Subcortical grey matter structures in multiple sclerosis: what is their role in cognition?

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Abstract

The present study aimed to investigate altered grey matter (GM) and functional connectivity (FC) in deep subcortical areas, like the thalamus and basal ganglia, and their relationship with cognitive impairment in multiple sclerosis (MS). Thirty-six patients were neuropsychologically assessed, classified as cognitive preserved (CP) and cognitive impairment (CI), and were compared with 18 healthy controls (HC). GM atrophy and FC were observed in 10 predefined functional areas of the thalamus and in six of basal ganglia. GM atrophy was prominent in the basal ganglia in CI patients compared to CP MS patients. Increased FC was observed between the right caudate and the bilateral orbitofrontal cortex in CI *vs.* CP patients. The discriminant and correlation analyses revealed that the enhanced FC observed between the right caudate and the orbitofrontal cortex was closely associated with cognitive impairment in MS patients. In conclusion, reduced GM volume and enhanced fronto-basal ganglia connectivity are related to cognition in MS patients.

Key words: thalamus, basal ganglia, multiple sclerosis (MS), cognition, grey matter (GM), functional connectivity (FC).

1. Introduction

Neuroimaging studies have revealed that grey matter (GM) atrophy is the main neuroanatomical correlate of cognitive disability in MS patients [1]. The relationship between these two variables has been assessed primarily in cortical areas [2], but more recent studies have suggested that GM loss plays a key role in subcortical structures, such as the thalamus [3]. In contrast, the relationship between GM atrophy in other subcortical areas, such as basal ganglia, has been less studied [3].

The thalamus and basal ganglia form part of cortico-subcortical circuits, and alterations of functional connectivity (FC) in these circuits might be as relevant as GM atrophy in MS patients' cognitive decline. Indeed some recent studies have shown that thalamic atrophy is accompanied by increased FC between the thalamus and several cortical regions, and that these thalamic dysfunctions correlate with MS patients' cognitive decline [4; 5]. Conversely, the existence and possible significance of similar alterations in basal ganglia remain largely unexplored.

In this background, and given the relevance of subcortical structures in cognition, we devised an exploratory study that aimed to: 1) describe the GM and FC differences between cognitive preserved (CP) and cognitive impaired (CI) MS patients in different thalamic subregions of functional significance; 2) describe GM and FC differences between CP and CI patients in basal ganglia; 3) identify which of these neuro-anatomical and neuro-functional subcortical alterations are associated with cognitive performance in MS patients.

Methods

Participants

Eighteen healthy controls (HC), and 36 patients with clinically defined relapsing-remitting (RR) MS, according to the revised Mc Donald criteria [6], were recruited from the Hospital General de Castellón and were assessed using the Brief Repeatable Battery of Neuropsychological Tests (BRB-N). Following the criteria used in previous studies [7], the patients who scored a standard deviation of 1.5 below the mean normative values in at least one BRB-N test were considered CI, while the rest were recruited as CP. Each participant obtained a global cognition Z score by averaging the Z scores that corresponded to all the subtests. *The Matrix Reasoning Subtest (WAIS-III* [8]) and *the Fatigue Severity Scale* [9] were also administered. The study was approved by the Ethics Standards Committees of the Hospital General and the Universitat Jaume I, both of Castellón.

Magnetic Resonance Imaging (MRI) acquisition

Anatomical high-resolution 3d sagittal MPRAGE T1 images were acquired using a 1.5 T scanner (Siemens Avanto, Erlangen, Germany, TR = 11 ms, TE = 4.9 ms, FOV = 24 cm, matrix = 256 x 224 x 176, voxel size = 1 x 1 x 1 mm). Functional (fMRI) resting-state 270 volumes were recorded over 9 min using a gradient-echo T2*-weighted echo-planar imaging sequence (TR/TE = 2000/30 ms, matrix = 64 x 64 x 30, voxel size = 3.5 x 3.5 x 4.02 mm, flip angle = 90º). During the resting sequence, participants were instructed to remain motionless and to relax with their eyes closed, to not fall asleep and to think of nothing in particular.

