Original Research Paper

Fronto-limbic disconnection in patients with multiple sclerosis and depression

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Abstract

Background: The biological mechanism of depression in multiple sclerosis (MS) is not well understood. Based on work in major depressive disorder, fronto-limbic disconnection might be important.

Objective: To investigate structural and functional fronto-limbic changes in depressed MS (DMS) and non-depressed MS (nDMS) patients.

Methods: In this retrospective study, 22 moderate-to-severe DMS patients (disease duration 8.2 ± 7.7 years), 21 nDMS patients (disease duration 15.3 ± 8.3 years), and 12 healthy controls underwent neuropsychological testing and magnetic resonance imaging (MRI; 1.5 T). Brain volumes (white matter (WM), gray matter, amygdala, hippocampus, thalamus), lesion load, fractional anisotropy (FA) of fronto-limbic tracts, and resting-state functional connectivity (FC) between limbic and frontal areas were measured and compared between groups. Regression analysis was performed to relate MRI measures to the severity of depression.

Results: Compared to nDMS patients, DMS patients (shorter disease duration) had lower WM volume ($p < 0.01$), decreased FA of the uncinate fasciculus ($p < 0.05$), and lower FC between the amygdala and frontal regions ($p < 0.05$). Disease duration, FA of the uncinate fasciculus, and FC of the amygdala could explain 48% of variance in the severity of depression. No differences in cognition were found.

Conclusion: DMS patients showed more pronounced (MS) damage, that is, structural and functional changes in temporo-frontal regions, compared to nDMS patients, suggestive of fronto-limbic disconnection.

Keywords: Multiple sclerosis, depression, cognition, limbic system, magnetic resonance imaging, functional magnetic resonance imaging, functional connectivity, diffusion tensor imaging

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Introduction

Depression is present in 30% of patients with multiple sclerosis (MS)[1] and thereby more common than in other chronic diseases among young adults (e.g. rheumatoid arthritis).[2] The clinical manifestation of depression in MS is very similar to that of patients with major depressive disorder (MDD) without MS.[3] The etiology of depression in MS is still poorly understood, but it could be hypothesized that specific MS pathology triggers the onset of depressive symptoms. In MDD, brain abnormalities such as limbic and frontal atrophy,[4,5] decreased fractional anisotropy (FA) of fronto-limbic tracts,[6–8] and decreased functional connectivity (FC) between limbic and frontal regions[9–11] have been found, suggestive of fronto-limbic disconnection.[11]

In MS, studies have shown that, compared to non-depressed MS (nDMS) patients, depressed MS (DMS) patients show more severe atrophy of frontal regions[12,13] and frontal white matter (WM) damage (atrophy and decreased FA).[14,15] Furthermore, higher scores on depression questionnaires have been related to decreased FC between the hippocampus and amygdala with frontal regions during emotional processing[16] and between the hippocampus with default mode network at rest.[17]

However, multimodal imaging studies investigating the fronto-limbic system and depression in MS are lacking. To better understand the etiology of depression in MS, combining these structural and functional measures within individual patients is essential, as is the inclusion of MS patients with profound depressive symptoms. Hence, we investigated structural and functional differences in the fronto-limbic system in DMS and nDMS patients and healthy controls (HCs). We hypothesize that DMS patients will show more severe structural and functional fronto-limbic disconnection compared to nDMS patients and HCs.

Methods

Participants

Participants were selected and matched for age, sex, and educational level from two different study cohorts. Both cohorts followed identical protocols for neuropsychology and magnetic resonance imaging (MRI). Cohort 1 consisted of DMS patients of a randomized controlled trial investigating the effect of cognitive behavioral therapy for depression.[18] Inclusion criteria were as follows: (1) aged ≥18 years, (2) scoring ≥20 on the Beck Depression Inventory, Second Edition (BDI-II) indicating moderate-to-severe depression,[19] (3) MS diagnosis (>3 months) confirmed by a neurologist, and (4) no contra-indications for MRI. Exclusion criteria were as follows: (1) elevated suicide risk, (2) psychotherapy, and (3) using antidepressants for <6 weeks. Note that these patients had not yet started with the cognitive behavioral therapy. Hence, no effects of this intervention are measured in this study. Cohort 2 consisted of MS patients and HCs that were prospectively recruited for a longitudinal study (data not published yet) but retrospectively added to the DMS sample to answer our post hoc research question. The inclusion criteria for this study were as follows: (1) aged 18–65 years, (2) no presence or history of neurological or psychiatric diseases (for patients other than MS), (3) scoring <8 on the Hospital Anxiety and Depression Scale—Depression (HADS-D),[20] indicative of absence of depression, (4) MS diagnosis according to the revised McDonald criteria, and (5) no contra-indications for MRI. Both study protocols were approved by the local ethical committee and conducted in accordance with the ethical standards laid down in the Declaration of Helsinki. All participants gave written informed consent prior to participation.

