STRUCTURAL AND FUNCTIONAL PAPEZ CIRCUIT INTEGRITY IN AMYOTROPHIC LATERAL SCLEROSIS

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Running title: Memory circuit in amyotrophic lateral sclerosis

Word count abstract: 240

Tables: 2

Figures: 1

Supplementary Material Tables:

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Abstract

Cognitive impairment in amyotrophic lateral sclerosis (ALS) is heterogeneous but now recognized as a feature in non-demented patients and no longer exclusively attributed to executive dysfunction. However, despite common reports of temporal lobe changes and memory deficits in ALS, episodic memory has been less explored. In the current study, we examined how the Papez circuit – a circuit known to participate in memory processes – is structurally and functionally affected in ALS patients (n=20) compared with healthy controls (n=15), and whether these changes correlated with a commonly used clinical measure of episodic memory. Our multimodal MRI approach (cortical volume, voxel-based morphometry, diffusion tensor imaging and resting state functional magnetic resonance) showed reduced gray matter in left hippocampus, left entorhinal cortex and right posterior cingulate as well as decreased white matter fractional anisotropy and increased mean diffusivity in the left cingulum bundle (hippocampal part) of ALS patients compared with controls. Interestingly, thalamus, mammillary bodies and fornix were preserved. Finally, we report a decreased functional connectivity in ALS patients in bilateral hippocampus, bilateral anterior and posterior parahippocampal gyrus and posterior cingulate. The results revealed that ALS patients showed statistically significant structural changes, but more important, widespread prominent functional connectivity abnormalities across the regions comprising the Papez circuit. The decreased functional connectivity found in the Papez network may suggest these changes could be used to assess risk or assist early detection or development of memory symptoms in ALS patients even before structural changes are established.

Keywords: Multimodal MRI, Papez circuit, episodic memory, cognitive deficits, amyotrophic lateral sclerosis.

Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease sharing clinical, pathological and genetic features with frontotemporal dementia (FTD), specifically with its behavioural variant presentation (bvFTD). This overlap between both diseases is now recognized to form a pathophysiological spectrum (Lillo & Hodges, 2009). In addition to motor symptoms, some ALS patients can present with full-blown bvFTD, while others can display some cognitive and behavioural deficits without meeting criteria for dementia (Raaphorst et al., 2015; van der Hulst et al., 2015; Hervieu-Begue et al., 2016; Mioshi et al., 2014).

Cognitive deficits in ALS occur in up to 30% of patients and are usually associated with shorter survival (Woolley & Strong, 2015; Beeldman et al., 2015; Abrahams et al., 2000). The deficits are commonly characterized by executive dysfunction in the form of verbal fluency deficits and as impairments of intrinsic response generation (Goldstein & Abrahams, 2013). However, cognitive dysfunction in ALS is heterogeneous, with the presence of social cognition and emotion processing deficits among others (Abrahams et al., 2000; Volpato et al., 2010).

Most ALS studies report working memory impairments (Hammer et al., 2011; Libon et al., 2012), but an increasing number of recent studies show semantic and episodic memory deficits (Hervieu-Begue et al., 2016; Sarro et al., 2011; Mantovan et al., 2003; Machts et al., 2014), while imaging studies report ALS patients can present temporal gray matter (GM) and white matter (WM) changes, with marked hippocampal atrophy correlating with memory performance (Raaphorst et al., 2015; Christidi et al., 2017; Kasper et al., 2015). However, most impairments are attributed to executive dysfunction (Consonni et al., 2015; Matuszewski et al., 2006). Interestingly, this mirrors interpretation of memory deficits in bvFTD (Hornberger et al., 2012), although there is evidence that a subgroup of bvFTD patients shows memory deficits due to Papez circuit pathology and

hippocampal atrophy (Bertoux et al., 2014; Flanagan et al., 2016; de Souza et al., 2013; Brooks et al., 2000). Nonetheless, to our best knowledge, the complete Papez circuit – the well-known circuit for episodic memory processing – and its contribution to episodic memory deficits in ALS have not yet been investigated.

In this study, we investigated the integrity of GM, WM and functional connectivity of the Papez circuit in non-demented ALS patients and healthy controls (HC). We conducted voxel-based morphometry (VBM), GM volumetric analysis, diffusion tensor imaging (DTI) and resting state functional MRI (rs-fMRI) analyses. Based on previous studies, and considering the link between ALS and bvFTD, we hypothesized that changes in GM, WM and functional connectivity would be present in ALS patients and correlated with a commonly used clinical measure of episodic memory.

