In vivo and post-mortem memory circuit integrity in frontotemporal dementia and Alzheimer’s disease

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Behavioural variant frontotemporal dementia can present with episodic memory deficits as severe as those in Alzheimer’s disease. Little is known of the integrity of grey matter areas and white matter tracts of the Papez memory circuit in these diseases. The integrity of the Papez circuit (hippocampus, fornix, mammillary bodies, anterior thalamus, cingulate cortex) was investigated in vivo and at post-mortem in behavioural variant frontotemporal dementia and Alzheimer’s disease cohorts using voxel-based morphometry, diffusion tensor imaging and manual volumetric tracing. Our findings indicate that behavioural variant frontotemporal dementia and Alzheimer’s disease show similar degrees of hippocampal atrophy in vivo, but patients with behavioural variant frontotemporal dementia show greater hippocampal atrophy at post-mortem, with the frontotemporal lobar degeneration with TDP-43 inclusions subtype being particularly affected. Cingulate cortex findings show an expected atrophy pattern with behavioural variant frontotemporal dementia being affected more anteriorly and Alzheimer’s disease showing more posterior atrophy. More importantly, subcortical Papez circuit regions (fornix and anterior thalamus) were affected in behavioural variant frontotemporal dementia only, with atrophy in these regions determining the degree of amnesia in behavioural variant frontotemporal dementia. Hippocampal atrophy does not appear to be an efficient diagnostic marker for underlying behavioural variant frontotemporal dementia or Alzheimer’s disease pathology, although for behavioural variant frontotemporal dementia, episodic memory deficits in conjunction with marked hippocampal atrophy emerge as potential biomarkers for frontotemporal lobar degeneration with TDP-43 inclusions pathology. Sub-regions of the Papez circuit were differentially affected in behavioural variant frontotemporal dementia and Alzheimer’s disease with subcortical regions determining the degree of episodic memory deficits in behavioural variant frontotemporal dementia. Subcortical atrophy should be taken into account when establishing whether the severe amnesia observed in a patient is likely to be due to behavioural variant frontotemporal dementia or Alzheimer’s disease pathology.

Keywords: behavioural variant frontotemporal dementia; Alzheimer’s disease; episodic memory; Papez circuit
Abbreviations: DTI = diffusion tensor imaging; FTD = frontotemporal dementia; FTLD = frontotemporal lobar dementia; VBM = voxel-based morphometry

Received March 21, 2012. Revised July 5, 2012. Accepted July 8, 2012
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Introduction

The medial temporal lobe, particularly the hippocampus, is widely regarded as the key memory-related region since its surgical removal has severe effects on memory performance (Milner and Penfield, 1955). The hippocampus is one of the earliest regions to degenerate in Alzheimer’s disease (Braak and Braak, 1991) and episodic memory deficits, a well-established early feature of Alzheimer’s disease (McKhann et al., 1984), are usually attributed to hippocampal pathology (de Leon et al., 1996; Jack et al., 1997). Mounting evidence shows that patients with behavioural variant frontotemporal dementia (FTD) also experience episodic memory impairment which, in some cases, may be of similar severity to that seen in Alzheimer’s disease (Graham et al., 2005; Hornberger et al., 2010). At present, little is known about the integrity of the episodic memory systems in behavioural variant FTD, although considerable post-mortem pathology occurs in the hippocampus in behavioural variant FTD, even in patients dying early in the course of the disease (Broe et al., 2003; Knl and Halliday, 2004). Medial temporal lobe atrophy has also been reported in behavioural variant FTD using neuroimaging (Rabinovici et al., 2007; Seeley et al., 2008; Whitwell et al., 2009). While initial studies reported a greater hippocampal atrophy in Alzheimer’s disease than behavioural variant FTD (Boccardi et al., 2003; Grossman et al., 2004), recent studies have shown a similar degree of hippocampal atrophy in behavioural variant FTD and Alzheimer’s disease (van de Pol et al., 2006; Rabinovici et al., 2007), as well as in surrounding cortices (entorhinal and perirhinal) (Pennington et al., 2011). Important, the hippocampus is but one region in a larger memory circuit, the so-called ‘Papez circuit’ (Fig. 1), which comprises hippocampus, fornix, mammillary bodies, anterior thalamus and cingulate cortex. Lesions affecting any region of the Papez circuit produce substantial memory deficits in animals and humans, even when sparing the hippocampus (for a review see Aggleton and Brown, 1999). Neuroimaging and neuropathological investigations have shown that all Papez circuit regions degenerate in Alzheimer’s disease, except the anterior cingulate (Hooper and Vogel, 1976; Callen et al., 2001), with hippocampal and posterior cingulate regions being particularly affected. In contrast, studies of behavioural variant FTD atrophy have focused exclusively on the hippocampus and cingulate regions, in particular the anterior cingulate cortex (Rabinovici et al., 2007; Seeley et al., 2008). To our knowledge, no study has investigated the integrity of the remaining relay stations of the Papez circuit in behavioural variant FTD.

