

1 **STRUCTURAL AND FUNCTIONAL PAPEZ CIRCUIT INTEGRITY IN**  
2 **AMYOTROPHIC LATERAL SCLEROSIS**

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41 **Abstract**

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

63

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

66 **Introduction**

67 Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease sharing  
68 clinical, pathological and genetic features with frontotemporal dementia (FTD),  
69 specifically with its behavioural variant presentation (bvFTD). This overlap between both  
70 diseases is now recognized to form a pathophysiological spectrum (Lillo & Hodges,  
71 2009). In addition to motor symptoms, some ALS patients can present with full-blown  
72 bvFTD, while others can display some cognitive and behavioural deficits without meeting  
73 criteria for dementia (Raaphorst et al., 2015; van der Hulst et al., 2015; Hervieu-Begue et  
74 al., 2016; Mioshi et al., 2014).

75 Cognitive deficits in ALS occur in up to 30% of patients and are usually associated  
76 with shorter survival (Woolley & Strong, 2015; Beeldman et al., 2015; Abrahams et al.,  
77 2000). The deficits are commonly characterized by executive dysfunction in the form of  
78 verbal fluency deficits and as impairments of intrinsic response generation (Goldstein &  
79 Abrahams, 2013). However, cognitive dysfunction in ALS is heterogeneous, with the  
80 presence of social cognition and emotion processing deficits among others (Abrahams et  
81 al., 2000; Volpato et al., 2010).

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

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

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

103

## 104 **Methods**

### 105 *Participants*

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

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

121

122 [Table 1 here]

123

#### 124 ***Brief memory assessment: ACE-R***

125 Patients underwent the Addenbrooke's Cognitive Examination-Revised (ACE-R),  
126 a battery of general cognitive tests (Mioshi et al., 2006), including a multidimensional  
127 assessment of episodic memory with five scores: immediate recall (measuring the ability  
128 to recall three previously learned words); anterograde memory (measuring the ability to  
129 learn and recall a postal address - delayed recall score); retrograde memory (measuring  
130 the recall of common knowledge acquired months/years earlier); and recognition  
131 (evaluating recognition abilities of the address previously learned, if delayed recall fail).

132 We subdivided the ALS patients according to their ages, considering the cut offs proposed  
133 by Mioshi and colleagues (2006) to evaluate their performance on the memory tests and  
134 used Mann-Whitney test to compare memory performance between groups. Spearman  
135 correlation was performed in SPSS to correlate memory performance with every structure  
136 presenting changes in structural and diffusion MRI and disease duration, with Bonferroni  
137 correction for multiple comparisons.

138

#### 139 ***MRI acquisition***

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

142 diffusion MRI (n=15) and rs-fMRI (n=11). T1-weighted images were acquired as follows:  
143 multi shot 256 TFE factor (TR/TE 5.4/2.4ms, 256x256 matrix, FOV 256x256 x180, flip  
144 angle 8°), slice thickness 1mm, coronal orientation, voxel size 1x1x1mm<sup>3</sup>. DTI-weighted  
145 images were acquired using a single shot echo-planar imaging (EPI) sequence, (TR/TE  
146 11595/78ms, 96x96 matrix size, FOV 240x240x137, flip angle 90°), 2.5mm transverse  
147 slices with no gaps, 61 gradient directions, b-value 0 and 2000s/mm<sup>2</sup>, voxel size  
148 2.5x2.5x2.5mm<sup>3</sup>. The following protocol was used for resting-state fMRI acquisition:  
149 T2\*-weighted images using single shot EPI (TR/TE 3000/30ms, 120x120 matrix, FOV  
150 240x240x140, flip angle 80°), 127 scans, 40 transverse slices with thickness 3.5mm and  
151 no gap, voxel size 2x2x3.5mm<sup>3</sup>.

