Prefrontal Cortical Structural Abnormalities Associated with Hippocampal atrophy following VGKCC-Ab Limbic encephalitis

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Abstract

Limbic encephalitis associated with anti-voltage-gated potassium channel complex antibodies (VGKCC-Ab-LE) is an autoimmune form of encephalitis. VGKCC-Ab-LE patients present well-documented residual episodic memory impairment and atrophy in the hippocampus (HPC). Nevertheless, it remains unclear whether HPC atrophy per se explains this amnestic syndrome. The present study addresses this gap of knowledge and sought to investigate if the structural brain abnormalities in these patients extend beyond the established HPC atrophy and whether these extra-HPC abnormalities are associated with patients pronounced memory impairment. The analysis comprised volume- (VBM) and surface-based morphometry (SBM), the latter being a novel method in the study of this specific disease and a more sensitive one in detecting and localizing abnormalities in the cortex. SBM revealed significant cortical thinning in several areas in the prefrontal cortex (PFC), along with some differences in patients' gyrification index in the bilateral insula. Furthermore, a correlation between the measures in the PFC and the bilateral insula with the memory performance was found. Moreover, VBM revealed bilateral HPC and also thalamic atrophy, along with a positive correlation between this atrophy and the areas presenting cortical thinning. This study demonstrates that VGKCC-Ab-LE not only results in bilateral HPC and thalamic atrophy, but also that this atrophy may result in the disruption of HPC-PFC network or cortico-thalamic-HPC circuits causing significant reductions in the cortical thickness of the PFC. This cortical thinning accompanied by the finding of gyrification reduction in the bilateral insula might be related to the accentuated memory deficits of the patients.

Keywords: Limbic Encephalitis, Cortical thickness, Hippocampus, Prefrontal Cortex, Amnesia

1. Introduction

Limbic encephalitis (LE) is a significant cause of morbidity worldwide characterized by the inflammation of the grey matter (GM) structures of the limbic system [1]. LE associated with anti-voltage-gated potassium channel complex antibodies (VGKCC-Ab-LE) is an autoimmune form of encephalitis in which the antibodies are mainly directed against two surface proteins: leucine-rich glioma inactivated (LGI1) and contactin-associated protein-like (CASPR2) [1]. However patients' neurobehavioral outcome has not been fully characterised, and reveals considerable variability, it is agreed that they are impaired in several reported cognitive domains, with episodic memory (the capacity to recollect past events that occurred at a particular time and place [2]) impairment being the most prominent characteristic [3, 4]. VGKCC-Ab-LE is a potentially reversible disease, thought to be responsive to immunotherapy. Substantial recovery can be made in processing speed and executive function (two impaired domains at the acute phase of the disease) [5, 6], although patients may be left with permanent anterograde amnesia (loss of the ability to create new memories after the onset of a particular event) [3, 5–7]. Although the majority of studies agree with the verbal memory impairment in these patients, the non-verbal memory impairment findings are not consistent and some studies report their impairment [6, 7] while others consider it as a spare domain [3, 5]. The conflicting nature of these reports should be seen in the light of the small number of patients reported in each study, as well as the limited range of neuropsychological tests often used (see Butler et. al [5] for discussion). Furthermore, only a subset of the studies considered have employed correction for multiple comparisons, which may further explain the inconsistencies noticed among the studies. Brain Morphometry, the study of the size and shape of the brain structures as well as their changes [8], employs several fully automated morphometric methods - voxel-based morphometry (VBM), surface-based morphometry (SBM) and tensor-based mor-

phometry (TBM) - which through statistical analysis, can not only identify and characterize structural differences (in GM volume, cortical thickness (CT), gyrification index and sulcus depth) among populations, but also allow correlations between structural properties and measures of interest. These methods complement the traditional brain morphometric approach of manually delineated structures, allowing an identification of more subtle differences, across the whole brain [8]. Furthermore, post-mortem studies demonstrated focal hippocampus (HPC) pathology in autoimmune LE patients [9]. It is a consistent finding the volume deficits presented in the medial temporal lobe (MTL), particularly in the HPC [3, 4, 10], in these patients, whereas their relationship with the memory impairment remains unclear. Although up to date, there are still no studies employing the SBM or TBM in this type of patients, through VBM, the atrophy in the HPC is identified and different correlations between this atrophy and deficits in memory were found: In both Miller et al. [10] and Finke et al.[4] a positive correlation between the CA3 volume and episodic memory is reported. On the other hand, Loane et al. [3] accounts for no correlation between the HPC atrophy and patients' memory performance despite of, a positive correlation between inter-HPC functional connectivity with both memory scores and thalamic volumes. Neuroimaging studies of amnesia, or more specifically episodic memory, have predominantly focused their attention on MTL. There is growing acknowledgment however, that regions outside the MTL can also support episodic memory processes [11–13]. Considering this growing acknowledgment and the existent controversy regarding the HPC atrophy and its relationship with the amnesia syndrome, this study aims to determine whether this atrophy suffices to explain this syndrome in these patients, or if there are other regions, not yet identified, which help to explain it. Furthermore, given the HPC-Thalamic volume relationship in the VGKCC-Ab-LE patients [3], that suggests that the thalamic atrophy may be an indirect result of the HPC atrophy, as well as the existing evidence that degeneration in remote regions of the brain following focal lesions might be an indirect effect of the Wallerian degeneration, it is of interest to explore the relationship between the possible cortical abnormalities and the thalamic atrophy and their respective association with the HPC atrophy. This will be carried out through different morphometry approaches compassing volume and surface based morphometry. It is hypothesized that the HPC atrophy may trigger more subtle structural abnormalities in remote regions that are connected with the HPC, which in turn, may provide a more adequate explanation of the variability of patients performance in tests of episodic memory.