The current study investigated whether Papez circuit grey matter regions (hippocampus, mammillary bodies, anterior thalamus, cingulate cortex) and the main white matter tract (fornix) are differentially atrophied in behavioural variant FTD and Alzheimer’s disease. We employed parallel in vivo and post-mortem volumetric methods to establish the intactness of the circuit with disease progression. For the in vivo study, we conducted region of interest voxel-based morphometry (VBM) and diffusion tensor imaging (DTI) analyses in a clinical cohort of patients with behavioural variant FTD and Alzheimer’s disease. We employed parallel in vivo and post-mortem volumetric methods to establish the intactness of the circuit with disease progression. For the in vivo study, we conducted region of interest voxel-based morphometry (VBM) and diffusion tensor imaging (DTI) analyses in a clinical cohort of patients with behavioural variant FTD and Alzheimer’s disease compared with controls. To confirm the clinical findings in a post-mortem sample, we conducted volumetric analysis of grey matter regions of interest in pathologically verified cases of behavioural variant FTD.

Figure 1 Schematic diagram of the Papez circuit regions.
Alzheimer’s disease and controls. Based on previous findings, we predicted that both groups would show similar atrophy of the hippocampus, with atrophy of the posterior cingulate cortex more substantial in Alzheimer’s disease and atrophy of the anterior cingulate cortex more substantial in behavioural variant FTD. We also predicted that the fornix, mammillary bodies and anterior thalamus would be atrophic in Alzheimer’s disease.

Materials and methods

In vivo cohort

Fifteen patients with a clinical diagnosis of behavioural variant FTD (Rascovsky et al., 2011) were recruited from the Frontotemporal Dementia Research Group (FRONTIER) at Neuroscience Research Australia (NeuRA) following study approval by the University of New South Wales Human Research Ethics Advisory Panel D (Biomedical, ref. 10035). The behavioural variant FTD group was contrasted with 15 patients with a diagnosis of probable Alzheimer’s disease (McKhann et al., 1984), as well as 18 healthy controls from the FRONITIER participant database. Patients with Alzheimer’s disease and controls were matched for clinical and demographic variables (Table 1) to the behavioural variant FTD group. Clinical, basic neuropsychological and demographic data (Table 1) as well as high-resolution coronal T1, and diffusion tensor magnetic resonance brain images were available for all participants. All participants underwent comprehensive neuropsychological testing (Supplementary Table 1), including the following episodic memory tests: Rey Auditory Verbal Learning Test immediate recall testing (Supplementary Table 1), including the following episodic tests (max score)

Table 1 Mean scores (SD) for patients with behavioural variant FTD, Alzheimer’s disease and controls on demographics and cognitive tests