Clinical measures

The HADS—Anxiety (HADS-A) questionnaire was used to assess the level of anxiety.[20] All DMS patients underwent the Composite International Diagnostic Interview to diagnose a depressive disorder.[21] Neuropsychological testing was performed in all subjects, covering the following domains: verbal memory, visuospatial memory, information processing speed, short-term and working memory, and verbal fluency (see Supplementary Methods for all tests). For each test, the raw score was converted into a Z score relative to HCs.

Physical disability was assessed using the telephone version of the Expanded Disability Status Scale (EDSS), consisting of 10 questions that can be used to estimate the EDSS score.[22] We excluded the subjective rating of the severity of MS-related symptoms from the questionnaire, in order to keep the estimated EDSS score as objective as possible. As a result, the EDSS score was categorized in the following bins: 0–1.5 (no restrictions by MS in daily life), 2–4 (some restrictions by MS in daily life), 4.5–6, and ≥6.5.

MRI acquisition

All participants were scanned on a 1.5-T whole-body MRI system (Siemens Avanto, Erlangen, Germany) using a 12-channel phased array head coil. The protocol included a three-dimensional T1-weighted magnetization prepared rapid acquisition gradient-echo sequence for brain volume measurements and an axial turbo spin-echo proton density (PD)/T2-weighted sequence for WM lesion detection. Diffusion-weighted echo-planar images were obtained with 60 volumes with noncollinear diffusion gradients (b value: 700 s/mm^2) and 10 volumes without directional weighting. For FC analysis, resting-state (RS) functional magnetic resonance imaging (fMRI) was performed (200 volumes of echo-planar images; see Supplementary Methods for acquisition parameters).

The fronto-limbic system

The fronto-limbic system was defined based on previous studies.[5–8] The amygdala, hippocampus, and thalamus were defined as key limbic subcortical gray matter (GM) structures.[5] Fronto-limbic WM connections included the bilateral anterior thalamic radiation, cingulum, superior longitudinal fasciculus, and uncinate fasciculus.[6–8] The corticospinal tract was selected as a control tract. Functional fronto-limbic connections included the FC between key limbic structures and frontal regions, namely, the anterior cingulate cortex, medial prefrontal cortex (PFC), dorsolateral PFC, and ventral PFC.[5]
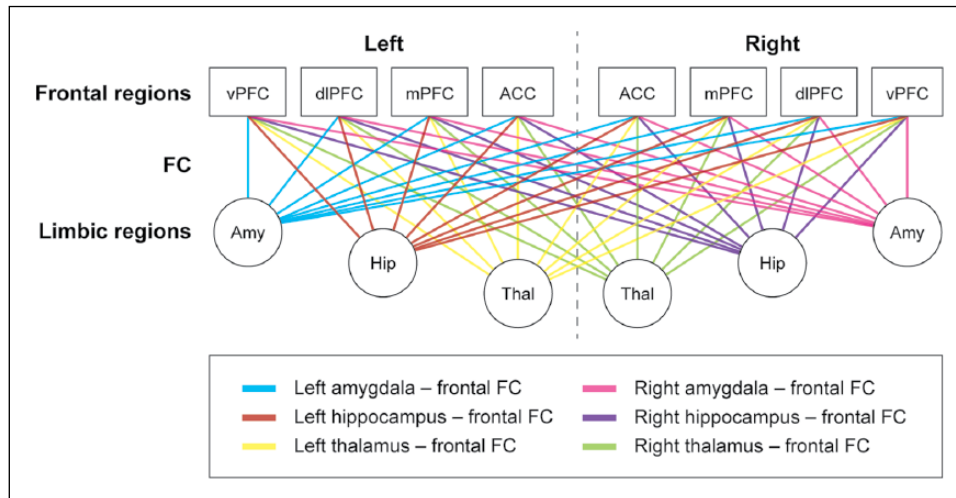
Figure 1. Schematic overview of functional connectivity between limbic and frontal regions.
All ipsi- and contralateral connections between key limbic and frontal regions were obtained. For each limbic region, its average FC with all frontal areas was calculated, which is represented by the different colors.
ACC: anterior cingulate cortex; Amy: amygdala; dlPFC: dorsolateral prefrontal cortex; FC: functional connectivity; Hip: hippocampus; mPFC: medial prefrontal cortex; Thal: thalamus; vPFC: ventral prefrontal cortex.

Volumetric measures

All MRI-processing steps were performed in FSL5.0 (FMRIB's Software Library, http://www.fmrib.ox.ac.uk/fsl). Lesion segmentation was applied using the PD/T2-weighted scans and subsequently filled as previously described.[23,24] Next, GM and WM volumes were obtained using SIENAx.[25] Volumes of key limbic regions were obtained using FIRST.[26] All volumetric measurements were normalized for head size.

