findings: The studies examined patients with (nonlesional) active inflammatory processes that led to edema in the thalamus, or the employed FA maps were not sufficiently corrected for thalamic atrophy, so that also (higher) FA values of surrounding WM confounded the thalamic FA estimates. Such confounding effect can be excluded for the present results (Fig. 2).

We employed a fully automated MR image analysis technique (FreeSurfer) for the calculation of absolute thalamic volume, ICV, WMLL, and grey matter volume. An inherent problem with such an approach is that segmentation errors can result in falsified volumetric measures.

However, the validity of our approach was recently shown for the thalamic volume by comparing FreeSurfer results with those derived from manual stereology, a technique that is considered to represent the “gold standard” for volumetric assessments of MRI [Keller et al., 2012]. Keller et al. demonstrated that stereological and FreeSurfer volume estimates yielded consistent thalamic volumes in healthy controls and patients with juvenile myoclonic epilepsy, and that the extent of agreeability between stereology and FreeSurfer is equal to the agreeability between two human anatomists estimating thalamic volume using stereological methods. Moreover they found that both techniques were equally sensitive in detecting bilateral thalamic atrophy in patients relative to healthy controls. Because up to now, there is no reliable automated and thus objective tool available for grey matter lesion detection—and filling, we did not correct our thalamic FA values for T2w lesion contribution. Potentially, reduced thalamic volume and FA in combination with cortical and WM volume changes could be an effect of lower MRI data quality. However, we could largely rule out this issue due to the following reasons. First, datasets of extremely reduced FA and thalamic volume were checked for movement artifacts (e.g., Figs. 2 and 3). Second, in the case of higher movement artifacts in more affected patients, RTV and FA would correlate in a much more spatially homogeneous fashion because movement artifacts usually affect an entire image. Instead we found a pattern of local FA reductions in WM portions of the thalamocortical network but not in other WM regions. WM not associated with a high lesion probability or part of predominant thalamocortical fibers was relatively less affected.

The utilization of the EDSS to illustrate the functional consequences of thalamic atrophy is another limitation of our study. The EDSS, which is used to classify the severity of disability of patients with MS, contains known deficits due to its subjective character, inter-rater variability and its disregard of important sections as cognition, attention, and fatigue [Horakova et al., 2012]. To prevent bias by inter-rater variability, the same neurologist estimated the EDSS. In future, we will apply neuropsychological tests to register effects of volumetric and microstructural thalamic alterations on cognition, attention, and alertness in patients with MS. In our study, we employed the FA to assess microstructure. There are also many other parameters that can be derived from DTI like mean, axial, and radial diffusivity, which we also evaluated. However, we reported here only the FA results as the other diffusivity parameters revealed not further insights.

This study was performed on a cross-sectional basis. Longitudinal studies are necessary to investigate whether early volumetric and microstructural thalamic alterations can serve as predictors of future disease course and as biomarkers for treatment effects. Moreover, the reason why some patients have already unquestionable and relevant very early thalamic atrophy and other are spared even after

years, remains enigmatic. It seems necessary to investigate potential factors that allow differentiating patients with early and silent thalamic degeneration from those without. In this context, the results shown in Figure 6 might be relevant. They demonstrate the general possibility to identify individual patients with “silent” thalamic atrophy by means of volumetry as performed here on 3D datasets or by DTI.

CONCLUSION

Early thalamic atrophy in patients with CIS and RRMS is mainly caused by silent microstructural destructive processes within the thalamus and not only by retrograde neuroaxonal degeneration or anterograde transsynaptic changes secondary to WM lesions. High-resolution structural and diffusion weighted MRI has—even in the absence of thalamic and extensive WM lesions—the potential to uncover early, functionally relevant thalamocortical damage. Our study further sets a new spotlight to the thalamus and the thalamocortical network as a site of pathological involvement in MS—even in the earliest phase of the disease.

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AUTHORS' CONTRIBUTIONS TO THE MANUSCRIPT:

Michael Deppe: Concept and idea of the study, recruitment of healthy control subjects, development of MRI data analysis software, diffusion MRI data analysis, confirmatory statistical analysis, interpretation of the data, drafting of the manuscript, over-all supervision, organization, and funding of the project.

Julia Krämer: Concept and idea of the study, recruitment, clinical examination, and treatment of patients with multiple sclerosis and clinically isolated syndrome, clinical data acquisition, structural MRI data analysis, confirmatory statistical analysis, interpretation of the data, and drafting of the manuscript.