Methods

Participants

ALS patients were recruited from the Forefront multidisciplinary ALS clinic in Sydney, Australia. Patients with ALS were evaluated by an experienced neurologist (MK) and classified according to the El Escorial (Brooks et al., 2000) and Awaji (de Carvalho et al., 2008) diagnostic criteria, as definite or probable ALS. Patients were an admixture of bulbar and limb onset. Respiratory function measured by forced vital capacity (FVC) was above 70% and there was no evidence of nocturnal hypoventilation for any patient. None of the patients reported depressive symptoms or had a diagnosis of clinical depression. Patients with a diagnostic of FTD were not included in the study. Patients were recruited consecutively and were not selected based on memory performance. Some of the patients were included in previous reports. Estimated disease duration was obtained from the date of reported symptoms onset to the date of MRI acquisition. Controls were

recruited from the community. Ethics approval was obtained from the Human Research Ethics Committee of South Eastern Sydney/Illawarra Area Health Service. Written consent was obtained from each participant or close relative. Table 1 summarizes demographic and neuropsychological data.

[Table 1 here]

Brief memory assessment: ACE-R

Patients underwent the Addenbrooke's Cognitive Examination-Revised (ACE-R), a battery of general cognitive tests (Mioshi et al., 2006), including a multidimensional assessment of episodic memory with five scores: immediate recall (measuring the ability to recall three previously learned words); anterograde memory (measuring the ability to learn and recall a postal address - delayed recall score); retrograde memory (measuring the recall of common knowledge acquired months/years earlier); and recognition (evaluating recognition abilities of the address previously learned, if delayed recall fail). We subdivided the ALS patients according to their ages, considering the cut offs proposed by Mioshi and colleagues (2006) to evaluate their performance on the memory tests and used Mann-Whitney test to compare memory performance between groups. Spearman correlation was performed in SPSS to correlate memory performance with every structure presenting changes in structural and diffusion MRI and disease duration, with Bonferroni correction for multiple comparisons.

MRI acquisition

Participants underwent whole-brain MRI on a 3T Philips. ALS patients (n=20) underwent structural, diffusion and rs-fMRI. Healthy controls underwent structural,

diffusion MRI (n=15) and rs-fMRI (n=11). T1-weighted images were acquired as follows: multi shot 256 TFE factor (TR/TE 5.4/2.4ms, 256x256 matrix, FOV 256x256 x180, flip angle 8º), slice thickness 1mm, coronal orientation, voxel size 1x1x1mm^3. DTI-weighted images were acquired using a single shot echo-planar imaging (EPI) sequence, (TR/TE 11595/78ms, 96x96 matrix size, FOV 240x240x137, flip angle 90º), 2.5mm transverse slices with no gaps, 61 gradient directions, b-value 0 and 2000s/mm^2, voxel size 2.5x2.5x2.5mm^3. The following protocol was used for resting-state fMRI acquisition: T2*-weighted images using single shot EPI (TR/TE 3000/30ms, 120x120 matrix, FOV 240x240x140, flip angle 80º), 127 scans, 40 transverse slices with thickness 3.5mm and no gap, voxel size 2x2x3.5mm^3.

MRI processing

Cortical volumetric analysis and VBM

Cortical and subcortical volumetric measures were obtained with Freesurfer software version 5.3.0 (http://surfer.nmr.mgh.harvard.edu). The preprocessing pipeline was performed using the fully-automated directive – the "recon-all" command. Briefly, the preprocessing included: intensity normalization, removal of non-brain tissues, Talairach transforms, segmentation of the GM and WM, and tessellation of the GM/WM boundary (technical details in Fischl et al., 2004). Once cortical models were complete, the cortical surface of each hemisphere was parcellated according to the atlas proposed by Desikan and colleagues (2006; with 34 cortical regions per hemisphere; "aparc" segmentation). Cortical volume was estimated multiplying cortical thickness (average shortest distance between the WM boundary and the pial surface) by area (Dale et al., 1999a; Dale et al., 1999b) . The subcortical volume measures were obtained via a whole brain segmentation procedure, using "aseg" segmentation (Fischl et al., 2004). A general

linear model (GLM) was performed in SPSS using regions of interest (ROIs) measures as dependent variables, age and gender as covariates, considering significance level as 5% (one-sided) and Bonferroni correction for multiple comparisons.