152

### 153 ***MRI processing***

#### 154 *Cortical volumetric analysis and VBM*

155 Cortical and subcortical volumetric measures were obtained with Freesurfer  
156 software version 5.3.0 (<http://surfer.nmr.mgh.harvard.edu>). The preprocessing pipeline  
157 was performed using the fully-automated directive – the “recon-all” command. Briefly,  
158 the preprocessing included: intensity normalization, removal of non-brain tissues,  
159 Talairach transforms, segmentation of the GM and WM, and tessellation of the GM/WM  
160 boundary (technical details in Fischl et al., 2004). Once cortical models were complete,  
161 the cortical surface of each hemisphere was parcellated according to the atlas proposed  
162 by Desikan and colleagues (2006; with 34 cortical regions per hemisphere; “aparc”  
163 segmentation). Cortical volume was estimated multiplying cortical thickness (average  
164 shortest distance between the WM boundary and the pial surface) by area (Dale et al.,  
165 1999a; Dale et al., 1999b) . The subcortical volume measures were obtained via a whole  
166 brain segmentation procedure, using “aseg” segmentation (Fischl et al., 2004). A general

167 linear model (GLM) was performed in SPSS using regions of interest (ROIs) measures  
168 as dependent variables, age and gender as covariates, considering significance level as  
169 5% (one-sided) and Bonferroni correction for multiple comparisons.

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

191

192 *Diffusion tensor imaging analysis*

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

211

212 *Functional magnetic resonance analysis*

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



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

227

## 228 **Results**

### 229 *Demographic and neuropsychological data*

230 ALS patients and HC did not statistically differ on age, but there was significant  
231 difference in gender distribution with higher proportion of females in the control group.  
232 To minimize possible influence of gender in the results, statistical analyses were  
233 implemented considering gender as a covariate. Mean education for the ALS group was  
234 12.5 years, and mean disease duration, 2.61 years. Years of education for the control  
235 group were not available.

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

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

248

### 249 *Gray matter analyses*

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

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

267

268 ***White matter analysis***

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

278

279 ***Resting-state functional connectivity***

280

281 [Figure 1 here]

282

283 Considering left hippocampus as seed, decreased functional connectivity was  
284 found in ALS patients compared with HC between posterior cingulate, left posterior  
285 parahippocampal gyrus, right anterior and posterior parahippocampal gyrus. Decreased  
286 functional connectivity was found between the right hippocampus and posterior  
287 cingulate, and between right hippocampus and left posterior parahippocampal gyrus. The  
288 posterior cingulate showed decreased functional connectivity between hippocampus  
289 bilaterally and right posterior parahippocampal gyrus. Decreased functional connectivity  
290 was found between the left posterior parahippocampal gyrus and hippocampus bilaterally  
291 and between left and right posterior parahippocampal gyrus. When the right posterior  
292 parahippocampal gyrus was the seed, decreased functional connectivity was observed

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

300

301 [Table 2 here]

302

### 303 **Discussion**

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

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

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

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

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

337         DTI has proven to be a reliable method to study ALS and FA measures emerge as  
338 a potential biomarker for the neuropathology (Hornberger & Kiernan, 2016; Müller et al.,  
339 2016). Microstructural WM damage in extra-motor areas is reported in ALS and  
340 correlated with cognitive impairment (Abrahams et al., 2005; Meoded et al., 2013), which  
341 corroborates our findings of increased FA and decreased MD in the left cingulum bundle.  
342 WM changes in the cingulum bundle were previously associated to phonemic fluency

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

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

359         Although our patients do not show an amnesic profile, there were correlations  
360 between structural changes and memory performance. After being underestimated in the  
361 past, memory impairments in ALS are recently highlighted in several studies (Abrahams  
362 et al., 2000; Machts et al., 2014). Previous studies have mostly considered impairments  
363 to follow frontal-executive damage (Consonni et al., 2015; Matuszewski et al., 2006),  
364 however recent works indicate the involvement of hippocampal atrophy (Raaphorst et al.,  
365 2015; Christidi et al., 2017; Kasper et al., 2015). Here, we report that abnormalities in  
366 different Papez circuit regions may affect memory performance in ALS beyond the sole  
367 hippocampus. GM atrophy of hippocampus significantly correlated with measures of