<table>
<thead>
<tr>
<th>Test</th>
<th>In vivo cohort (max score)</th>
<th>Post-mortem cohort (max score)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Behavioural variant FTD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>n</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>10/5</td>
<td>13/6</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>59 (8)</td>
<td>64 (8)</td>
</tr>
<tr>
<td>Duration of disease (years)</td>
<td>3.7 (2.6)</td>
<td>2.7 (1.7)</td>
</tr>
<tr>
<td>Path stage (4a or 6b)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ACE (100)</td>
<td>75.6 (11.4)*</td>
<td>80.1 (11.4)*</td>
</tr>
<tr>
<td>RAVLT A6 (15)</td>
<td>3.2 (3.3)*</td>
<td>3.9 (2.9)*</td>
</tr>
<tr>
<td>RAVLT recognition (15)</td>
<td>11.7 (5.5)</td>
<td>10.1 (3.6)</td>
</tr>
<tr>
<td>Rey 3 min recall (36)</td>
<td>5.6 (5.2)*</td>
<td>5.1 (4.5)*</td>
</tr>
<tr>
<td>Doors and People A (12)</td>
<td>7.8 (2.5)*</td>
<td>7.9 (2.4)*</td>
</tr>
</tbody>
</table>

*Pathological staging for FTD (Broe et al., 2003).

**Pathological staging for Alzheimer’s disease and aged controls.

*P < 0.001 disease groups different from controls.

ACE = Addenbrooke’s Cognitive Examination; RAVLT = Rey Auditory Verbal Learning Test.

In vivo cohort: magnetic resonance imaging acquisition and analyses

Imaging acquisition

All patients and controls in the clinical cohort underwent the same imaging protocol with whole-brain T1 and DTI-weighted images using a 3T Philips MRI scanner with standard quadrature head coil (eight channels). The 3D T1-weighted sequences were acquired as follows: coronal orientation, matrix 256 x 256, 200 slices, 1 x 1 mm² in-plane resolution, slice thickness 1 mm, echo time/repetition time = 2.6/5.8 ms. The DTI-weighted sequences were acquired as follows: 32 gradient
direction DTI sequence (repetition time/echo time/inversion time: 8400/68/90 ms; \( b \)-value = 1000 s/mm\(^2\); 55 2.5-mm horizontal slices, end resolution: 2.5 \( \times \) 2.5 \( \times \) 2.5 mm\(^3\); field of view 240 \( \times \) 240 mm, 96 \( \times \) 96 matrix; repeated twice). Two DTI sequences were acquired for each participant, which were in a first step then averaged and corrected for eddy current distortions. The diffusion tensor models were then fitted at each voxel via the FDT toolbox in FSL (http://www.fmrib.ox.ac.uk/fsl/fdt/index.html), resulting in maps of three eigenvalues (L1, L2, L3) which allowed calculation of fractional anisotropy maps for each subject.

**Voxel-based morphometry analysis**

Three-dimensional T\(_1\)-weighted sequences were analysed with FSL-VBM, a VBM analysis (Ashburner and Friston, 2000; Good et al., 2001), which is part of the FSL software package http://www.fmrib.ox.ac.uk/fsl/fslvbm/index.html. First, tissue segmentation was carried out using FMRIB’s Automatic Segmentation Tool (FAST) from brain extracted images. The resulting grey matter partial volume maps were then aligned to the Montreal Neurological Institute standard space (MNI 152) using the non-linear registration approach using FNIRT, which uses a b-spline representation of the registration warp field. The registered partial volume maps were then modulated (to correct for local expansion or contraction) by dividing them by the Jacobian of the warp field. The modulated images were then smoothed with an isotropic Gaussian kernel with a standard deviation of 3 mm (full-width at half-maximum: 8 mm). A voxel-wise general linear model was applied and permutation-based non-parametric testing was used to form clusters with the Threshold-Free Cluster Enhancement method, tested for significance at \( P < 0.001 \), corrected for False Discovery Rate (FDR) with a voxel threshold of at least 20 contiguous voxels. Finally, regions of interests for the hippocampus, mammillary bodies, thalamus and cingulate were created based on the alveus and white matter of the hippocampus are defined by the alveus and white matter of the parahippocampal gyrus, respectively.