Region of interest (ROI) creation

Sixteen independent ROIs were created in the MNI space. 6 ROIs corresponded to basal ganglia, specifically the left and right caudate, putamen and pallidum, were obtained by WFU Pickatlas (wfu_pickatlas:www.fmri.wfubmc.edu/download.htm). Thalamic ROIs were obtained with Zhang thalamic connectivity atlas included in the LEAD-DBS toolbox (http://www.lead-dbs.org/). This atlas divides the thalamus into five regions according to the cortical areas that are highly correlated with specific thalamic areas, obtained in a previous resting-state fMRI study [10]. We separated these thalamic ROIs into left-right to finally obtain 10 ROIs. All the ROIs were binarized and used in subsequent analyses (see Figure, 1. A).

Voxel-Based Morphometry (VBM) analysis

In all patients, T1-hypointense lesions were identified and filled with the Jim software (Version 5.0, Xinapse Systems, Northants., UK; http://www.xinapse.com) to reduce the influence of MS lesions and to exclude the misclassified pixels from the statistical analysis following the previously described steps [11].

Then lesion-filled images were reoriented along the AC-PC and pre-processed following an optimized VBM protocol with the Diffeomorphic Anatomical Registration Through Exponential Lie Algebra (DARTEL) included in SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK). Pre-processing included image segmentation into GM, WM, and CFS tissues in a native space, spatial normalization to a population-specific template and modulation (to ensure that the overall amount of GM tissue was preserved). GM volumes (in milliliters -ml-) from all the ROIs were extracted in each participant through the intersection between the GM modulated map and the binary map that corresponded to each one.

Seed-Functional Connectivity (FC) analysis

The resting-state fMRI images were pre-processed using the DPARSF V4.3 tool [12] and included: discarding the first 10 functional volumes to achieve a signal equilibrium, slice timing correction, realignment to the first scan of each session, head motion correction, coregister, nuisance covariates regression to remove nonspecific sources of variance (including the 24-parameter head motion model, scrubbing regressors, white matter signal, CSF and global signal), spatial normalization with a resampled voxel size of 3 mm3 to the Montreal Neurological Institute (MNI) space, and spatial smoothing with an isotropic Gaussian kernel of 4-mm full width at half maximum (FWHM). Next temporal filtering (0.01 Hz - 0.08 Hz) was applied to the time series of each voxel to reduce the effect of low-frequency drifts and high-frequency noise.

Subsequent seed-FC analyses were performed by averaging the time series from each ROI and correlating with the time series of each voxel in the brain.

Statistical Analyses

Between-group comparisons were made with the demographic, clinical and cognitive variables by one-way ANOVAs and Bonferroni's *post hoc* test using SPSS v.23. The GM and FC differences between groups were assessed with separate analyses of variance (ANOVA) in SPSS and SPM8 by setting the significance level at $p < 0.05$, and were FWE cluster-corrected for the multiple comparisons in combination with a threshold of $p < 0.001$ at the uncorrected level. The effect size for each statistically significant comparison was estimated by calculating the corresponding Cohen's d. Finally, SPSS v.23 was used to run a discriminant analysis to identify the single volumetric/FC variable that best characterised each experimental group (HC, CP or CI). Pearson's correlation index was used to identify the most relevant predictors of cognitive status (global Z score) in MS patients.

Results

The demographic, clinical and neuropsychological results are presented in Table 1. The between-group differences in GM volume and FC are described in Table 2 and Figure 1. B, C, D. To sort according to importance, many effect sizes of the between-group differences were used. Thus, the main differences in GM volume in the thalamic regions between HC and CP patients were observed in the left parietal-occipital nucleus (d=2.31) and in the left temporal nucleus (d=2.04), whereas the differences between groups HC and CI reached their largest size in the left somatosensorial (d=2.40), the left temporal nucleus (d=2.34) and the left parietal occipital nucleus (d=2.18). Finally, the most prominent differences found between groups CP and CI were observed in the FC between the right caudate and the bilateral orbitofrontal cortex (d=1.81) and, to a lesser extent, in the volumes of the right (d=1.16) and left (d=1.15) putamen. Therefore, the main differences between HC and MS patients seem to involve a reduced GM volume in specific thalamic regions of the left hemisphere, where the parietal occipital nucleus is the most relevant anatomical area. Conversely, increased FC between the right caudate and the bilateral orbitofrontal cortex was the most salient characteristic of the CI group compared to the CP one.