VBM analysis was performed with Statistical Parametric Mapping 12 software (SPM12; http://www.fil.ion.ucl.ac.uk/spm). First, the anterior commissure of all images was set as the origin of the spatial coordinates. Next, the segmentation algorithm bias-corrected the raw T1-weighted images for inhomogeneities and generated rigid-body aligned GM and WM images of the subjects. Then, we used the DARTEL algorithm (Ashburner, 2007) to estimate the nonlinear deformations that best aligned all our images together by iteratively registering the imported images with their average. The created mean template was registered to the ICBM template in the Montreal Neurological Institute (MNI) space. Finally, we obtained the normalized and modulated tissue probability map of GM image (with isotropic voxel size of 1.5 mm) that were smoothed with a 3mm full-width at half-maximum (FWHM) smoothing kernel. ROI masks were generated using the Harvard-Oxford Atlas (http://www.cma.mgh.harvard.edu/fsl_atlas.html) for anterior cingulate, posterior cingulate, parahippocampal gyrus (anterior and posterior division), thalamus and hippocampus. For mammillary bodies and entorhinal cortex, we used the WFU PickAtlas (http://www.nitrc.org/projects/wfu_pickatlas). The mean modulated tissue probability of GM was extracted for the ROIs. Computational Anatomy Toolbox 12 (CAT12; http://www.neuro.uni-jena.de/cat) was used to calculate TIV. The processed data was fit to a GLM in the SPSS software, considering ROIs as dependent variables, and age, gender and TIV as covariates, considering significance level as 5% (one-sided) and Bonferroni-corrected for multiple comparisons.

Diffusion tensor imaging analysis

Diffusion weighted images preprocessing was performed in the FSL platform version 5.0.9, including eddy current correction (Andersson & Sotiropoulos, 2016) and brain-tissue extraction (Smith, 2002). Then, a diffusion tensor model was fit using FDT (FMRIB's Diffusion Toolbox). Tract-based spatial statistics (TBSS; Smith et al., 2006) was employed to perform a skeletonized analysis on fractional anisotropy (FA) maps, through an inter-subject registration (-n flag), resulting in the mean FA skeleton image (a group FA skeleton). Tracts of each subject were projected onto this skeleton employing a threshold of 0.2. The same skeleton projection was applied to mean diffusivity (MD) maps, following the non-FA images pipeline. Statistical analyses were carried out in the whole-brain analysis in TBSS and at ROI level. Specific matrices were generated to test group differences, considering age and gender as covariates. Randomise was performed with 10000 permutations using a threshold-free cluster enhancement (TFCE) analysis (FWE corrected). For ROI analysis, specific masks were created based on the probabilistic JHU White-Matter Tractography Atlas for the fornix, anterior thalamic radiations and cingulum. Mean FA and MD values were extracted for the ROIs and considered as dependent variables to perform a GLM with the SPSS software, considering age and gender as covariates and significance level as 5% (one-sided). Bonferroni test was used for correction for multiple comparisons.

Functional magnetic resonance analysis

fMRI data was preprocessed with CONN toolbox version 17.a (https://www.nitrc.org/projects/conn). The first four scans were dropped to achieve the steady state condition. Preprocessing steps included a standard pipeline (realignment and unwarping, slice-timing correction, segmentation, normalization, outlier detection, and

smoothing), resulting in both functional and structural images in MNI-space; denoising (simultaneous option) consisting on removal of WM and CSF noise (with 5 dimensions each), scrubbing (no subjects excluded), motion regression (12 regressors: 6 motion parameters + 6 first-order temporal derivatives) and band-pass filtering. ROI-to-ROI analyses considered two sided-effects with p-FDR analysis and permutation tests (10000 permutations) for hippocampus, parahippocampal gyrus (anterior and posterior divisions, anterior and posterior cingulate, and thalamus with masks from the Harvard-Oxford Atlas (http://www.cma.mgh.harvard.edu/fsl_atlas.html). A second-level GLM was obtained in CONN for population-level estimates and inferences with FDR-corrected p-values ≤ 0.05 at ROI level, considering age, gender and memory scores as covariates.