2. Methods

2.1. Participants

The data used were obtained in the scope of the University of Oxfords Memory and Amnesia Project (MAP) and the patients were recruited between 2013 and 2017. The study included 22 VGKCC-Ab-LE patients tested positive for serum VGKC antibodies ((mean±SD: 63.89± 11.82 years; range: 20.71 - 83.1) and 67 healthy controls ((mean±SD): 61.08±13.59 years; range: 22.68 - 86). All patients were recruited in the post-acute phase of the illness (mean = 5.02 years; SD = 3.76 years). For further clinical details, see Loane et al. [3].

2.2. Neuropsychological Evaluation

The Neuropsychological assessment was conducted using a battery of standardized neuropsychological tests for the purpose of the current study. Episodic memory scores for healthy controls and patients were recovered from the MAP database. The raw scores from individual tests for each patient were converted to z-scores and, by averaging these z-scores of each patient's test, it was possible to derive four composite memory scores (visual / verbal x recall / recognition). It is important to note that the controls used for the calculation of the z-scores (Table 1), are not exactly the same controls used for the structural analysis, since some controls were only scanned and did not perform the neuropsychological tests - 41 controls along with 22 patients were assessed with these tests.

Table 1: Summary of neuropsychological assessment of neurotypical controls and VGKCC-Ab-LE patients; D&P: Doors and People Test [14]; RMT: Recognition Memory Test [15]; ROCFT: Rey-Osterrieth Complex Figure Test [16]; WMS-III: Wechsler Memory Scale III [17]; t: = Students t-test; χ^2: Pearson Chi Square; p*: p values are adjusted for Bonferroni correction for multiple comparisons across all composite scores.

		Controls		Patients		Comparison		
Demographics		**Mean**	**SD**	**Mean**	**SD**	***test***	***value***	***p***
Age	**years**	59.44	13.36	63.89	11.82	*t*	-1.312	0.195
Sex	**M:F**	27:14		18:4		χ^2	1.788	0.181
Domain								
Test	**Measure**							***p****
Episodic Memory								
Overall Composite						*W-t*	5.840	<.0001
Recall Composite	Recall Verbal	WMS-III		Logical Memory immediate Logical Memory delayed Word List immediate Word List delayed		*t*	5.840	<.0001
		D&P		People				
	Recall Visual	ROCFT		Immediate Delayed		*W-t*	4.396	.00085
		D&P		Shapes				
Recognition Composite	Recognition Verbal	D&P		Names		*t*	3.732	.0021
		RMT		Words				
	Recognition Visual	RMT		Faces		*W-t*	2.612	.075
				Scenes				
		D&P		Doors				

2.3. MRI acquisition

Structural MRI data were recovered from MAP's database for controls and patients. For the present research, the data was acquired using a Siemens 3T Trio system equipped with a 32-channel head coil located at the University of Oxford Centre for Clinical Magnetic Resonance Research. A 3D T1-weighted magnetization-prepared rapid gradient echo (MPRAGE) sequence was used with the following parameters: Repetition Time (TR) = 2040 ms, Echo time (TE) = 4.7 ms, flip angle = 8°, FOV = 192 mm, voxel size = 1 x 1 x 1 mm;

2.4. Manual Segmentation of the HPC

Through an established and validated protocol (to access the protocol: `https://www.ndcn.ox.ac.uk/files/research/segmentation_protocol_medial_temporal_lobes.pdf`) manual segmentation of the HPC was performed using the manual segmentation tools in ITK-SNAP 3.6 (`http://www.itksnap.org`). Thereafter it was normalized for total intra-cranial volume (TIV) derived from the Computational Anatomy Toolbox (CAT12, `http://dbm.neuro.uni-jena.de/cat/`) an extension toolbox of the Statistical Parametric Mapping software (SPM12, `http://www.fil.ion.ucl.ac.uk/spm/software/spm12`.

2.5. Preprocessing of Neuroimaging Data

The images were first manually examined for scanner artifacts and reoriented to have the same point of origin (anterior commissure) and spatial orientation. The VBM, SBM and TBM analysis were conducted using the CAT12, running in Matlab R2017b. The default settings for each analysis are described in detail in the manual of the CAT12 toolbox (`http://dbm.neuro.uni-jena.de/cat12/CAT12Manual.pdf`). The T1-weighted images were bias-corrected to remove intensity non-uniformities, segmented and registered to a common template in MNI stereotactic space by iteratively registering segmented images via DARTEL (Diffeomorphic Anatomical Registration using Exponentiated Lie algebra) toolbox to CAT12's default template (IXI555_MNI152).