Fronto-limbic damage: WM integrity and lesion load

Processing of diffusion tensor imaging (DTI) data included motion and eddy current correction, after which a diffusion tensor was fitted for each voxel using FMRIB's Diffusion Toolbox. From the tensor, FA and mean diffusivity (MD) images were obtained. For atlas registration purposes, all participants' FA images were non-linearly registered to the FMRIB58_FA using the standard tract-based spatial statistics pipeline. Next, the JHU white matter tractography atlas[27] in standard space was thresholded, so that voxels with a probability ≤15% were excluded to measure tracts with more spatial accuracy. Each tract was then non-linearly registered to the native space FA and MD images. SIENAx's WM mask and the FIRST segmentation were used to only select WM voxels for each tract. Subsequently, average FA and MD values for each tract were obtained. For each patient, the lesion mask was linearly registered to native DTI space, and for each WM tract, the number of lesioned voxels was calculated (expressed as a percentage of the total tract size).

RS FC analysis

For a detailed description of the FC pipeline, see Supplementary Methods. In short, the subject's fMRI data were preprocessed in Melodic. The Automated Anatomical Labeling (AAL) atlas[28] was registered to each subject's fMRI scan and complemented with the subcortical areas segmented by FIRST. For each region (92 in total), the average time series was obtained and imported into MATLAB R2012a. Pearson correlation coefficients were used to correlate activity between all regions and subsequently normalized for each participant's average correlation strength and standard deviation of the entire correlation matrix. Finally, average FC between each limbic region with all frontal areas was calculated (see Figure 1).

Relationship between depression and MRI

To combine data from the two cohorts (HADS-D and BDI-II), we calculated a depression rank score. Subjects were assigned a rank score starting from 1 (subject with lowest depression score) with incremental steps of 1 toward the subject with the highest depression score. Subjects with identical depression scores were assigned similar rank scores (maximum score: 24).

Statistical analyses

The statistical analyses were performed in SPSS 20 (Chicago, IL, USA). Normality of data was assessed with visual inspection of histograms. Demographic data were analyzed using univariate analysis of variance (for normally distributed data) or a Mann–Whitney *U* test

Table 1. Demographics and clinical data of depressed and non-depressed patients with MS and healthy controls.

	DMS (n=22)	nDMS (n=21)	HCs (n=12)	p value
Age	44.35 (10.84)	49.12 (8.14)	52.42 (9.05)	0.06
Sex (female/male)	17/5	14/7	9/3	0.72
Educational level[a] on a scale from 1 (low) to 7 (high)	6.00 (5.75–6.00)	6.00 (5.00–6.00)	6.00 (5.00–6.75)	0.39
MS type (RRMS/SPMS/ PPMS/missing)	17/4/1/0	17/3/0/1	–	0.59
Disease duration[a]	5.50 (2.00–13.00)	16.00 (8.00–22.50)	–	<0.01
EDSS groups (%)				0.16
Missing	0 (0)	1 (5)	–	
0–1.5	0 (0)	1 (5)	–	
2–4	16 (72)	13 (62)	–	
4.5–6	3 (14)	6 (28)	–	
≥6.5	3 (14)	0 (0)	–	
BDI-II	29.09 (6.82)	–	–	–
HADS-A[a]	9.50 (7.00–11.00)	4.0 (3.00–6.50)	3.50 (1.00–5.80)	<0.001[b,c]
HADS-D[a]	–	2.0 (2.00–4.50)	1.00 (0.00–2.00)	<0.01

BDI-II: Beck Depression Inventory Second Edition; DMS: depressed multiple sclerosis patients; EDSS: Expanded Disability Status Scale; HADS: Hospital Anxiety and Depression Scale; A: Anxiety; D: Depression; HCs: healthy controls; MS: multiple sclerosis; nDMS: non-depressed multiple sclerosis patients; PPMS: primary progressive multiple sclerosis; RRMS: relapsing remitting multiple sclerosis; SPMS: secondary progressive multiple sclerosis.
Displayed data are mean (standard deviation) values.
[a]Not normally distributed data for which median (interquartile range) values are provided.
[b]Significant difference between DMS and HCs.
[c]Significant difference between DMS and nDMS.

(two groups) or Kruskal–Wallis test (three groups) for not normally distributed data. The main analysis consisted of group comparisons in cognitive performance and imaging measures, analyzed using linear regression with two dummy variables to contrast all three groups, corrected for age and sex. In addition, a direct comparison was made between DMS and nDMS patients with correction for disease duration instead of age to better control for disease-specific changes. Hierarchical regression analysis was performed to investigate the added value of FC on top of structural MRI (including all MRI measures in the model that showed group differences between DMS and nDMS patients in the main analysis) to predict the depression rank score (covariate: disease duration). Benjamini–Hochberg false discovery rate (FDR)-corrected p values for each family of multiple test statistics are reported in the tables and main text, and a corrected p value of 0.05 was considered statistically significant.