Results

Demographic and neuropsychological data

ALS patients and HC did not statistically differ on age, but there was significant difference in gender distribution with higher proportion of females in the control group. To minimize possible influence of gender in the results, statistical analyses were implemented considering gender as a covariate. Mean education for the ALS group was 12.5 years, and mean disease duration, 2.61 years. Years of education for the control group were not available.

Ten percent of the patients scored at the most lower limit of the normal range (controls' mean minus two standard deviation), therefore considered as having a subnormal performance according to what was expected for their age, but were not counted as impaired. Another ten percent scored below the normative scores according to their age, evidencing memory impairment. However, Mann-Whitney test revealed no significant difference in memory performance between ALS patients and controls and the

groups did not differ on the other ACE-R domains (attention/orientation, fluency and visuospatial; p=0.03) however there was a significant difference in language (Supplementary material Table 1 shows the ACE-R results). Spearman correlation coefficients showed no significant correlations between disease duration and memory scores, but a negative correlation between disease duration and atrophy in the right posterior cingulate (rho= -0.43; p= 0.03) was found.

Gray matter analyses

Cortical volume: ALS patients showed GM differences in an asymmetric pattern, with significant decreased GM volume in the left entorhinal cortex (p=0.02) and left hippocampus (p=0.03) compared with HC. In the right hemisphere, significant difference was present in the posterior cingulate (isthmus), with ALS showing decreased volume compared with HC (p=0.02). However, none of the results survived correction for multiple comparisons. Supplementary Material Table 2 shows the structures of the Papez circuit and its respective p-values and mean ± sd for cortical volumes. Spearman correlation analysis displayed significant positive association between all memory tests and cortical volume of left hippocampus (immediate recall: rho=0.42; anterograde memory: rho=0.44; retrograde memory: rho=0.45; delayed recall: rho=0.47; recognition: rho=0.55; all $p \leq 0.03$). Positive correlation between left entorhinal cortex volume and delayed recall (rho=0.38; p=0.04) and recognition scores (rho=0.53; p=0.008) was also significant (Supplementary Material Table 3). These correlations did not survive Bonferroni correction.

VBM: structures of the Papez circuit displayed no significant difference in GM between ALS patients and HC. Supplementary Material Table 4 shows the structures, its respective p-values and mean ± sd.

White matter analysis

ALS patients showed increased FA (p=0.04) and decreased MD (p=0.02) in the left cingulum bundle (hippocampal part) compared with HC. None of the results survived after correction for multiple comparisons. Anterior thalamic radiations and fornix did not reach significance. Supplementary Material Table 5 shows the tracts and its respective p-values and mean ± sd, related to FA and MD. Spearman correlation analyses indicated MD value of the left cingulum bundle had significant negative correlation with immediate recall (rho= -0.55; p=0.005), anterograde memory (rho= -0.42; p=0.03), delayed recall (rho= -0.66; p=0.001) and recognition scores (rho= -0.51; p=0.01; Supplementary Material Table 6).

Resting-state functional connectivity

[Figure 1 here]

Considering left hippocampus as seed, decreased functional connectivity was found in ALS patients compared with HC between posterior cingulate, left posterior parahippocampal gyrus, right anterior and posterior parahippocampal gyrus. Decreased functional connectivity was found between the right hippocampus and posterior cingulate, and between right hippocampus and left posterior parahippocampal gyrus. The posterior cingulate showed decreased functional connectivity between hippocampus bilaterally and right posterior parahippocampal gyrus. Decreased functional connectivity was found between the left posterior parahippocampal gyrus and hippocampus bilaterally and between left and right posterior parahippocampal gyrus. When the right posterior parahippocampal gyrus was the seed, decreased functional connectivity was observed

between the seed and left hippocampus, posterior cingulate, left anterior and posterior parahippocampal gyrus. Decreased functional connectivity was found between the right anterior parahippocampal gyrus and the left hippocampus. Figure 1 shows the connectivity map of the Papez circuit comparing ALS patients with HC, and Table 2 shows the statistical analyses with p-FDR values (all p-FDR=0.04). Memory measures did not show significant correlations with decreased functional connectivity using p-FDR analysis.