2.5.1 Surface-based Morphometry (SBM)

The estimation of CT and central surface for each hemisphere is based on the Projection-based Thickness(PBT) method and is fully automated [18]. It uses tissue segmentation to estimate the white matter (WM) distance and its local maxima (the CT) is then projected to other GM voxels by using a neighbor relationship described by the WM distance [18]. Moreover, the surface pipeline includes the implementation of topology correction, spherical mapping and spherical registration, by this order, to the surface mesh. The Gyrification Index, extracted from central surface data based on absolute mean curvature, and the Sulcus Depth, based on the Euclidean distance between the central surface and its convex hull, were also extracted as additional surface parameters [19]. The CT and the Gyrification Index/Sulcus Depth images of the left and right hemispheres were smoothed with a 15-mm and 20-mm FWHM Gaussian kernel, respectively. In order to verify the effectiveness of the pre-processing and to avoid error propagation, a quality control of all images was conducted throughout the pipeline through the toolbox's provided ratings.

ROI Extraction: For the three measures under study, the mean values were extracted for 180 ROIs defined by the HCP_MMP1.0 based atlas - in which the anatomical boundaries of each individual region are described by Glasser et al. [20]-, using the standard procedure for ROI extraction provided.

2.5.2 Volume-based Morphometry (VBM)

A VBM analysis was used to allow a voxel-wise study of GM volume abnormalities in the brain. After the segmentation and registration, the GM images are modulated and smoothed (using an isotropic Gaussian kernel of 4mm FWHM). The image data quality was assessed and analysed through the toolbox's provided ratings.

2.5.3 Voxel Asymmetries

In order to infer about the structural asymmetries of the brain between patients and controls, a voxel-wise GM asymmetry analysis was performed according to the fully automated VBM-based approach protocol[21]. The T1-weighted segmented images were flipped at midline. A symmetric DARTEL template from the original and flipped GM and WM segments was subsequently created and both the original and flipped segments were warped to this template and modulated. In order to limit the analysis to the right hemisphere, a binary mask that covers the right hemisphere (using the previous template) was created using MRIcron (`http://people.cas.sc.edu/rorden/mricron/index.html`). The asymmetry index images were finally calculated, using both the warped original and warped flipped images, and smoothed using two different smoothing kernels (8mm and 4mm). The quality of each step across the pipeline was visual analyzed to avoid error propagation.

2.5.4 Tensor-based Morphometry (TBM)

To localize regions of shape differences between groups, a tensor-based analysis approach was used to compare relative volumes of different brain structures. After the segmentation and the registration a Jacobian matrix field was derived from the gradients of the deformation field that aligned an

individual brain to the previous created template. The local Jacobian determinant of the deformation field, to characterize the local volume differences, is then written, for each subject, in normalized space. The Jacobian determinats were smoothed with an isotropic Gaussian kernel with FWHM of 4mm.

2.6. Statistical Analysis

For all the analyses in this study, normal distribution of the data was checked by the ShapiroWilk test. Data with normal distribution was analyzed by the Student's t-test, when the assumption of homogeneity of variance (assessed using the Levene's test) was not violated, otherwise, the Welch t-test would be used. On the other hand, not normally distributed data were subjected to the MannWhitney U test. To study the relationship between the composite scores and the reported areas in which significant differences were found, a bivariate correlation was performed, using SPSS (version 25.0, SPSS Inc) for Macintosh, and the Pearson correlation coefficient (r) and the Spearman correlation coefficient (rho) were used as well as the p values corrected for multiple comparisons using the Bonferroni correction method. To address multiple comparisons, all statistical maps were assigned thresholds at voxel/vertex-level uncorrected and voxel and cluster-level FWE corrected $p < 0.05$. The surviving clusters were reported.

2.6.1 Surface-based Morphometry (SBM)

A two-sample t-test (contrast: controls>patients) was conducted to detect which brain regions showed abnormalities in CT, gyrification index and sulcus depth (maps of the left and right hemispheres were independently analyzed), with age and sex as nuisance covariates. With the results from the previous analysis, a bivariate correlation was performed to study the association between the reduction in the previous measures and aspects of episodic memory impairment. Through the ROI extraction, the estimated mean values, for each measure, for each ROI, in the previous created SPM design were then transferred and analyzed in SPSS Software (version 25.0, SPSS Inc).

2.6.2 Volume-based Morphometry (VBM)

The GM volume was assessed by voxel-wise two-sample t-tests (contrast: controls>patients) using age, sex, and TIV as nuisance covariates. The resultant clusters were reported and extracted as a binary mask. A multiple regression model between GM volume images (with the explicit mask from the previous step) and the average CT per ROI of the areas that showed reduction in CT in patients was applied (same nuisance covariates). Two separate bivariate correlations between the composite scores and the reported clusters and between the composite scores and the right and left manual HPC volume were also performed.

2.6.3 Voxel Asymmetries

A two-sample t-test (applying the created right hemisphere mask as an explicit mask) was conducted, with age, sex, and TIV as nuisance covariates. A multiple regression model was applied in order to investigate associations between asymmetry index in patients (explicit masking for right hemisphere) and HPC Volume as a regressor of interest in a GLM.

2.6.4 Mediation Analysis

An indirect mediation analysis (Figure 1) was performed hypothesizing that the HPC volume would indirectly influence the CT in predetermined areas through causally linked the thalamic volume as a mediator.

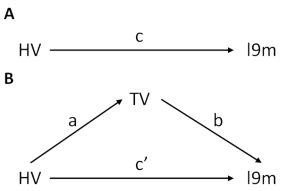

Figure 1: Path diagram illustrating (A) the direct effects and (B) causal paths linking Hippocamapal volume (HV) → Thalamic volume (TV) → CT in area l9m.

2.6.5 Tensor-based Morphometry (TBM)

Replication of the tests used in the VBM analysis.

