DYNAMIC CHANGES OF HIPPOCAMPAL SUBFIELDS IN CIS PATIENTS: A 3-MONTH MRI LONGITUDINAL STUDY

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INTRODUCTION

Lesional and atrophy data[1,2] support the evidence of gray matter (GM) involvement in patients with clinically isolated syndrome (CIS) suggestive of multiple sclerosis (MS). Such a GM affection has shown dynamic modifications over time.[3]

The hippocampus is affected early in MS, with characteristic subregional patterns of involvement at different stages of the disease and in different disease clinical phenotypes.

The subgranular layer of the dentate gyrus (DG), in the C4 region of the Cornus Ammonis, experiences neurogenesis and there is *in-vivo* evidence of its expansion in relapsing-remitting (RR) MS patients.[4]

Whether such an hippocampal involvement is also present in a population of patients with CIS at a very early stage of the disease has not been investigated yet.

OBJECTIVES

To evaluate short-term patterns of regional hippocampal volume variations in CIS patients early in the course of the disease.

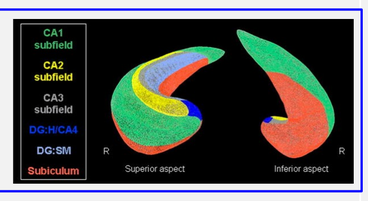
METHODS

20 CIS patients within 2 months from the first clinical episode. Appropriate investigations were performed as necessary to exclude alternative diagnoses, and all patients were interviewed carefully to rule out possible previous events. Exclusion and inclusion criteria, MRI acquisition and hippocampal analysis are explained in **Table 1**.

Table 1. Summary of exclusion and inclusion criteria of the study population, MRI acquisition and hippocampal analysis.

Study population	MRI acquisition	Hippocampal analysis
Exclusion criteria (CIS patients and HC) History of neurological/psychiatric illness/substances abuse and steroid administration during the month before study inclusion. **Inclusion criteria (CIS patients)** CIS patients within 2 months from the first clinical episode who underwent: 1)Clinical evaluation with Expanded Disability Status Scale (EDSS) 2)MRI acquisition At baseline and after 3 months.	**Structural MRI (1.5 T scanner)** Axial dual-echo fast spin-echo Sagittal 3D-T1 Post-gadolinium Sagittal 3D-T1 **MRI analysis** Quantification of T2 lesion volume (LV) **(Jim6.0 software)** Refilling of T1-hypointense lesions[5], quantification of normalized brain volume (NBV), GM and white (WM) matter volumes **(SIENAx software)**	**Segmentation** Manual tracing of the hippocampus on contiguous coronal slices,[6] and volume computation of the traced hippocampi controlling for head size (MultiTracer software). **Radial atrophy mapping** Creation of 3D surface models of each hippocampus with functions available in the library LONI Shape Tools (http://www.loni.ucla.edu/Software/ShapeTools version 1.3.11). Medial 3D curve derived from each individual hippocampus and calculation of the distance of each surface point from the centerline, measuring the radial size of each hippocampus.[7] Hippocampal subfields were superimposed on the average hippocampus obtained from a HC group (**Figure 1**[8]) .

Figure 1. Abbreviations: CA=cornu ammonis; DG:H=hilus of the dentate gyrus; DG:SM=stratum molecolare of the dentate gyrus; R=right; L=left.



Statistical analysis: age and disease duration adjusted.

Between-group comparisons (SPSS): non-parametric Mann-Whitney and Pearson $\chi 2$ for HC vs CIS (demographic, clinical and conventional MRI data). Wilcoxon test for paired data for longitudinal analysis of the CIS population.

Radial mapping analysis (LONI Rshape library): Matching of homologous hippocampal surface points across individuals and analysis of variance to evaluate local differences in radial distance between HC and CIS patients at two time points. Pearson correlation at each surface point to map correlation between radial size variation (at month 3, month 3 *vs* baseline, baseline) in CIS patients and T2-, T1- and gadolinium LV at baseline.

RESULTS

Demographic and coventional MRI data

At baseline, no significant differences were observed between HC and CIS patients. During the follow-up, an increase of brain volume in CIS patients was observed (**Table 2**).

Table 2. Main clinical, demographic and radiological data of HC and CIS at baseline.