[Table 2 here]

Discussion

In this study, we investigated the integrity of the Papez network in non-demented ALS patients using a multimodal MRI approach. Although most previous studies attribute memory deficit in ALS to frontal-executive damage, recent studies report episodic memory impairment not solely attributed to executive dysfunction (Machts et al., 2014). In our study, we show structural and functional changes in the entire Papez circuit in ALS, with these changes associated with episodic memory performance.

Structural, diffusion and functional MRI explored the pattern of changes in the Papez circuit of ALS patients compared with healthy controls. Our findings show the Papez network presented consistent functional abnormalities in our ALS sample, with GM and WM changes present, although to a lesser degree. Specifically, we found decreased functional connectivity and GM atrophy in left hippocampus. Hippocampal atrophy in ALS has been previously shown by Raaphorst and colleagues (2015). It is worth mentioning that functional alterations of the right hippocampus suggest that functional changes may take place before structural damage is detectable. This

assumption is corroborated by imaging studies in neurodegeneration reporting functional abnormalities before structural or cognitive changes appear (Dennis et al., 2010; Trojsi et al., 2015; Li et al., 2014).

Along with the hippocampus, the left anterior parahippocampal gyrus, encompassing the entorhinal cortex, showed functional connectivity and volumetric GM decrease. This corroborates findings by Loewe and colleagues (2017) showing bilateral parahippocampal decreased functional connectivity in non-demented ALS patients with minor cognitive deficits, suggesting a pattern of temporal dysfunction in ALS, similar to that in FTD. Although we did not find increased activity in any region as found in their study, we corroborate their findings of decreased functional connectivity in parahippocampal gyrus. Importantly, in our sample, functional abnormalities are present bilaterally before cell loss.

Further, a recent study reported decreased fluctuations in the posterior cingulate of ALS patients (Trojsi et al., 2015). Of interest was the fact that the fluctuation was increased in the bvFTD group, suggesting although these two groups share commonalities, they may differ in some characteristics. In our study, decreased functional connectivity was present in the posterior cingulate cortex of ALS patients. In fact, the right posterior cingulate cortex, which connects the cingulate to the parahippocampal gyrus, showed GM atrophy in ALS. Mammillary bodies and thalamus were preserved.

DTI has proven to be a reliable method to study ALS and FA measures emerge as a potential biomarker for the neuropathology (Hornberger & Kiernan, 2016; Müller et al., 2016). Microstructural WM damage in extra-motor areas is reported in ALS and correlated with cognitive impairment (Abrahams et al., 2005; Meoded et al., 2013), which corroborates our findings of increased FA and decreased MD in the left cingulum bundle. WM changes in the cingulum bundle were previously associated to phonemic fluency

deficits and executive dysfunction (Sarro et al., 2011). The caudal part of the cingulum bundle entering the temporal lobe and connecting with parahippocampal gyrus and entorhinal area presented functional abnormalities and GM atrophy in our study. Interestingly, despite the changes in temporal regions, the fornix was preserved. Fornix integrity was unexpected given hippocampal abnormal functional connectivity and atrophy present, as well as reports of fornix abnormalities in the literature (Mantovan et al., 2003; Christidi et al., 2014). Its preservation may contribute to the relatively good memory performance in our patients, given the area is closely associated with memory processes (Rudebeck et al., 2009). Anterior thalamic radiations did not present changes.

In sum, although primary motor cortex degeneration is the hallmark of ALS, with studies demonstrating significant structural and functional changes in motor areas (Fekete et al., 2013; Mezzapesa et al., 2013), our results show that ALS patients presented significant changes in the Papez circuit. Functional abnormalities, although controversial, are documented in the ALS literature, reporting both decreased and increased functional connectivity (Douaud et al., 2011; Agosta et al., 2013). Decreased functional connectivity in our study was consistent with structural changes.

Although our patients do not show an amnesic profile, there were correlations between structural changes and memory performance. After being underestimated in the past, memory impairments in ALS are recently highlighted in several studies (Abrahams et al., 2000; Machts et al., 2014). Previous studies have mostly considered impairments to follow frontal-executive damage (Consonni et al., 2015; Matuszewski et al., 2006), however recent works indicate the involvement of hippocampal atrophy (Raaphorst et al., 2015; Christidi et al., 2017; Kasper et al., 2015). Here, we report that abnormalities in different Papez circuit regions may affect memory performance in ALS beyond the sole hippocampus. GM atrophy of hippocampus significantly correlated with measures of

memory. Similarly, left entorhinal atrophy correlated with delayed recall and recognition. Finally, the MD of the left cingulum bundle also correlated with memory performance. While being consistent with previous works focusing on hippocampus atrophy to explain memory impairments, our findings show a more general involvement of the Papez circuit in ALS.