Post hoc analysis: impact of MDD diagnosis

In order to be increasingly sensitive to detect differences between DMS and nDMS patients, all analyses were repeated in a subsample consisting of DMS patients with a current (i.e. past 6 months) MDD diagnosis (MSD[MDD]) and nDMS patients with an HADS-D score (≤4) that did not exceed those of HCs (nDMS[lowHADS]). These analyses were corrected for disease duration and sex.

Results

Demographics and clinical measures

For information on demographic data for DMS patients (n=22), nDMS patients (n=21), and HCs (n=12), see Table 1. In total, 18 DMS patients had a lifetime MDD diagnosis, and in 9 of these patients, the onset was 14.6 ± 10.9 years before MS diagnosis. In addition, 14 DMS patients had a current MDD diagnosis. No significant group differences were found for age (p=0.06) and sex (p=0.72). Disease duration in nDMS patients was shorter (8.2 ± 7.7 years) than in DMS patients (15.3 ± 8.3 years; p<0.01), while physical disability (EDSS) was similar (p=0.16). Performance on verbal memory was lower in both patient groups compared to HCs (p<0.01). In addition, DMS patients performed worse on information processing speed and working memory compared to HCs (p=0.01 and p=0.05, respectively; see Supplementary Table 1).

MRI variables

Brain volumes. Compared to HCs, lower GM, WM, and limbic volume were observed in both patient groups (Table 2), except for volume of the WM and left amygdala which did not significantly differ between nDMS and HCs ($p = 0.06$ and $p = 0.08$, respectively). In DMS compared to nDMS patients, WM volume was lower ($p < 0.01$), whereas lesion load and GM volume were similar.

Fronto-limbic WM integrity and lesion load. DMS patients displayed decreased FA compared to HCs in the left cingulum ($p = 0.04$) and left uncinate fasciculus ($p = 0.03$), whereas no differences were found between nDMS patients and HCs (Table 3). Compared to nDMS patients, DMS patients showed lower FA in the left uncinate fasciculus ($p = 0.05$). No differences were found for the corticospinal tract between DMS and nDMS patients. Furthermore, no differences between both MS groups were found for MD and lesion load of fronto-limbic tracts (Supplementary Table 2).

Fronto-limbic FC. Compared to HCs, DMS and nDMS patients did not show differences in FC between limbic and frontal regions (Table 4). However, FC of the right amygdala with frontal regions was lower in DMS compared nDMS patients ($p = 0.04$).

DMS vs nDMS with correction for disease duration. Direct comparisons between DMS and nDMS patients with correction for disease duration and sex revealed significant group differences for WM volume ($p < 0.001$) and FC of the right amygdala ($p = 0.05$; see Supplementary Tables 3–5).

Relationship between depression and MRI

A hierarchical regression analysis was performed in all patients to investigate the added value of FC of the right amygdala on top of structural MRI measures (WM volume and FA of the left uncinate fasciculus) in predicting depression rank score. Structural MRI measures could explain 41% of variance in depression rank score, with disease duration ($\beta = -0.48$, $p < 0.001$), WM volume ($\beta = -0.32$, $p = 0.02$), and FA of the left uncinate fasciculus ($\beta = -0.28$, $p = 0.04$) as significant predictors. Adding FC of the right amygdala to the model increased the explained variance in depression rank score with 7% to a total of 48%. The significant predictors in this model were as follows: disease duration ($\beta = -0.35$, $p = 0.01$), FA of the left uncinate fasciculus ($\beta = -0.35$, $p = 0.01$), and FC of the right amygdala ($\beta = -0.32$, $p = 0.02$).

The impact of MDD diagnosis

Compared to nDMS[lowHADS] patients ($n = 16$; average disease duration 15.0 ± 8.7 years), DMS[MDD] patients ($n = 14$; average disease duration 6.5 ± 6.7 years) had lower WM volume ($p < 0.001$). However, no difference was found for FA of the left uncinate fasciculus ($p = 0.64$). In addition, DMS[MDD] patients showed a borderline significant decrease in FC between the right amygdala and frontal areas, as well as between the right hippocampus and frontal areas ($p = 0.06$ and $p = 0.07$, respectively). For all differences, see Supplementary Tables 6–10.

Discussion

In this study, we investigated structural and functional disconnection between limbic and frontal regions and depression in MS, using a multimodal approach. Both patients groups were statistically similar on several variables, including demographics, cognitive performance, GM volume and lesion load. However, in comparison with nDMS patients, DMS patients, with nearly half the disease duration of nDMS patients, had decreased WM volume, decreased FA of the uncinate fasciculus, and lower FC between the amygdala and frontal regions. Patients with a current MDD diagnosis did not show significant differences on FA of fronto-limbic WM tracts, but did display lower WM volume and a borderline significant decrease in FC between the right hippocampus and amygdala with frontal regions compared to nDMS[lowHADS] patients ($p = 0.06$). Together, these findings support our hypothesis that DMS patients have more severe structural and functional disconnection than nDMS patients in areas associated with depression and that combining structural and functional measures increases the explained variance in depressive symptoms.