3. Results

3.1. Surface-based Morphometry (SBM)

Cortical thickness: Compared with controls, the VGKCC-Ab-LE exhibited significantly reduced CT in 15 different areas (HPC-MMP1.0 based atlas), mainly compassing the frontopolar areas (Figure 2). A stepwise discriminant function analysis was applied and the best predictor (that best discriminate between the groups) areas among the 15 were: l9a, l9m, la9-46v, la47r, rp10p and r8BL. These areas correspond to the bilateral Anterior Cingulate and Medial Prefrontal Cortex (mPFC), Left inferior frontal cortex and Orbital and polar frontal cortex [20](Figure 2). When correlating the CT in these areas with the patients' composite memory scores a positive correlation between the area l9a (Left Anterior Cingulate and mPFC) and the recognition memory composite score was found (rho=0.649, p=0.005) after correction for multiple comparisons.
Gyrification Index: Compared with controls, the VGKCC-Ab-LE exhibited significantly reduced gyrification index in bilateral insula. When correlating these areas with the patients' composite memory scores the left insula correlated with

visual recognition (r=0.539, p=0.024) and recall (r=0.552,p=0.018) composite scores while the right correlated with the verbal recognition (r=0.485, p=0.044). ***Sulcus depth:*** No differences found when correcting for multiple comparisons.

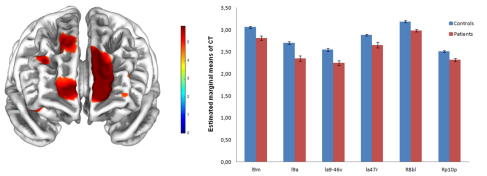

Figure 2: Structural differences in CT. Controls >VGKCC-Ab-LE patients; nuisance covariates: age, sex. Color bar: t-values with the cortical surface map IXI555_MNI152 (from CAT12) as an underlay. FWE-correction (p≤0.05). (B) Estimated marginal means of CT in each areas between groups(±1 SEM). Patients showed reduced CT across all regions relative to controls (l9m:p<.0001; l9a:p<.0001; la9-46v:p<.0001; la47r:p=.003; r8bl:p<.0001; rp10p:p<.0001).

3.2. Volume-based Morphometry (VBM)

A decrease in GM volume in patients was found in bilateral hippocampi and thalami (Fig. 3-Top). Furthermore, a positive correlation between these two bilateral areas and the areas that previously showed a reduction in CT is established (Fig. 3-Bottom) in the left HPC and right thalamus. No significant correlations between the clusters found in the left HPC and right thalamus and the memory composite scored were found neither between the left and right manual HPC volumes and the same composite scores. ***Mediation Analysis:*** Results indicated that HPC volume was a significant predictor of the thalamic volume, p = .0013, and that, the thalamic volume was a significant predictor of the CT in area l9m, p = .0008, but not in area r8BL, p= .0992. A significant overall indirect effect (mediated by the Thalamic volume) was observed for the l9m (b= 0.1304; BCa bootstrapped CI [0.0431, 0.2734]) with an indirect effect ratio (what % of total effect is indirect effect) of 98% but not in area r8BL (b=.0334; BCa bootstrapped CI [-.0025, .0853]). ***Hemispheric asymmetry:*** No significant differences between hemispheric asymmetries were identified in patients when compared to controls but a negative correlation between the patients' asymmetry index and HPC volume demonstrated that asymmetry in the mPFC was associated with the extent of HPC atrophy in patients.

3.3. Tensor-based Morphometry (TBM)

As in VBM, this analysis revealed bilateral reduction in the relative volume of the bilateral HPC and thalamus in patients (Fig. 4). It exposed a significant positive correlation between the CT areas and the volume in bilateral HPC and left thalamus.

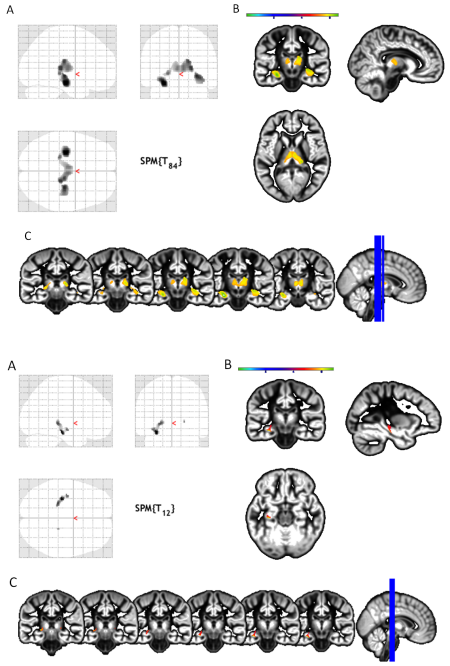

Figure 3: Top: Controls >Patients;Bottom: Clusters of significant positive correlation of GM volume with CT areas; nuisance covariates: age, sex and TIV (p<.05, FWE-corrected at peak-level). (A) maximum intensity projection, (B) slice /(C) multislice overlay on the Dartel template in MRIcron.