Jan-Gerd Tenberge: Development of MRI data analysis software and patient database.

Jasmin Marinell: Organization of MRI experiments, clinical data analysis, MRI data analysis, and data integration.

Wolfram Schwindt/Katja Deppe: Radiological assessment of MRIs.

Sergiu Groppa: Organization of MRI experiments, clinical data analysis, MRI data analysis, and data integration.

Heinz Wiendl: Overall organization of funding and supervision of the study, organization, and support for advanced MRI, and revising the manuscript for intellectual content.

Sven G. Meuth: Concept and idea of the project, overall supervision, organization, and funding of the project, and drafting and revising the manuscript for intellectual content.

REFERENCES

- Al-Radaideh AM, Wharton SJ, Lim SY, Tench CR, Morgan PS, Bowtell RW, Constantinescu CS, Gowland PA (2013): Increased iron accumulation occurs in the earliest stages of demyelinating disease: An ultra-high field susceptibility mapping study in clinically isolated syndrome. *Mult Scler* 19:896–903.
- Audoin B, Davies GR, Finisku L, Chard DT, Thompson AJ, Miller DH (2006): Localization of grey matter atrophy in early RRMS: A longitudinal study. *J Neurol* 253:1495–1501.
- Audoin B, Zaaraoui W, Reuter F, Rico A, Malikova I, Confort-Gouny S, Cozzone PJ, Pelletier J, Ranjeva JP (2010): Atrophy mainly affects the limbic system and the deep grey matter at the first stage of multiple sclerosis. *J Neurol Neurosurg Psychiatry* 81:690–695.
- Batista S, Zivadinov R, Hoogs M, Bergsland N, Heininen-Brown M, Dwyer MG, Weinstock-Guttman B, Benedict RH (2012): Basal ganglia, thalamus and neocortical atrophy predicting slowed cognitive processing in multiple sclerosis. *J Neurol* 259: 139–146.
- Benedict RH, Hulst HE, Bergsland N, Schoonheim MM, Dwyer MG, Weinstock-Guttman B, Geurts JJ, Zivadinov R (2013): Clinical significance of atrophy and white matter mean diffusivity within the thalamus of multiple sclerosis patients. *Mult Scler* 19:1478–1484.
- Bergsland N, Horakova D, Dwyer MG, Dolezal O, Seidl ZK, Vaneckova M, Krasensky J, Havrdova E, Zivadinov R (2012): Subcortical and cortical gray matter atrophy in a large sample of patients with clinically isolated syndrome and early relapsing-remitting multiple sclerosis. *AJNR Am J Neuroradiol* 33:1573–1578.
- Calabrese M, Rinaldi F, Mattisi I, Bernardi V, Favaretto A, Perini P, Gallo P (2011): The predictive value of gray matter atrophy in clinically isolated syndromes. *Neurology* 77:257–263.
- Ceccarelli A, Rocca MA, Pagani E, Colombo B, Martinelli V, Comi G, Filippi M (2008): A voxel-based morphometry study of grey matter loss in MS patients with different clinical phenotypes. *Neuroimage* 42:315–322.
- Ciccarelli O, Werring DJ, Wheeler-Kingshott CA, Barker GJ, Parker GJ, Thompson AJ, Miller DH (2001): Investigation of MS normal-appearing brain using diffusion tensor MRI with clinical correlations. *Neurology* 56:926–933.
- Cifelli A, Arridge M, Jezard P, Esiri MM, Palace J, Matthews PM (2002): Thalamic neurodegeneration in multiple sclerosis. *Ann Neurol* 52:650–653.
- Dale A, Fischl B, Sereno MI (1999): Cortical surface-based analysis: I. Segmentation and surface reconstruction. *Neuroimage* 9:179–194.
- Deppe M, Marinell J, Krämer J (2014): Increased cortical curvature reflects white matter atrophy in individual patients with early multiple sclerosis. *Neuroimage Clin*
- Deppe M, Muller D, Kugel H, Ruck T, Wiendl H, Meuth SG (2013): DTI detects water diffusion abnormalities in the thalamus that correlate with an extremity pain episode in a patient with multiple sclerosis. *Neuroimage Clin* 2:258–262.