		HC	CIS	p
Clinical/ Demographic data	Number	10	20	-
	Female/Males	8/2	17/3	0.1[1]
	Mean age (range)[years]	34.1 (23-49)	28.8 (18-42)	0.09[2]
	Median EDSS (range)	-	2.5 (0-6)	-
	Median DD (range) [days]	-	22.5 (8-60)	-
MRI data	Median T2LV (range) [ml]	-	2.28 (0.04-22.5)	-
	Median T1LV (range) [ml]	-	0.41 (6.68-0.00)	-
	Median GDLV (range) [ml]	-	0.00 (0.00-0.53)	-
	Mean NBV (SD) [ml]	1605 (90)	1549 (94)	0.45[2]
	Mean GMV (SD) [ml]	750 (55)	732 (51)	0.79[2]
	Mean WMV (SD) [ml]	855 (54)	817 (58)	0.08[2]
	Mean PBVC (SD) [%]	-0.1 (0.4)	+ 0.3 (0.6)	0.04[2]
	Mean PGMVC (SD) [%]	-1.8 (1.9)	-3.5 (4.6)	0.2[2]
	Mean PWMVC (SD) [%]	-0.7 (3.2)	-2.7 (4.9)	0.4[2]

[1]Pearson χ^2, [2]Mann-Whitney. Abbreviations: DD=disease duration; SD=standard deviation; LV=lesion volume; NBV=normalized brain volume; GMV=gray matter volume; WMV=white matter volume.; PBVC=percentage brain volume change; PGMVC=percentage gray matter volume change, PWMVC=percentage white matter volume change.

During the follow-up, significant amelioration of EDSS was detected in CIS patients (**Table 3**).

Table 3. Clinical and MRI data in CIS patients.at baseline and after 3 months.

	CIS Baseline	CIS 3-month follow-up	p
Median EDSS (range)	2.5 (0.0-6.0)	1.0 (0.0-3.0)	<0.001
Median T2LV (range) [ml]	2.28 (0.04-22.5)	1.66 (0.07-21.49)	0.94
Median T1LV (range) [ml]	0.41 (0.00-6.68)	0.47 (0.00-7.78)	0.18
Median GDLV (range) [ml]	0.00 (0.00-0.53)	0.00 (0.00-0.48)	0.32

Wilcoxon test for paired data. Abbreviations: SD=standard deviation, LV=lesion volume; GD= gadolinium.

At baseline, hippocampal volumes did not differ between HC and CIS patients. Moreover, in CIS patients, global hippocampal volume remained stable during follow-up (**Table 4**).

Both in HC and CIS patients, significant difference between right and left hippocampal volume was found (p=0.04 in HC, p=0.03 in CIS), which is in line with previous studies.

Table 4. Global hippocampal volumes in HC and CIS patients. [1]Mann-Whitney, sex and age-adjusted, [2]Wilcoxon test for paired data. Abbreviations: R=right, L=left.

	HC Baseline	CIS Baseline	p	CIS 3-month follow-up	p
Mean R-Hippocampus (SD) [ml]	3.81 (0.36)	3.78 (0.40)	0.37[1]	3.79 (0.40)	0.14[2]
Mean L-Hippocampus (SD) [ml]	3.71 (0.37)	3.62 (0.42)	0.42[1]	3.64 (0.42)	0.14[2]

[1]Mann-Whitney, sex and age-adjusted, [2]Wilcoxon test for paired data. Abbreviations: R=right, L=left.

Radial mapping analysis

At baseline, CIS patients had a reduced radial distance (RD) (p<0.05) involving the lateral CA1 subfield of both the right and left hippocampal tail and of the right hippocampal head. At month 3, an expansion of the right hippocampus DG subfield was observed (p<0.01), associated with homolateral CA1 and subiculum volume loss in the tail (p<0.01) and a partial volume recovery in the head.

In the left hippocampus, only a CA1 RD increase was found, with no DG expansion (**Figure 2**).