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

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

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

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

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

406

#### 407 **Compliance with Ethical Standards**

408

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415

#### 416 **Conflicts of interest**

417 All authors report no conflict of interest.



418

419 **Ethical approval**

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

424

425 **Informed consent**

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

428

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435 **References**

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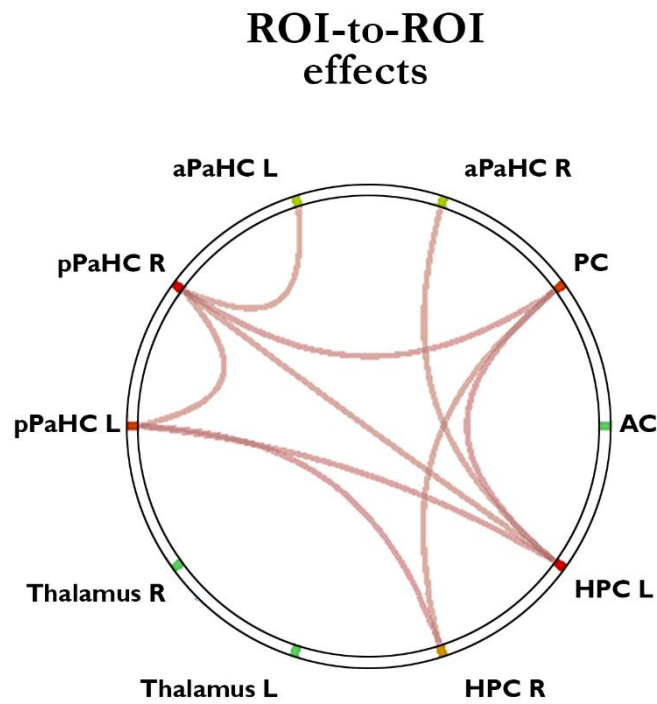
**Table 1 – Demographic.**

Demographic	Mean ± SD		p-value
	HC	ALS	
n	15	20	-
Age	60 ± 7.2	63.8 ± 12.2	0.2
Gender (male, female)	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

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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.

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



626 AC= anterior cingulate; PC= posterior cingulate; aPaHC r= right anterior parahippocampal; aPaHC l= left anterior  
627 parahippocampal; pPaHC r= right posterior parahippocampal; pPaHC l= left posterior parahippocampal. Map refers to  
628 two-side effects. Positive results meaning decreased functional connectivity found in anterior cingulate, hippocampus  
629 and parahippocampal gyrus of ALS patients compared with HC. No negative effects were found, meaning no increased  
630 functional connectivity in ALS patients compared with HC. All p-FDR at ROI-level. Data did not show correlation  
631 with memory measures.  
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635 **Table 2 – Functional connectivity of the Papez circuit in ALS patients compared**  
 636 **with controls.**  
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<b>Analysis Unit</b>	<b>Statistic</b>	<b>p-FDR</b>
<b>Seed Hippocampus l</b>	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
<b>Seed pPaHC l</b>	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
<b>Seed aPaHC l</b>	F(7)(22) = 1.79	0.1991
aPaHC l -pPaHC r	T(28) = 2.82	0.0438
<b>Seed PC</b>	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
<b>Seed pPaHC r</b>	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
<b>Seed aPaHC r</b>	F(7)(22) = 2.09	0.1778
aPaHC r -Hippocampus l	T(28) = 2.83	0.0438
<b>Seed Hippocampus r</b>	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

638 AC= anterior cingulate; PC= posterior cingulate; aPaHC l= left anterior parahippocampal gyrus; aPaHC r= right  
 639 anterior parahippocampal gyrus; pPaHC l= left posterior parahippocampal gyrus; pPaHC r= right posterior  
 640 parahippocampal gyrus.  
 641