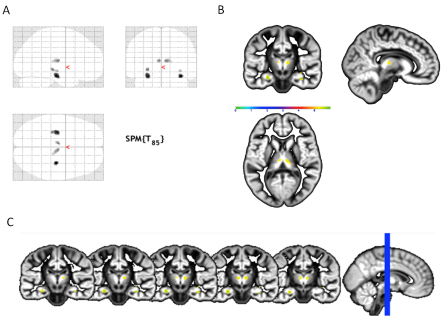

Figure 4: Contrast: Controls >Patients; nuisance covariates: age and sex (p<.05, FWE-corrected at peak-level). (A) maximum intensity projection, (B) slice and (C) multislice overlay on the Dartel template in MRIcron.

4. Discussion

To the best of the author's knowledge, this is the first study to use SBM analysis and combine it with VBM analysis for evaluating structural alterations of the cortical mantle and the respective GM in VGKCC-Ab-LE patients. This study sought to assess if these structural alterations go beyond the established HPC atrophy and if other regions outside the MTL can help to support episodic memory

processes.

4.1. SBM Analysis in VGKCC-Ab-LE patients and neuropsychological assessment

Through the SBM approach three complementary indicators of brain structure (CT, gyrification and sulcus depth) were used to map differential effects. The present data demonstrate CT reduction, in VGKCC-Ab-LE patients when compared to healthy controls, in different areas of the PFC. These areas were also shown to be good predictors of group membership, suggesting that they are able to correctly classify the VGKCC-Ab-LE patients. Although some isolated case studies of single patients with LE mentioned some abnormalities in the intensity or metabolism in prefrontal regions [22, 23] this has not being further studied. Up to date, there have been no reports of structural alterations in these areas, neither in this specific group of patients nor in the broader spectrum of autoimmune LE, which can be explained by the current approaches used in order to perform this analysis. VBM is the approach that mainly has been employed. It allows to infer regional GM loss and, although it is a reliable technique, it lodges some limitations regarding the identification of more subtle changes in the cortical mantle since it does not account for the cortical folding [24]. On the other hand, SBM can be a higher sensitivity measure since it not only improves the accuracy of cortical registration but also allows other type of analyses concerning the cortical complexity [25]. Thus, the SBM results suggest that other areas outside of the MTL, particularly in the PFC, may also be compromised since a reduction in CT is present. Moreover, differences in gyrification were identified in bilateral insula in patients when compared to healthy controls. Although the exact functional implications of the cortical gyrification remain to be established, the insular atrophy has been implicated in several diseases [26, 27] and its activity thought to be related with behavioral and emotional states [28]. Reductions in insular volume were found in Alzheimer's disease [27] and schizophrenia [29] and this atrophy related to the cognitive deficits shown. A few studies had report not only that autoimmune encephalitis affects symmetrical regions in the brain [23] but also the involvement of the insular cortex in this disease including bi-insular cortical abnormalities on MRI and F18-FDG-PET [23, 28, 30, 31]. However, the influence that this area has in the VGKCC-Ab-LE is unclear and in order to ascertain its role, further research with a higher magnetic field strength and larger patients' sample is needed. No abnormalities were seen in terms of sulcus depth. Summarily, the structural analyses in both groups revealed possible differences in patients in the CT as well as in the gyrification index in the PFC and bilateral insula, respectively. These findings are novel and suggest that other areas outside the MTL are also possible to be affected by the disease.

4.2. Structural atrophy related with memory impairments

The patient group under study demonstrated salient episodic memory deficit with a significant impairment of verbal/non-verbal recall memory and verbal recognition memory relative to controls and also a marginal impairment in the recognition memory for visual material. Although there is a high variability in neuropsychological findings these results seems to be in line with earlier reports [5–7, 32]. Regions outside the MTL can also support episodic memory processes and the specific involvement of the left and right prefrontal cortices have been emphasized [12, 33]. Furthermore, the areas in the PFC that showed a reduction in CT were tested for correlations in these domains. Consistent with the previous hypothesis, a significant positive correlation between CT in area l9a (Left Anterior Cingulate and mPFC) and verbal recognition memory was found. Left lateralization of the region in which CT was associated with verbal recognition memory scores is consistent with the evidence for hemispheric specialization in processing stimuli of different modalities, such that the left hemisphere plays a greater role in processing verbal material [34]. Moreover, Warrington et al. [15] reported that patients with left frontal lesions obtained poor scores in recognition memory tests for words. The lack of other correlations can be explained by the strict corrections for multiple comparisons approach used as well as the size of the sample. Such evidence, seen in the light of the present findings, suggests that cortical thinning in the mPFC may help, at some degree, to explain some aspects of the memory impairment. The left insula correlated mainly with the visual recall and recognition scores while the right side of this structure correlated with the verbal recognition scores. These correlations have also been reported in a few different studies that associate the insula with declarative memory, in particular with the recognition [35, 36], encoding and recall [37, 38] of episodic memories. Bermudez-Rattoni [35] proposed the contribution of this structure for visual recognition memory while Tsukiura et al. [38] suggested that the insula plays a role in the processing of visual impressions (i.e attractiveness, trustworthiness) and through the insular-HPC interaction this processing contributes to the recall and recognition of this visual stimuli. These studies are in accordance with the obtained recall and recognition visual correlations. However, a correlation with the verbal recognition memory score was found, the insula is not traditionally associated with verbal recognition memory, and there is cur-

rently little evidence to support this finding. Only Buckner et al.[36] report its possible role in verbal recall being hard to infer on the verbal recognition correlation since there is no literature supporting it. Nevertheless is important to further assess if the proven influence of the insula in the recognition process is also related to the verbal component. These findings mainly suggest that the visual processing of stimuli in insula could be affected by this areas reduced gyrification which may be related with the impairment shown by patients. This can help to explain the lack of correlations between the HPC volume and the memory scores for visual stimuli. One possible interpretation of this result is that might be the insula atrophy and posterior disruption of the insular-HPC interaction instead of the HPC atrophy that predicts the level of this impairment. However, further work, related with the insula structural morphometry and its influence in specific aspects of episodic memory, is necessary to explore these findings. Moreover, no significant correlations were found between sulcus depth in VGKCC-Ab-LE patients and composite memory scores. Summarily, the correlations between the areas with reduction in CT and gyrification index with the memory composite scores, might help to explain these impairments and give some insights in the lack of correlations between the HPC volume and the memory scores.