These conclusions were confirmed by a linear discriminant analysis (see Text, Supplemental Digital Content 1, Figure, Supplemental Digital Content 2 and Table, Supplemental Digital Content 3), which yielded a first significant discriminant function that distinguished HC from the two patient subgroups (79.1% explained variance; Wilks' lambda (12)= 0.136, $p<0.000$) which, in turn, identified the left Parietal-Occipital nucleus as its best single proxy (total structure coefficient=0.603). The second discriminant function was also statistically significant (20.9% explained variance; Wilks' lambda (5)= 0.552, $p<0.000$) and distinguished CP from CI. The FC between the

right caudate and the bilateral orbitofrontal cortex was the variable that showed the highest bivariate correlation with the second discriminant function (total structure coefficient =0.772).

Finally, the relationship between the neuroanatomical and neurofunctional variables included in this study (Table 2) and MS patients' cognitive status (global Z score) was assessed by Pearson's correlation index. The FC between the right caudate and the bilateral orbitofrontal cortex emerged as both the most prominent (see Figure 2) and the inverse neural correlate of cognitive performance. Interestingly, the GM volumes of almost all the basal ganglia subregions, but not those of the thalamus, also correlated significantly and directly with the global Z scores.

Discussion

We confirmed the results of previous studies, which indicate that GM loss in the thalamus is involved with worse cognition in MS [3]. However, we extend these results by 1) also providing a functional subregional analysis of the thalamus and 2) showing that both the thalamus and basal ganglia are important subcortical areas to determine MS patients' cognitive status. Specifically, both patient groups (the CI and also the CP group) showed structural and functional differences in the thalamus compared to HC. However, while the GM volume was significantly reduced in each thalamic subregion at the bilateral level in CI patients, these effects were statistically significant only in the left, but not the right, hemisphere in CP patients. The direct comparisons made between CI and CP patients yielded only a significant difference at the right somatosensorial nucleus level, which suggests that the subregional analyses of thalamic atrophy could uncover the subtle neuroanatomical alterations associated with cognitive decline in MS patients.

The differences between the two patient subgroups became more evident in basal ganglia. We highlight two results that we found between CP and CI patients: 1) we observed GM atrophy in the bilateral caudate and the putamen in CI compared to CP; 2) the reduced GM in the right caudate was accompanied by increased FC between this area and the bilateral orbitofrontal cortex in CI *versus* CP patients. Finally, we found that increased FC was the best variable to distinguish between CP and CI patients, and it also correlated negatively with cognitive performance (global Z score). As a whole, these results suggest that cognitive decline in MS patients is associated with alterations to the specific modules of previously described fronto-basal loops. In line with this, it is worth noting that current models conceive cortico-striatal function as being modular and hierarchically organised, which confers the caudate nucleus a primarily cognitive role [13; 14]. The reason for this is, while the putamen is connected primarily to primary sensory and motor areas, the caudate is connected to the frontal areas involved in the regulation of emotional and cognitive functions [15], and also because non-invasive measures of anatomical and functional connectivity in humans have demonstrated that caudate and executive frontal areas are clearly linked [14]. Therefore, the results of the present study agree with the proposed cognitive role of the caudate-frontal loop and highlight its importance in understanding cognitive decline in MS patients.

In conclusion, this is the first study to reveal that deficiencies in basal ganglia and, more specifically, enhanced FC in the fronto-basal ganglia network, are related to cognitive impairment in MS. Future studies are needed to confirm these previous exploratory results.

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Table 1. Main demographic, clinical and neuropsychological characteristics of all participants.