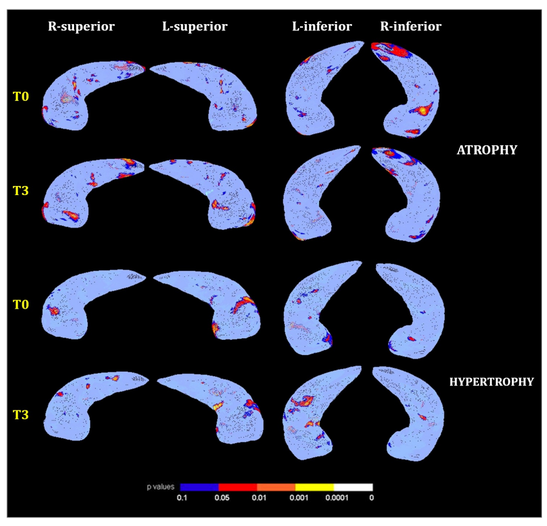
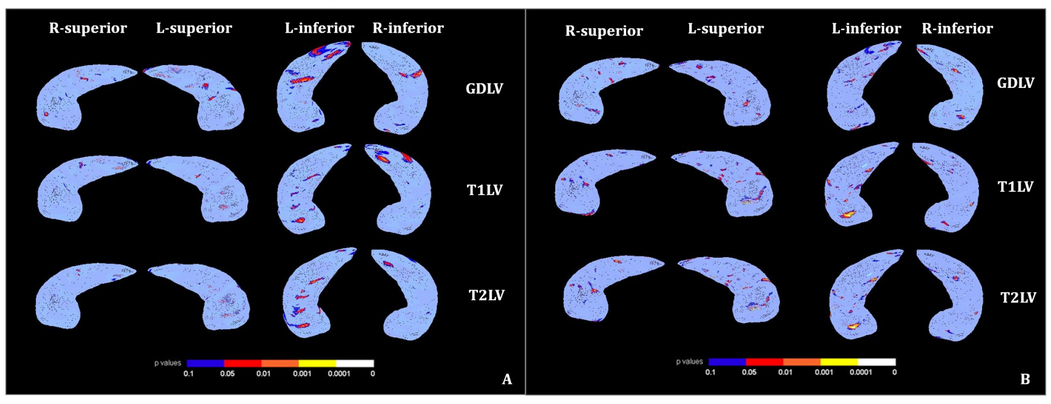


Figure 2.
Surface distribution of regions of significant local atrophy and ipertrophy in CIS *vs* HC at baseline and after 3 months.
p value ranges are shown in the color bar.
Abbreviations: T0=baseline, T3=month 3, R= right, L= left.

In the right hippocampus, RD in CA1 and subiculum subfields was negatively correlated with GD, T1 and T2LV at baseline (p<0.05, R>-0.5) (**Figure 3A**).

DG increase of the right hippocampus during the follow-up correlated positively with baseline GD, T1 and T2LV (p<0.05) (R>0.5). A positive correlation between DG increase and baseline GDLV was observed also in the left hippocampus (**Figure 3B**).

Figure 3. A) Negative correlation between RD at baseline in CIS patients and GDLV, T1LV and T2LV. B) Positive correlation between RD variation during the follow-up in CIS patients and GDLV, T1LV and T2LV.



Abbreviations: LV=lesion volume; GD= gadolinium; R = right; L = left.

CONCLUSIONS

Regional hippocampal volume abnormalities occur in CIS patients, with higher susceptibility to damage of CA1 and subiculum.

After an acute inflammatory event, hippocampal volume abnormalities are dynamic and modulated by the burden of inflammation, as suggested by the correlation between DG expansion and lesional measures.

The lateralization of our data, predominantly in the right side, might be explained by an higher vulnerability to damage of this hemisphere.[9] This is in agreement with previous works reporting a more marked volume loss of the right hippocampus with aging and in MS patients.[10]

REFERENCES

[1]**Calabrese** et al., Neurology 2011; [2] **Kolber** et al., Journal of Neurology 2015; [3]**Rocca** et al., Radiology 2016; [4]**Rocca** et al., Human Brain Mapping 2015; [5]**Chard** et al., Journal of Magnetic Resonance Imaging 2010; [6]**Pruessner** et al., Cerebral Cortex 2000; [7]**Thompson** et al., Neuroimage 2007; [8]**Longoni** et al., Brain Struct Funct 2015; [9]**Dolcos** et al., Neurosci Biobehav Rev 2002; [10] **Roosendaal** et al., Radiology 2010.