4.3. Connection between the VBM and SBM analyses in VGKCC-Ab-LE patients

Through the VBM analysis the same results reported in Loane et al. [3] were found, as expected: a GM volume reduction in bilateral HPC and thalami when comparing the two groups. Thalamic atrophy was interpreted by Loane et al. [3] as result of the Wallerian degeneration following HPC atrophy, given the correlation of HPC and thalamic volumes in patients. Along the same line of thought, the results of the GM volume reduction in VBM and the CT reduction in SBM were combined and a significant correlation was found. The GM volume reduction in left HPC and bilateral thalami positively correlated with the CT reduction in specific areas of the PFC. These findings are in accordance with the previous hypothesis that HPC atrophy may compromise the structural integrity of regions beyond the MTL that communicate with the HPC - here, through the disruption of the HPC-PFC pathway. Although the mechanisms underlying the interactions between these areas are not fully understood, there are several studies that could facilitate the interpretation of this correlation since they support the idea that the neuronal projections from the HPC, either directly or indirectly, to the PFC (HPC-PFC circuit) play a critical role in cognitive and emotional regulation [39–41]. This circuit holds a fundamental role in memory, as demonstrated by hemodynamic changes in task-based fMRI studies in neurotypical adults [30, 42] as well as in structural and functional connectivity in neurodegenerative diseases, e.g. Alzheimers disease [40]. The involvement of this network in encoding and retrieval of episodic-like memories [39] as well as in recognition memory processes [41]- previously confirmed to be severely impaired in VGKCC-Ab-LE patients - have been reported. In this study, patients memory scores were neither associated with their manual HPC volume, nor with the volume of the HPC clusters that correlated with the CT areas. These results were expected and they are in accordance with Loane et al. [3] which also accounts for no correlation between the HPC atrophy and patients' memory performance. A positive correlation between the CA3 volume and episodic memory and CA2/3 volume with verbal memory, is respectively reported [4, 10] however, in the current study, due to the limitations of the resolution of the acquired data, subfield-specific relationships were not possible to explore. Moreover, no GM asymmetries were found between VGKCC-Ab-LE patients and controls. This lack of asymmetry may be explained by the size of the sample of patients and it is predicted that with a broader and larger sample some GM asymmetries may be found. However, the fact that HPC atrophy in this specific group of patients was bilateral is also important to consider. This might perhaps suggest that groups with strong lateralization of HPC atrophy may have corresponding volumetric asymmetries in extra-MTL regions. Furthermore, in a whole-brain voxel-based regression, a negative correlation between the volumes of the manually delineated HPC with patients asymmetry index was found in the mPFC. This finding suggests that when HPC volume decreases, the GM asymmetry between hemispheres in patients in the PFC increases. This also supports the idea of an important relationship between the HPC and the PFC in this specific group of patients. Although the latest results are considered consistent with the literature, a TBM analysis was also conducted, in order to help to cross-validate them. As expected, the results of a bilateral volume reduction in HPC and thalamus previously found in VBM when comparing the two groups were also found. Moreover, the areas that presented a reduction in CT were correlated with GM volume of the previous clusters and a positive correlation with the GM volume in the bilateral HPC and left thalamus was found. In the VBM analysis, the average CT of these prefrontal areas correlated with GM volume in the left HPC and bilateral thalamus and it is consistent with the above. The clusters disclosed by the two analyses spanned across identical regions and the previous

differences are due to the fact that SPM only shows the maximum peak voxels coordinates of each cluster which does not mean that the clusters do not extend to adjacent areas. This may suggest that both bilateral HPC and thalamus are positively correlated with the areas that show a CT reduction. Overall, the results obtained suggest that the structural abnormalities found in the PFC, more specifically in the Anterior Cingulate and mPFC, may be driven by the HPC atrophy present in the patients in this study. Damage in the broader PFC-HPC may exacerbate amnesia.

4.4. Mediation Analysis

The mediation analysis performed suggests that a significant overall indirect effect (mediated by the Thalamic volume) was observed for area l9m (Left anterior Cingulate and Medial Prefrontal Cortex). This suggests that a considerable proportion of variance in cortical thinning in this area is explained by the variance in the HPC atrophy through its correlative thalamic atrophy. This is consistent with earlier findings of thalamic atrophy in VGKCC-Ab-LE patients that was correlative to HPC atrophy [3]. It is also in line with evidence for an indirect pathway from the HPC to the mPFC via thalamus [43, 44]. This result then implies that HPC atrophy may trigger thalamic atrophy, which may in turn compromise CT in some PFC areas. Although, some investigation is required to determine the exact areas that are affected by this causal effect since, apparently, some are only affected by the HPC atrophy such as the area r8BL (Right anterior Cingulate and mPFC) in which no mediation occurred but a strong correlation with the HPC atrophy was found.