Microstructural WM changes

Similar to findings in MDD (without MS),[6–8] decreased FA of the uncinate fasciculus was found in DMS compared to nDMS patients. The uncinate fasciculus connects the temporal lobe, in which the amygdala and hippocampus are located, with the PFC, and is thought to be involved in cognitive and social-emotional functions.[29] Previously, lower FA in the anterior temporal lobe has been linked to depression in MS,[14] and structural network alterations including the amygdala, hippocampus, and frontal regions have been found in DMS compared to nDMS patients and HCs.[30] In addition, a higher depression rank score was related to lower FA of the left uncinate fasciculus. FA of the corticospinal tract was not

Table 2. Structural MRI data from depressed and non-depressed patients with MS and healthy controls.

	DMS (n = 22)	nDMS (n = 21)	HCs (n = 12)	DMS vs nDMS			DMS vs HCs			nDMS vs HCs		
				Difference (CI)	p value	p corr.	Difference (CI)	p value	p corr.	Difference (CI)	p value	p corr.
NWMV (mL)	667.68 (34.89)	706.98 (47.90)	739.46 (31.59)	−38.08 (−63.21 to −12.95)	<0.01	<0.01	−68.53 (−99.02 to −38.03)	<0.001	<0.001	−30.45 (−60.00 to −0.90)	0.04	0.07
NGMV (mL)	745.76 (56.61)	715.45 (50.63)	767.45 (37.74)	13.75 (−8.81 to 36.31)	0.23	0.31	−42.56 (69.94 to −15.18)	<0.01	<0.01	−56.30 (−82.84 to −29.77)	<0.001	<0.01
T2LL (mL)	5.69 (5.92)	7.45 (5.43)	—	−1.57 (−5.07 to 1.92)	0.37	0.46	—	—	—	—	—	—
Hippocampus L (mL)	4.74 (0.68)	4.66 (0.69)	5.60 (0.61)	−0.01 (−0.41 to 0.40)	0.97	0.97	−0.96 (−1.45 to −0.47)	<0.001	<0.01	−0.96 (−1.43 to −0.48)	<0.001	<0.01
Hippocampus R (mL)	4.80 (0.76)	4.51 (0.73)	5.36 (0.86)	0.19 (−0.28 to 0.65)	0.42	0.48	−0.67 (−1.24 to −0.11)	0.02	0.03	−0.86 (−1.40 to −0.31)	<0.01	<0.01
Amygdala L (mL)	1.55 (0.36)	1.59 (0.32)	1.82 (0.24)	−0.08 (−0.28 to 0.11)	0.40	0.47	−0.31 (−0.54 to −0.07)	0.01	0.02	−0.22 (−0.45 to 0.00)	0.06	0.08
Amygdala R (mL)	1.62 (0.41)	1.50 (0.29)	1.96 (0.27)	0.10 (−0.10 to 0.30)	0.32	0.42	−0.31 (−0.55 to −0.07)	0.01	0.02	−0.41 (−0.65 to −0.18)	<0.01	<0.01
Thalamus L (mL)	9.61 (1.00)	9.39 (1.39)	10.82 (0.85)	0.03 (−0.60 to 0.67)	0.91	0.95	−1.35 (−2.12 to −0.58)	<0.001	<0.01	−1.39 (−2.13 to −0.64)	<0.001	<0.01
Thalamus R (mL)	9.33 (1.05)	9.06 (1.20)	10.35 (0.58)	0.07 (−0.50 to 0.64)	0.80	0.86	−1.21 (−1.90 to −0.51)	<0.001	<0.01	−1.28 (−1.95 to −0.61)	<0.001	<0.01

CI: confidence interval; corr.: FDR-corrected *p* value; DMS: depressed multiple sclerosis patients; HCs: healthy controls; L: left; MS: multiple sclerosis; MRI: magnetic resonance imaging; NGMV: normalized gray matter volume; nDMS: non-depressed multiple sclerosis patients; NWMV: normalized white matter volume; R: right; T2LL: T2-weighted lesion load.
Displayed data are mean (standard deviation) values.

Table 3. Fractional anisotropy and mean diffusivity for each white matter tract of depressed and non-depressed patients with MS and healthy controls.