Taken together, our results show that ALS patients presented functional and structural changes in the Papez circuit. In addition, the anatomical changes were linked to memory performance, similarly to what is observed in bvFTD (Bertoux et al., 2014). Sub-regions of the Papez network are indeed impaired in different degrees in bvFTD, with marked atrophy of the hippocampus and cingulate cortex (Bertoux et al., 2014; Irish et al., 2014). Although the fornix seemed to be spared in our non-demented ALS population, while being a site of atrophy in bvFTD, our findings bring evidence of common Papez changes in ALS and bvFTD, and these changes might contribute to cognitive decline in ALS. These results corroborate the contemporary view that ALS and FTD may be part of a disease continuum (Lillo et al.,2016; Bueno et al., 2017). However, the question remains, if fornix, mammillary bodies and thalamus, which showed no structural changes in our ALS group, but shows significant changes in bvFTD, would be altered in later disease stages.

Some limitations must be acknowledged. Although our structural results do not survive correction for multiple comparisons, they suggest an involvement of structures that are corroborated in other studies. Future studies should replicate these findings in a larger sample to confirm our findings and bring more insights into the discussion. However, while our patient sample size was relatively small, such group sizes are common in neurodegenerative studies (Agosta et al., 2013; Irish et al., 2014; Mioshi et al., 2013). In addition, to overcome the limitation of the memory test applied in this study,

the use of more sensitive neuropsychological tests and specific to temporal lobe impairment will help to refine our results and better describe the extent and nature of impairments in ALS. Importantly, to evaluate executive dysfunction impact on memory performance, specific assessments are recommended, similarly to what has been performed in bvFTD (Bertoux et al., 2016).

In conclusion, ALS patients exhibited denoting functional changes in the Papez circuit and structural damage, the latter being linked to memory performance. Functional connectivity abnormalities of the Papez circuit may turn out to be useful to assess risk or assist early detection of cognitive impairment in ALS patients, before structural changes are established. Since cognitive impairment has a negative impact on the prognosis of ALS patients, early detection of cognitive changes and improvement of diagnosis may be important for disease management. Future studies investigating longitudinal changes of the Papez circuit are warranted to explore this further.

Compliance with Ethical Standards

Funding

This work was supported by the National Health and Medical Research Council of Australia Program Grant to Forefront (1037746) and the Brain Foundation Australia grant to MH. MH is further supported by Alzheimer's Research UK and the Wellcome trust. AB is supported by FAPESP. Grant 2016/19376-9, São Paulo Research Foundation (FAPESP). RR is supported by the Motor Neuron Disease Association (MNDA).

Conflicts of interest

All authors report no conflict of interest.

Ethical approval

All procedures performed in this study were in accordance with the ethical standards of the institutional and national research committee (Human Research Ethics Committee of South Eastern Sydney/Illawarra Area Health Service) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Written informed consent was obtained from all individual participants included in the study or from a close relative.

Acknowledgements

The authors gratefully acknowledge the contribution of the patients and their families. The authors thank Prof. Paulo Caramelli for his valuable comments on early versions of the manuscript.

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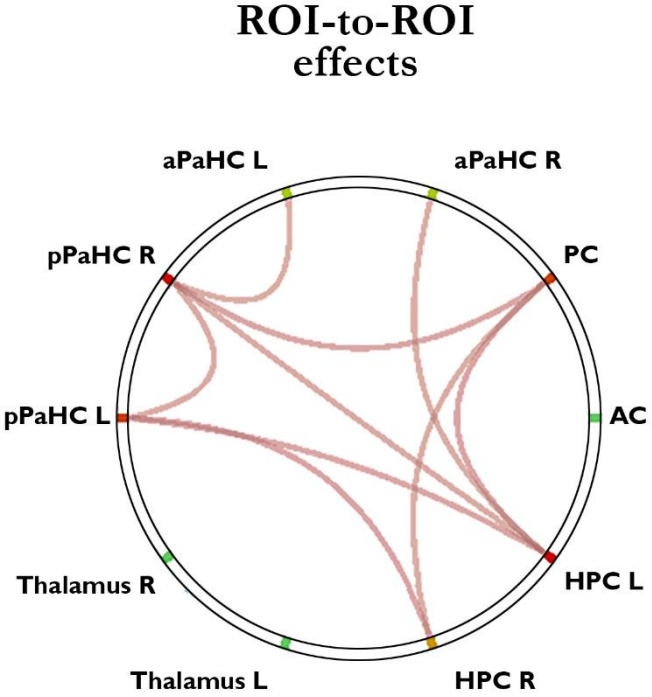
Table 1 – Demographic.