5. Conclusions

This study has demonstrated that VGKCC-Ab-LE not only results in bilateral HPC and thalamic atrophy, as already reported in literature, but also that this atrophy may result in the HPC-PFC network disruption and trigger significant cortical thinning in the mPFC. Such CT reduction, accompanied by the finding of gyrification reduction in the bilateral insula might be related to the accentuated memory deficits of the patients. These findings are of significance in three important aspects. Firstly they reveal structural variation in the VGKCC-Ab-LE patients in different structures, namely the PFC and the insula, and due to this, it is hypothesized that there are other areas beyond the MTL that are also possible to be affected by this disease. Secondly, the structure-behaviour correlations provide shed light on the contributions of these structures in the different memory processes captured by the different memory tests here. Thirdly, consistent with previous studies that report an important pathway between the HPC-PFC, structural covariance between these two structures in patients was demonstrated, which suggests that cortical thinning in the PFC is associated with HPC atrophy. Moreover, a mediation analysis implied that the atrophy in the HPC is directly related with the atrophy in thalamus which will then affect CT in some PFC areas. It is then hypothesized that the structural abnormalities found in the PFC may be driven by HPC atrophy directly, or indirectly via its effect on the thalamus. These abnormalities may exacerbate amnesia and explain some of the patients deficits, which do not correlate with HPC volume. Furthermore, the relationship between different morphometric measures across brain populations has not received much attention so far, and this research can also demonstrate the importance of the use of diverse structural approaches in order to accurately study the cortical differences and their association with the diseases.

5.1. Limitations and Future Work

There are several shortcomings of this study which must be considered. First, although it is hard to find this specific type of patients, a larger sample is needed in order to increase the statistical power. Secondly, it is important to take into account the resolution of the data acquired at a 3-Tesla and its limitations in the accuracy of a structural analysis and in the study of subfields that can be related with some findings and help to predict others. Moreover, this study followed an exploratory approach, considering many factors, and should be pursued by a more confirmatory approach in order to validate and further study all of the obtained results and confirm that no false positives were presented. It is also important to address the lack of a function in CAT12 which allows the direct extraction of the cortical measures in the significant clusters upon a certain contrast. This, although the data was carefully examined and extracted in order to correctly correspond to the significant clusters, may lead to small inaccuracies. Finally, it is worth acknowledging the possibility that the observed memory impairment and the structural abnormalities found occur as products of the interaction of HPC atrophy with other factors (e.g. structural and functional dysconnectivity) that have yet to be identified. It is also important to resume some possibilities that should be addressed by future work:

1. A 7-Tesla study should be carried out in order to assess if more regions show reduction in cortical thickness and gyrification and if any difference in the sulcus depth is noticeable. In conjunction would also be very interesting if the size of the sample increases.

2. A deeper study relating the bilateral insula

with the VGKCC-Ab-LE patients and the influence of this structure in the correspondingly impaired composite scores, more specifically with the recognition verbal scores since they are the least discussed in the literature.

3. Investigate further the relationship between the HPC-PFC pathway and the neuropsychological scores and also the exact areas affected by the disruption not only of this network but also of the HPC-thalamus-PFC one.

4. Study the integrity of the structural connectivity of the HPC with the thalamus and the prefrontal cortex in patients, by way of tract-based spatial statistics on DTI data.

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References

[1] Alessandra Dodich et al. Neuropsychological and FDG-PET profiles in VGKC autoimmune limbic encephalitis. *Brain and Cognition*, 2016.

[2] Adam J. O. Dede et al. Autobiographical memory, future imagining, and the medial temporal lobe. *Proceedings of the National Academy of Sciences*, 2016.

[3] C. Loane et al.(under review). Structural and functional brain imaging following autoimmune limbic encephalitis. 2018.

[4] Carsten Finke et al. Evaluation of cognitive deficits and structural hippocampal damage in encephalitis with leucine-rich, glioma-inactivated 1 antibodies. *JAMA Neurology*, 2017.

[5] Christopher R. Butler et al. Persistent anterograde amnesia following limbic encephalitis associated with antibodies to the voltage-gated potassium channel complex. *Journal of Neurology, Neurosurgery and Psychiatry*, 2014.

[6] C. Frisch et al. Neuropsychological course of voltage-gated potassium channel and glutamic acid decarboxylase antibody related limbic encephalitis. *European Journal of Neurology*, 2013.

[7] Angela Vincent et al. Potassium channel antibody-associated encephalopathy: A potentially immunotherapy-responsive form of limbic encephalitis. *Brain*, 2004.

[8] Douglas N Greve. An absolute beginner's guide to surface-and voxel-based morphometric analysis. In *ISMRM 19th Annual Meeting & Exhibition, Montréal, Québec, Canada*, 2011.

[9] N. L. Khan et al. Histopathology of VGKC antibody-associated limbic encephalitis. *Neurology*, 72(19):1703–5, 2009.

[10] Thomas D. Miller et al. Focal CA3 hippocampal subfield atrophy following LGI1 VGKC-complex antibody limbic encephalitis. *Brain*, 2017.

[11] Marco Sandrini et al. The role of prefrontal cortex in verbal episodic memory: rTMS evidence. *Journal of Cognitive Neuroscience*, 2003.