	DMS (n=22)	nDMS (n=21)	HCs (n=12)	DMS vs nDMS			DMS vs HCs			nDMS vs HCs		
				Difference (CI)	p value	p corr.	Difference (CI)	p value	p corr.	Difference (CI)	p value	p corr.
FA												
Anterior thalamic radiation L	0.380 (0.021)	0.385 (0.018)	0.394 (0.017)	−0.005 (−0.017 to 0.007)	0.40	0.56	−0.013 (−0.028 to 0.002)	0.08	0.20	−0.008 (−0.022 to 0.006)	0.27	0.41
Anterior thalamic radiation R	0.368 (0.020)	0.375 (0.014)	0.383 (0.017)	−0.008 (−0.019 to 0.003)	0.16	0.33	−0.016 (−0.029 to −0.003)	0.02	0.08	−0.008 (−0.021 to 0.004)	0.19	0.37
Cingulum cingulate L	0.389 (0.024)	0.400 (0.022)	0.410 (0.022)	−0.014 (−0.028 to 0.001)	0.06	0.18	−0.025 (−0.042 to −0.007)	0.01	0.04	−0.011 (−0.028 to 0.006)	0.20	0.37
Cingulum cingulate R	0.360 (0.025)	0.367 (0.023)	0.376 (0.022)	−0.010 (−0.025 to 0.005)	0.20	0.37	−0.020 (−0.038 to −0.001)	0.04	0.12	−0.010 (−0.028 to 0.008)	0.26	0.41
Cingulum hippocampus L	0.351 (0.023)	0.342 (0.035)	0.360 (0.021)	0.010 (−0.007 to 0.028)	0.25	0.41	−0.006 (−0.028 to 0.015)	0.56	0.67	−0.016 (−0.037 to 0.004)	0.12	0.25
Cingulum hippocampus R	0.357 (0.029)	0.352 (0.033)	0.363 (0.025)	0.005 (−0.014 to 0.024)	0.59	0.69	−0.004 (−0.027 to 0.019)	0.73	0.80	−0.009 (−0.032 to 0.013)	0.42	0.58
Superior longitudinal fasciculus L	0374 (0.018)	0.374 (0.020)	0.374 (0.021)	−0.002 (−0.014 to 0.011)	0.78	0.84	−0.003 (−0.018 to 0.012)	0.69	0.78	−0.001 (−0.016 to 0.013)	0.86	0.86
Superior longitudinal fasciculus R	0.378 (0.016)	0.370 (0.023)	0.377 (0.020)	0.007 (−0.006 to 0.019)	0.30	0.44	−0.002 (−0.017 to 0.014)	0.84	0.87	−0.008 (−0.023 to 0.007)	0.27	0.41
Uncinate fasciculus L	0.360 (0.016)	0.373 (0.017)	0.376 (0.013)	−0.014 (−0.024 to −0.004)	<0.01	0.05	−0.018 (−0.030 to −0.006)	<0.01	0.03	−0.004 (−0.016 to 0.008)	0.47	0.59
Uncinate fasciculus R	0.371 (0.017)	0.379 (0.016)	0.386 (0.012)	−0.008 (−0.018 to 0.002)	0.11	0.25	−0.016 (−0.028 to −0.003)	0.01	0.06	−0.007 (−0.019 to 0.005)	0.22	0.38
Corticospinal tract L	0.494 (0.018)	0.496 (0.016)	0.492 (0.016)	−0.003 (−0.014 to 0.008)	0.57	0.67	0.002 (−0.012 to 0.015)	0.81	0.85	0.005 (−0.008 to 0.017)	0.46	0.60
Corticospinal tract R	0.479 (0.018)	0.479 (0.022)	0.481 (0.018)	0.001 (−0.011 to 0.014)	0.85	0.88	−0.001 (−0.017 to 0.014)	0.86	0.87	−0.003 (−0.017 to 0.012)	0.73	0.80

(Continued)

Table 3. (Continued)