	HC (n=18)	**CP patients (n=18)**	**CI patients (n=18)**	**HC-CP**	**HC-CI**	**CP -CI**
Age (SD) [range]	33.33 (9.49) [22-54]	33.39 (7.03) [23-49]	38.78 (9.74) [20-58]	1.000	.211	.219
Gender (Male/Female)	9/9	5/13	5/13	-	-	-
Mean Education Years (SD) [range]	13.61 (2.64) [8-16]	12.22 (2.32) [8-16]	11.67 (2.87) [8-15]	.353	.091	1.000
EDSS (SD) [range]	-	1.56 (0.76) [0-3.5]	3.14 (1.28) [1-6.5]	-	-	**.000 ****
Mean years disease duration (SD) [range]	-	5.94 (4.53) [1-15]	10.72 (7.69) [1-29]	-	-	.031*
FSS	31.78 (10.15)	31.33 (16.85)	37.67 (13.92)	1.000	.629	.534
Manipulative IQ (Matrix WAIS-III)	107.22 (9.74)	103.89 (10.92)	99.17 (8.79)	.945	.053	.470
SDMT	59.06 (11.22)	60.61 (6.81)	39.72 (11.79)	1.000	**.000 ****	**.000 ****
PASAT 3 sec.	46.44 (9.87)	49.78 (6.44)	35.17 (13.24)	.999	**.005 ****	**.000 ****
SRT Long Term Storage	52.67 (12.08)	50.00 (6.79)	33.22 (11.12)	1.000	**.000 ****	**.000 ****
SRT Consistent Long Term Retrieval	42.28 (10.46)	42.00 (8.29)	21.89 (9.77)	1.000	**.000 ****	**.000 ****
SRT Delayed Recall	9.94 (1.39)	9.78 (1.21)	5.89 (2.19)	1.000	**.000 ****	**.000 ****
10/36 SPART Long-Term Storage	22.28 (4.55)	22.56 (3.33)	16.39 (4.23)	1.000	**.000 ****	**.000 ****
10/36 SPART Delayed Recall	7.94 (2.15)	7.61 (1.61)	5.61 (2.17)	1.000	**.003 ****	.012*
Phonetic Fuency	12.89 (3.97)	14.56 (4.54)	10.72 (3.29)	.639	.322	.016*

Abbreviations**:** HC = Healthy Controls; CP = cognitive preserved; CI = cognitive impaired; EDSS = Expanded Disability Status Scale; FSS = Fatigue Severity Scale; IQ = Intelligence Quotient; SDMT = Symbol Digit Modalities Test; PASAT = Paced Auditory Serial Addition Test; SRT = Selective Reminding Test; SPART = Spatial Recall Test; ** = significantly different at $p < 0.01$; * = significantly different at $p < 0.05$.

Table 2: Between-group differences in basal ganglia and thalamic volume. Descriptive data are expressed as means and standard deviations (in brackets). For the between-groups comparisons, the p values associated with the Bonferroni *post hoc* comparisons and the Cohen's d values are provided.