[12] S. M. Daselaar, D. J. Veltman, S. A. R. B. Rombouts, J. G. W. Raaijmakers, and C. Jonker. Neuroanatomical correlates of episodic encoding and retrieval in young and elderly subjects. *Brain*, 2003.

[13] Vijeth Iyengar. *Contributions Of the Human Medial Prefrontal Cortex To Associative Recognition Memory: Evidence From Functional Neuroimaging.* PhD thesis, Duke University, 2016.

[14] I. Baddeley, A. D., Emslie, H., & Nimmo-Smith. The Doors and People Test: A test of visual and verbal recall and recognition. *BurySt-Edmunds, UK: Thames Valley Test Company*, 1994.

[15] E.K. Warrington. The Recognition Memory Test. *Windsor, UK: NFER-Nelson*, 1984.

[16] A Rey. Manuel du test de copie d'une figure complexe de A. Rey. *Paris: Les Editions du Centre de Psychologie Appliquée*, 1959.

[17] D. Wechsler. Wechsler Memory Scale Third edition administration and scoring manual. *San Antonio, TX: Pearson Assessments*, 1997.

[18] Robert Dahnke et al. Cortical thickness and central surface estimation. *NeuroImage*, 2013.

[19] Christian Gaser and Florian Kurth. CAT12 Manual, 2018.

[20] Matthew F. Glasser et al. A multi-modal parcellation of human cerebral cortex. *Nature*, 2016.

[21] Florian Kurth et al. A 12-step user guide for analyzing voxel-wise gray matter asymmetries in statistical parametric mapping (SPM). *Nature Protocols*, 2015.

[22] Vincent Navarro et al. Motor cortex and hippocampus are the two main cortical targets in LGI1-antibody encephalitis. *Brain*, 2016.

[23] SadanandavalliRetnaswami Chandra et al. Progressive limbic encephalopathy: Problems and prospects. *Annals of Indian Academy of Neurology*, 2014.

[24] Alan Tucholka et al. Surface-based versus volume-based fMRI group analysis : a case study. *MICCAI 2009 Functional NeuroImaging Workshop*, 2013.

[25] Nikolaas N. Oosterhof et al. A comparison of volume-based and surface-based multi-voxel pattern analysis. *NeuroImage*, 2011.

[26] Jeremy D. Cohen et al. Morphometry of human insular cortex and insular volume reduction in Williams syndrome. *Journal of Psychiatric Research*, 2010.

[27] A. L. Foundas et al. Atrophy of the hippocampus, parietal cortex, and insula in Alzheimer's disease: a volumetric magnetic resonance imaging study, 1997.

[28] Philip A. Kelly et al. Cortical thickness, surface area, and gyrification abnormalities in children exposed to maltreatment: Neural markers of vulnerability? *Biological Psychiatry*, 2013.

[29] Benedicto Crespo-Facorro et al. Insular cortex abnormalities in schizophrenia: a structural magnetic resonance imaging study of first-episode patients. *Schizophrenia Research*, 2000.

[30] R L Buckner, W M Kelley, and S E Petersen. Frontal cortex contributes to human memory formation. *Nature Neuroscience*, 1999.

[31] Harald Hegen et al. Bi-insular cortical involvement in anti-NMDA-receptor encephalitis - a case report. *BMC Neurology*, 2016.

[32] M Lad, S Mullally, and TD Griffiths. 18Recall deficits with preserved recognition memory in limbic encephalitis. *Journal of Neurology, Neurosurgery & Psychiatry*, 2017.

[33] Patrizia Turriziani et al. The role of the prefrontal cortex in familiarity and recollection processes during verbal and non-verbal recognition memory: An rTMS study. *NeuroImage*, 2010.

[34] James B. Brewer et al. Making memories: Brain activity that predicts how well visual experience will be remembered. *Science*, 1998.

[35] Federico Bermudez-Rattoni. The forgotten insular cortex: Its role on recognition memory formation, 2014.

[36] R L Buckner et al. Functional anatomic studies of memory retrieval for auditory words and visual pictures. *The Journal of Neuroscience*, 1996.

[37] S. M. Daselaar et al. When less means more: Deactivations during encoding that predict subsequent memory. *NeuroImage*, 2004.

[38] Takashi Tsukiura et al. Insular and hippocampal contributions to remembering people with an impression of bad personality. *Social Cognitive and Affective Neuroscience*, 2013.

[39] Jingji Jin and Stephen Maren. Prefrontal-Hippocampal Interactions in Memory and Emotion. *Frontiers in Systems Neuroscience*, 2015.

[40] Ming Li, Cheng Long, and Li Yang. Hippocampal-prefrontal circuit and disrupted functional connectivity in psychiatric and neurodegenerative disorders, 2015.

[41] Bill P. Godsil et al. The hippocampal-prefrontal pathway: The weak link in psychiatric disorders?, 2013.

[42] Alison R. Preston and Howard Eichenbaum. Interplay of hippocampus and prefrontal cortex in memory. *Current Biology*, 23(17):1–21, 2013.

[43] Lezio S. Bueno-Junior et al. Interaction between hippocampal-prefrontal plasticity and thalamic-prefrontal activity. *Scientific Reports*, 2018.

[44] Lezio S. Bueno-Junior and Joao P. Leite. Input Convergence, Synaptic Plasticity and Functional Coupling Across Hippocampal-Prefrontal-Thalamic Circuits. *Frontiers in Neural Circuits*, 2018.