	DMS (n = 22)	nDMS (n = 21)	HCs (n = 12)	DMS vs nDMS			DMS vs HCs			nDMS vs HCs		
				Difference (CI)	p value	p corr.	Difference (CI)	p value	p corr.	Difference (CI)	p value	p corr.
MD (mm²/s) (*10⁻³)												
Anterior thalamic radiation L	0.869 (0.038)	0.856 (0.030)	0.827 (0.021)	0.012 (−0.008 to 0.032)	0.24	0.40	0.039 (0.014 to 0.063)	<0.01	0.05	0.027 (0.003 to 0.050)	0.03	0.10
Anterior thalamic radiation R	0.868 (0.038)	0.853 (0.031)	0.829 (0.019)	0.014 (−0.006 to 0.034)	0.17	0.34	0.038 (0.014 to 0.063)	<0.01	0.03	0.024 (0.000 to 0.048)	0.05	0.14
Cingulum cingulate L	0.824 (0.039)	0.814 (0.024)	0.798 (0.031)	0.010 (−0.010 to 0.031)	0.31	0.45	0.027 (0.002 to 0.052)	0.04	0.12	0.016 (−0.008 to 0.040)	0.18	0.36
Cingulum cingulate R	0.800 (0.032)	0.789 (0.025)	0.776 (0.025)	0.010 (−0.008 to 0.028)	0.25	0.41	0.023 (0.001 to 0.045)	0.04	0.12	0.013 (−0.008 to 0.034)	0.23	0.39
Cingulum hippocampus L	0.861 (0.041)	0.863 (0.039)	0.796 (0.028)	−0.005 (−0.028 to 0.018)	0.68	0.68	0.064 (0.036 to 0.092)	<0.001	<0.01	0.069 (0.041 to 0.096)	<0.001	<0.001
Cingulum hippocampus R	0.860 (0.043)	0.865 (0.040)	0.814 (0.037)	−0.008 (−0.034 to 0.018)	0.54	0.66	0.041 (0.010 to 0.073)	0.01	0.06	0.049 (0.019 to 0.080)	<0.01	0.05
Superior longitudinal fasciculus L	0.812 (0.034)	0.805 (0.026)	0.785 (0.029)	0.007 (−0.012 to 0.026)	0.46	0.61	0.028 (0.005 to 0.051)	0.02	0.08	0.021 (−0.002 to 0.043)	0.07	0.18
Superior longitudinal fasciculus R	0.806 (0.034)	0.813 (0.029)	0.785 (0.028)	−0.006 (−0.026 to 0.014)	0.54	0.67	0.023 (−0.001 to 0.047)	0.06	0.16	0.029 (0.006 to 0.053)	0.01	0.07
Uncinate fasciculus L	0.853 (0.035)	0.834 (0.028)	0.814 (0.029)	0.017 (−0.002 to 0.037)	0.08	0.20	0.038 (0.013 to 0.062)	<0.01	0.03	0.020 (−0.003 to 0.044)	0.09	0.21
Uncinate fasciculus R	0.855 (0.037)	0.846 (0.025)	0.819 (0.024)	0.007 (−0.012 to 0.026)	0.45	0.60	0.033 (0.010 to 0.056)	<0.01	0.04	0.026 (0.003 to 0.048)	0.03	0.10
Corticospinal tract L	0.819 (0.027)	0.815 (0.022)	0.790 (0.016)	0.006 (−0.009 to 0.020)	0.44	0.59	0.028 (0.010 to 0.045)	<0.01	0.04	0.022 (0.005 to 0.039)	0.01	0.06
Corticospinal tract R	0.825 (0.026)	0.838 (0.036)	0.803 (0.018)	−0.014 (−0.032 to 0.005)	0.14	0.30	0.020 (−0.002 to 0.043)	0.08	0.19	0.034 (0.012 to 0.056)	<0.01	0.03

CI: confidence interval; corr.: FDR-corrected *p* value; DMS: depressed multiple sclerosis patients; FA: fractional anisotropy; HCs: healthy controls; L: left; MD: mean diffusivity; MS: multiple sclerosis; nDMS: non-depressed multiple sclerosis patients; R: right.
Displayed data are mean (standard deviation) values.

Table 4. Functional connectivity between limbic and frontal brain regions of depressed and non-depressed patients with MS and healthy controls.

	DMS (n=22)	nDMS (n=21)	HCs (n=12)	DMS vs nDMS			DMS vs HCs			nDMS vs HCs		
				Difference (CI)	p value	p corr.	Difference (CI)	p value	p corr.	Difference (CI)	p value	p corr.
Amygdala L—frontal FC	-1.066 (0.435)	-0.711 (0.678)	-0.540 (0.573)	-0.353 (-0.717 to 0.010)	0.06	0.26	-0.533 (-0.974 to -0.091)	0.02	0.11	-0.179 (-0.607 to 0.248)	0.40	0.91
Amygdala R—frontal FC	-1.094 (0.398)	-0.516 (0.655)	-0.472 (0.614)	-0.564 (-0.919 to -0.209)	<0.01	0.04	-0.585 (-1.015 to -0.154)	<0.01	0.08	-0.020 (-0.438 to 0.397)	0.92	0.92
Hippocampus L—frontal FC	-0.608 (0.535)	-0.535 (0.620)	-0.737 (0.433)	-0.061 (-0.412 to 0.289)	0.73	0.93	0.170 (-0.255 to 0.596)	0.43	0.77	0.232 (-0.181 to 0.644)	0.27	0.68
Hippocampus R—frontal FC	-0.681 (0.454)	-0.498 (0.517)	-0.406 (0.696)	-0.224 (-0.564 to 0.117)	0.19	0.58	-0.348 (-0.761 to 0.065)	0.10	0.35	-0.125 (-0.525 to 0.276)	0.54	0.88
Thalamus L—frontal FC	0.047 (0.482)	0.098 (0.580)	0.085 (0.496)	-0.039 (-0.365 to 0.287)	0.81	0.97	0.030 (-0.366 to 0.425)	0.88	0.93	0.069 (-0.314 to 0.452)	0.72	0.99
Thalamus R—frontal FC	0.073 (0.514)	-0.047 (0.590)	0.044 (0.559)	-0.144 (-0.203 to 0.491)	0.41	0.82	0.107 (-0.314 to 0.529)	0.61	0.92	-0.036 (-0.445 to 0.372)	0.86	0.97

CI: confidence interval; corr.: FDR-corrected *p* value; DMS: depressed multiple sclerosis patients; FC: functional connectivity; HCs: healthy controls; L: left; MS: multiple sclerosis; nDMS: non-depressed multiple sclerosis patients; R: right.
Displayed data are mean (standard deviation) of *Z* scores.

different between the three groups, suggesting that the observed FA differences might be specific for depression (in MS) and perhaps do not reflect "general" changes due to MS.