- Fischl B, Salat D, Busa E, Albert M, Dieterich M, Haselgrove C, van der Kouwe A, Killiany R, Kennedy D, Klaveness S, Montillo A, Makris N, Rosen B, Dale A (2002): Whole brain segmentation: Automated labeling of neuroanatomical structures in the human brain. *Neuron* 33:341–355.
- Fischl B, Dale AM (2000): Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc Natl Acad Sci USA* 97:11050–11055.
- Fischl B, Sereno MI, Tootell RBH, Dale AM (1999): High-resolution intersubject averaging and a coordinate system for the cortical surface. *Hum Brain Mapp* 8:272–284.
- Geurts JJ, Calabrese M, Fisher E, Rudick RA (2012): Measurement and clinical effect of grey matter pathology in multiple sclerosis. *Lancet Neurol* 11:1082–1092.
- Geurts JJ, Reuling IE, Vrenken H, Uitdehaag BM, Polman CH, Castelijns JA, Barkhof F, Pouwels PJ (2006): MR spectroscopic evidence for thalamic and hippocampal, but not cortical, damage in multiple sclerosis. *Magn Reson Med* 55:478–483.
- Glauche V, Mohammadi S, Deppe M (2010): SPM normalisation toolbox for diffusion weighted images. *Proc Intl Soc Mag Reson Med* 18: Abstract 1648 (http://cds.ismrm.org/protected/10MProceedings/files/1648_5504.pdf).
- Hagemeyer J, Weinstock-Guttman B, Bergsland N, Heininen-Brown M, Carl E, Kennedy C, Magnano C, Hojnacki D, Dwyer MG, Zivadinov R (2012): Iron deposition on SWI-filtered phase in the subcortical deep gray matter of patients with clinically isolated syndrome may precede structure-specific atrophy. *AJNR Am J Neuroradiol* 33:1596–1601.
- Hagemeyer J, Yeh EA, Brown MH, Bergsland N, Dwyer MG, Carl E, Weinstock-Guttman B, Zivadinov R (2013): Iron content of the pulvinar nucleus of the thalamus is increased in adolescent multiple sclerosis. *Mult Scler* 19:567–576.
- Hannoun S, Durand-Dubief F, Confavreux C, Ibarrola D, Streichenberger N, Cotton F, Guttmann CR, Sappey-Marini D (2012): Diffusion tensor-MRI evidence for extra-axonal neuronal degeneration in caudate and thalamic nuclei of patients with multiple sclerosis. *AJNR Am J Neuroradiol* 33:1363–1368.
- Henry RG, Shieh M, Okuda DT, Evangelista A, Gorno-Tempini ML, Pelletier D (2008): Regional grey matter atrophy in clinically isolated syndromes at presentation. *J Neurol Neurosurg Psychiatry* 79:1236–1244.
- Horakova D, Kalincik T, Dusankova JB, Dolezal O (2012): Clinical correlates of grey matter pathology in multiple sclerosis. *BMC Neurol* 12:10
- Houtchens MK, Benedict RH, Killiany R, Sharma J, Jaisani Z, Singh B, Weinstock-Guttman B, Guttmann CR, Bakshi R (2007): Thalamic atrophy and cognition in multiple sclerosis. *Neurology* 69:1213–1223.
- Hulst HE, Geurts JJ (2011): Gray matter imaging in multiple sclerosis: What have we learned? *BMC Neurol* 11:153.
- Keller SS, Gerdes JS, Mohammadi S, Kellinghaus C, Kugel H, Deppe K, Ringelstein EB, Evers S, Schwindt W, Deppe M (2012): Volume estimation of the thalamus using freesurfer and stereology: Consistency between methods. *Neuroinformatics* 10:341–350.
- Llinas RR, Ribary U, Jeanmonod D, Kronberg E, Mitra PP (1999): Thalamocortical dysrhythmia: A neurological and neuropsychiatric syndrome characterized by magnetoencephalography. *Proc Natl Acad Sci USA* 96:15222–15227.
- McCormick DA (1999): Are thalamocortical rhythms the Rosetta Stone of a subset of neurological disorders? *Nat Med* 5:1349–1351.
- Mesaros S, Rocca MA, Absinta M, Ghezzi A, Milani N, Muiola L, Veggiotti P, Comi G, Filippi M (2008): Evidence of thalamic gray matter loss in pediatric multiple sclerosis. *Neurology* 70: 1107–1112.