	HC (n=18)	CP (n=18)	CI (n=18)	HC - CP	HC - CI	CP - CI
Basal Ganglia GM volume (ml)						
L Caudate	4.70 (0.48)	4.66 (0.49)	4.07 (0.84)	1.000	.013 * (d=0.92)	.020* (d=0.85)
R Caudate	5.01 (0.59)	4.80 (0.62)	4.03 (0.91)	1.000	.000 ** (d=1.23)	.008 ** (d=0.99)
L Putamen	5.04 (0.85)	4.16 (0.97)	2.99 (1.07)	0.027* (d=0.96)	.000 ** (d=2.12)	.002 ** (d=1.15)
R Putamen	4.94 (0.76)	4.63 (1.01)	3.42 (1.07)	.986	.000 ** (d=1.63)	.001 ** (d=1.16)
L Pallidum	0.31 (0.11)	0.22 (0.07)	0.18 (0.06)	0.009** (d=0.98)	.000 ** (d=1.47)	.334
R Pallidum	0.50 (0.11)	0.39 (0.11)	0.30 (0.12)	0.009** (d=0.99)	.000 ** (d=1.74)	.050
***Thalamus GM volume* (ml)**						
L Motor-Premotor nucleus	3.21 (0.39)	2.26 (0.66)	1.73 (0.82)	.000** (d=1.75)	.000 ** (d=2.30)	.055
R Motor-Premotor nucleus	2.55 (0.41)	2.17 (0.60)	1.72 (0.65)	.151	.000 ** (d=1.53)	.057
L Parietal-Occipital nucleus	2.04 (0.20)	1.59 (0.19)	1.57 (0.23)	.000 ** (d=2.31)	.000 ** (d=2.18)	1.000
R Parietal-Occipital nucleus	2.16 (0.24)	1.94 (0.32)	1.79 (0.37)	.113	.002** (d=1.18)	.456
L Prefrontal nucleus	3.39 (0.39)	2.73 (0.44)	2.47 (0.57)	.000 ** (d=1.59)	.000 ** (d=1.88)	.313
R Prefrontal nucleus	2.28 (0.27)	1.99 (0.40)	1.79 (0.39)	.068	.001 ** (d=1.46)	.295
L Somatosensorial nucleus	0.31 (0.05)	0.19 (0.10)	0.12 (0.10)	.000 ** (d=1.52)	.000 ** (d=2.40)	.057
R Somatosensorial nucleus	0.27 (0.06)	0.23 (0.09)	0.16 (0.08)	.374	.000 ** (d=1.56)	.023* (d=0.82)
L Temporal nucleus	1.91 (0.21)	1.44 (0.25)	1.37 (0.25)	.000 ** (d=2.04)	.000 ** (d=2.34)	1.000
R Temporal nucleus	1.26 (0.19)	1.15 (0.23)	1.00 (0.30)	.573	.008 ** (d=1.03)	.213
Functional connectivity						
FC-R caudate- B orbitofrontal cortex	0.08 (0.09)	0.04 (0.11)	0.21 (0.06)	0.53	.001** (d=1.57)	.000* (d=1.81)
FC-R premotor-L cerebellum	0.16 (0.12)	0.04 (0.10)	-0.01 (0.07)	.002** (d=1.23)	.000** (d=1.81)	.591
FC-R premotor-B cingulate	0.18 (0.10)	0.05 (0.08)	0.01 (0.11)	.002** (d=1.44)	.000** (d=1.65)	.571
FC R pallidum- R caudate	0.23 (0.11)	0.11 (0.10)	0.04 (0.11)	0.008** (d=1.14)	0.000** (d=1.72)	.163

Abbreviations: ml = millilitres; HC = healthy controls; CP = cognitive preserved; C =: cognitive impaired; L = left; R = right; B=Bilateral ** = significantly different at $p < 0.01$; * = significantly different at $p < 0.05$.

Figure 1. Figure illustrating the regions of interest (ROIs) and between-group seed rs-FC significant differences. A: ROIs corresponding to basal ganglia and the thalamus. B: reduction in FC between the right pallidum and the right caudate in CI patients *versus* HC; C: increased FC between the right caudate and the medial orbitofrontal cortex in CI patients *versus* CP patients; D: reduction in FC between the right motor/premotor thalamic nucleus and the bilateral middle cingulate gyrus and the left cerebellum in CI *versus* HC.

Figure 2. Correlational heat map between the global Z score and the MRI structural and Functional Connectivity variables. Colours denote significance level and Pearson r coefficient values are provided in cells.

List of Supplemental Digital Content

- Supplemental Digital Content 1. Text includes information Linear Discriminant analyses.
- Supplemental Digital Content 2. Figure illustrating the two-function plot of the group centroids and individual cases. Dotted lines depict the calculated boundaries of the classificatory territories of each group.
- Supplemental Digital Content 3. Table showing the standardised and total structure coefficients for each variable included in the obtained discriminant functions. The highest total structure coefficients of each function are highlighted in bold and correspond to the first two entries of the step-wise discriminating model. Note that although no strict correspondence between these two sets of coefficients exists, both indicate that the GM volume of the left parietal-occipital nucleus of the thalamus and the FC between the caudate and the orbitofrontal cortex are the most relevant variables of the obtained discriminant functions. Abbreviations: L: left; R: right, FC: functional connectivity.