Volumetric differences

Despite a shorter disease duration, DMS patients showed more severe WM atrophy than nDMS patients, but similar lesion load. In addition, WM atrophy was related to a higher depression rank score (when FC of the right amygdala was not included in the model). This is in line with previous studies that reported more severe central atrophy[31] and WM atrophy[14] in DMS compared to nDMS patients, with spatial preference for frontal and temporal regions.[14,31] Lesion and GM volume were not significant predictors for depression rank score (data not shown), which is not in line with previous studies reporting higher lesion volume and more severe GM atrophy in DMS compared to nDMS patients.[32,33] This is probably due to differences in sample characteristics (i.e. early or late MS and MS type).

The volume of subcortical limbic structures was similar between patient groups and lower than that of HCs, although the left amygdala of nDMS patients did not differ from that of HCs. One longitudinal study in MS showed that volume loss of the thalamus over a period of 17 months was related to more depressive symptoms in MS.[34] Thalamic atrophy in this study was observed in both patient groups relative to HCs, suggesting it to be an MS-related abnormality not specific for depression.

Functional alterations

Previous studies in patients with MDD have led to the hypothesis that FC between limbic and frontal regions is abnormal.[11] In line with this hypothesis, we observed decreased FC between the amygdala and frontal regions in DMS compared to nDMS patients, which was related to a higher depression rank score. Our results are in line with a previous study that found higher depression scores in MS to be related to lower RS FC between the hippocampus and orbitofrontal cortex.[17] Also during emotional processing, decreased FC between the amygdala and hippocampus can be observed relative to HCs, while within MS patients, lower FC between the amygdala with dorsolateral PFC and the hippocampus with orbitofrontal cortex was related to higher BDI scores.[16] Taken together, decreased FC between the amygdala with frontal regions might be a fingerprint of MS with comorbid depression.

Cognitive functioning

Although depression can be a confounder of cognitive performance in MS,[13] DMS and nDMS patients performed similar on cognitive tests. In the post hoc analysis, DMS^MDD patients performed worse than nDMS^lowHADS patients on verbal memory. This is in line with a previous study in MS that related higher BDI scores with worse performance on verbal memory.[13] Future studies should follow DMS patients over time to see whether fronto-limbic changes make them more prone to develop cognitive impairment.

While our study has several strengths (i.e. multi-modal approach, inclusion of moderate-to-severe DMS patients), our results have to be interpreted carefully due to sample sizes. We cannot conclude whether DMS patients already had a predisposition to develop a depression (nine DMS patients had a lifetime diagnosis of MDD with the onset prior to MS diagnosis) or whether MS pathology in fronto-limbic regions resulted in a depression. On average, in the group of nine patients with MDD diagnosis before MS diagnosis, the MDD diagnosis was 15 years prior to MS diagnosis. Furthermore, the first MS-related complaints (self-report) in this group were on average 7 years before MS diagnosis, thereby not completely coinciding with the time of MDD diagnosis. This might suggest that the MDD is an unrelated condition in this group. However, a previous study also suggested that depression might be a prodromal symptom of MS.[35] Ideally, we would have included MDD patients (without MS), in order to determine whether the changes we observed in DMS patients are similar to that in MDD (suggestive of a primary MDD) or whether the changes are more pronounced in our MS patients (suggestive of an interaction effect). Another limitation of the study is that the nDMS patients and HCs were prospectively recruited in the context of another study (no prior publications) but retrospectively added to the DMS patients in this study. As both patient groups differed in disease duration, we corrected for age (significantly correlated to disease duration; $\rho = 0.59$) and performed additional analyses with direct comparison between DMS and nDMS patients and correction for disease duration. Although we applied an FDR correction, the subgroup analyses are still prone to possible false-positive results given the small group sizes, and therefore, the findings have to be interpreted carefully. The results from the subgroup analyses were rather similar to that of the main analyses, except for FA of the left uncinate fasciculus that did not survive the correction for multiple testing. Finally, the depression rank score has to be interpreted carefully, as two different questionnaires were used.

To conclude, DMS patients showed more severe WM atrophy, decreased FA of the uncinate fasciculus, and decreased FC between the amygdala and frontal areas than nDMS patients, suggestive of fronto-limbic disconnection. The latter two measures were complementary predictors for the severity of depression and explained almost 50% of the variance, thereby highlighting the importance of a multimodal approach in understanding the etiology of depression in MS.

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