- Mesaros S, Rocca MA, Pagani E, Sormani MP, Petrolini M, Comi G, Filippi M (2011): Thalamic damage predicts the evolution of primary-progressive multiple sclerosis at 5 years. *AJNR Am J Neuroradiol* 32:1016–1020.
- Minagar A, Barnett MH, Benedict RH, Pelletier D, Pirko I, Sahraian MA, Frohman E, Zivadinov R (2013): The thalamus and multiple sclerosis: Modern views on pathologic, imaging, and clinical aspects. *Neurology* 80:210–219.
- Mohammadi S, Keller SS, Glauche V, Kugel H, Jansen A, Hutton C, Floel A, Deppe M (2012): The influence of spatial registration on detection of cerebral asymmetries using voxel-based statistics of fractional anisotropy images and TBSS. *PLoS One* 7:e36851.
- Mohammadi S, Moller HE, Kugel H, Muller DK, Deppe M (2010): Correcting eddy current and motion effects by affine whole-brain registrations: Evaluation of three-dimensional distortions and comparison with slice-wise correction. *Magn Reson Med* 64:1047–1056.
- Patenaude B, Smith SM, Kennedy DN, Jenkinson M (2011): A Bayesian model of shape and appearance for subcortical brain segmentation. *Neuroimage* 56:907–922.
- Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, Fujihara K, Havrdova E, Hutchinson M, Kappos L, Lublin FD, Montalban X, O'Connor P, Sandberg-Wollheim M, Thompson AJ, Waubant E, Weinshenker B, Wolinsky JS (2011): Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol* 69:292–302.
- Ramasamy DP, Benedict RH, Cox JL, Fritz D, Abdelrahman N, Hussein S, Minagar A, Dwyer MG, Zivadinov R (2009): Extent of cerebellum, subcortical and cortical atrophy in patients with MS: A case-control study. *J Neurol Sci* 282:47–54.
- Rocca MA, Mesaros S, Pagani E, Sormani MP, Comi G, Filippi M (2010): Thalamic damage and long-term progression of disability in multiple sclerosis. *Radiology* 257:463–469.
- Schmidt P, Gaser C, Arsic M, Buck D, Forschler A, Berthele A, Hoshi M, Ilg R, Schmid VJ, Zimmer C, Hemmer B, Muhlau M (2012): An automated tool for detection of FLAIR-hyperintense white-matter lesions in Multiple Sclerosis. *Neuroimage* 59:3774–3783.
- Schoonheim MM, Popescu V, Rueda Lopes FC, Wiebenga OT, Vrenken H, Douw L, Polman CH, Geurts JJ, Barkhof F (2012): Subcortical atrophy and cognition: Sex effects in multiple sclerosis. *Neurology* 79:1754–1761.
- Sepulcre J, Sastre-Garriga J, Cercignani M, Ingle GT, Miller DH, Thompson AJ (2006): Regional gray matter atrophy in early primary progressive multiple sclerosis: A voxel-based morphometry study. *Arch Neurol* 63:1175–1180.
- Tovar-Moll F, Evangelou IE, Chiu AW, Richert ND, Ostuni JL, Ohayon JM, Auh S, Ehrmantraut M, Talagala SL, McFarland HF, Bagnato F (2009): Thalamic involvement and its impact on clinical disability in patients with multiple sclerosis: A diffusion tensor imaging study at 3T. *AJNR Am J Neuroradiol* 30:1380–1386.
- Vercellino M, Masera S, Lorenzatti M, Condello C, Merola A, Mattioda A, Tribolo A, Capello E, Mancardi GL, Mutani R, Giordana MT, Cavalla P (2009): Demyelination, inflammation, and neurodegeneration in multiple sclerosis deep gray matter. *J Neuropathol Exp Neurol* 68:489–502.
- Wylezinska M, Cifelli A, Jezard P, Palace J, Alecci M, Matthews PM (2003): Thalamic neurodegeneration in relapsing-remitting multiple sclerosis. *Neurology* 60:1949–1954.
- Zivadinov R, Heininen-Brown M, Schirda CV, Poloni GU, Bergsland N, Magnano CR, Durfee J, Kennedy C, Carl E, Hagemeier J, Benedict RH, Weinstock-Guttman B, Dwyer MG (2012): Abnormal subcortical deep-gray matter susceptibility-weighted imaging filtered phase measurements in patients with multiple sclerosis: A case-control study. *Neuroimage* 59:331–339.