Demographic	Mean ± SD		p-value
	HC	ALS	
n	15	20	-
Age	60 ± 7.2	63.8 ± 12.2	0.2
Gender (male, famale)	2/13	10/10	0.02
Mean disease duration (years)	-	2.6 ± 2.1	-
Years of education	-	12.5 ± 3.5	-
Immediate Recall (3)	2.9 ± 0.3	2.4 ± 0.9	0.1
Memory - Anterograde (7)	7.0 ± 0.0	6.8 ± 0.5	0.2
Memory - Retrograde (4)	3.0± 0.8	3.4 ± 0.9	0.1
Delayed Recall (7)	6.0 ± 1.3	5.4 ± 2.1	0.4
Recognition (5)	4.7 ± 0.6	4.7 ± 0.4	0.8

ALS – Amyotrophic lateral sclerosis; HC – health controls; ACE-R – Addenbrooke´s Cognitive Examination - Revised; sd – standard deviation. p-value refers to ALS compared with controls.

Fig. 1 - Map of functional connectivity of the Papez circuit in ALS patients compared with controls.



AC= anterior cingulate; PC= posterior cingulate; aPaHC r= right anterior parahippocampal; aPaHC l= left anterior parahippocampal; pPaHC r= right posterior parahippocampal; pPaHC l= left posterior parahippocampal. Map refers to two-side effects. Positive results meaning decreased functional connectivity found in anterior cingulate, hippocampus and parahippocampal gyrus of ALS patients compared with HC. No negative effects were found, meaning no increased functional connectivity in ALS patients compared with HC. All p-FDR at ROI-level. Data did not show correlation with memory measures.

Table 2 – Functional connectivity of the Papez circuit in ALS patients compared with controls.

Analysis Unit	Statistic	p-FDR
Seed Hippocampus l	F(7)(22) = 2.63	0.1778
Hippocampus l-PC	T(28) = 3.40	0.0411
Hippocampus l-pPaHC l	T(28) = 3.18	0.0411
Hippocampus l-pPaHC r	T(28) = 3.04	0.0416
Hippocampus l-aPaHC r	T(28) = 2.83	0.0438
Seed pPaHC l	F(7)(22) = 2.35	0.1778
pPaHC l -Hippocampus r	T(28) = 3.36	0.0411
pPaHC l -Hippocampus l	T(28) = 3.18	0.0411
pPaHC l -pPaHC r	T(28) = 3.00	0.0416
Seed aPaHC l	F(7)(22) = 1.79	0.1991
aPaHC l -pPaHC r	T(28) = 2.82	0.0438
Seed PC	F(7)(22) = 2.09	0.1778
PC -Hippocampus l	T(28) = 3.40	0.0411
PC -pPaHC r	T(28) = 3.17	0.0411
PC -Hippocampus r	T(28) = 2.86	0.0438
Seed pPaHC r	F(7)(22) = 2.09	0.1778
pPaHC r -PC	T(28) = 3.17	0.0411
pPaHC r -Hippocampus l	T(28) = 3.04	0.0416
pPaHC r -pPaHC l	T(28) = 3.01	0.0416
pPaHC r -aPaHC l	T(28) = 2.82	0.0438
Seed aPaHC r	F(7)(22) = 2.09	0.1778
aPaHC r -Hippocampus l	T(28) = 2.83	0.0438
Seed Hippocampus r	F(7)(22) = 1.82	0.1991
Hippocampus r-pPaHC l	T(28) = 3.36	0.0411
Hippocampus r-PC	T(28) = 2.86	0.0438

AC= anterior cingulate; PC= posterior cingulate; aPaHC l= left anterior parahippocampal gyrus; aPaHC r= right anterior parahippocampal gyrus; pPaHC l= left posterior parahippocampal gyrus; pPaHC r= right posterior parahippocampal gyrus.