**Diffusion tensor imaging analysis**

Tract-based Spatial Statistics from FSL were used to perform a skeleton-based analysis of white matter fractional anisotropy. Fractional anisotropy maps of each individual subject were eddy current corrected and co-registered using non-linear registration using FNIRT to the MNI standard space using the FMRIB58 fractional anisotropy template, which is available as part of the FSL software. The template was sub-sampled at 2 \( \times \) 2 \( \times \) 2 mm\(^3\) due to the coarse resolution of native DTI data (i.e. 2.5 \( \times \) 2.5 \( \times \) 2.5 mm\(^3\)). After image registration, fractional anisotropy maps were averaged to produce a group mean fractional anisotropy image. A skeletonization algorithm was applied to the group mean fractional anisotropy image to define a group template of the lines of maximum fractional anisotropy, assumed to correspond to centres of white matter tracts. Fractional anisotropy values for each individual subject were then projected onto this group template skeleton. Clusters were tested using permutation-based non-parametric testing as described for the VBM analysis. Clusters reported have significance at \( P < 0.05 \), corrected for multiple comparisons across family-wise error (FWE) correction space, unless otherwise stated. Similar to the VBM analysis, masks for the regions of interest (fornix, cingulate cingulum) were created based on the probabilistic JHU White-Matter Tractography Atlas (Mori et al., 2005).

**Post-mortem preparation and volume analyses**

**Brain preparation**

The volumetric methods used in this study have been published in detail elsewhere (Halliday et al., 2003). Briefly, following a 14-day fixation in 15% neutral buffered formalin, each brain was weighed and the volume determined by fluid displacement. The cerebellum and brainstem were separated from the cerebrum by sectioning through the cerebral peduncles. Each cerebrum was embedded in 3% agar and sectioned in 3-mm coronal slices. Slices were photographed and printed at \( \times 1 \) magnification. Average slice thickness for each brain was determined by dividing the hemisphere length by the total number of slices.

The regions of interest (hippocampus, mammillary bodies, thalamus and cingulate) were quantified in the left and right hemispheres of each brain. In accordance with human brain atlases, gyral boundaries and anatomical structures most consistently associated with cytoarchitectonic boundaries were used to identify regions across all cases.

The anterior border of the hippocampus is determined by the alveus, which separates the hippocampus from the amygdala. Its posterior limit is where the hippocampal ovoid grey matter entirely disappears. The CSF of the lateral ventricle is used as an external marker for the lateral border of the hippocampus. The superior and inferior borders of the hippocampus are defined by the alveus and white matter of the parahippocampal gyrus, respectively.

The mammillary bodies are two spherical structures that lie inferior to the hypothalamus and anterior to the crus cerebri. The lateral border is defined by the points of the ‘mammillary notches’.

The anterior tubercle of the thalamus lies posterior to the interven- tricular foramen and anterior commissure. The superior border of the anterior thalamus forms part of the floor of the lateral ventricles. It is laterally and inferiorly bound by the internal medullary lamina. The anterior thalamus narrows towards its posterior pole, which occurs where the anterior tubercle no longer protrudes from the superior border of the thalamus.

The cingulate cortex was defined as the cortex in the cingulate gyrus surrounding the corpus callosum and defined by the cingulate sulcus. The anterior cingulate cortex includes subgenual and pregennal regions as well as cingulate cortex above the corpus callosum in coronal slices anterior to the pallidum. The posterior cingulate cortex was determined as the cingulate cortex posterior to the central lobule of the central sulcus and commenced in coronal slices posterior to the pallidum.

**Volume determination**

The volumes of the regions of interest were determined by a point counting procedure with two raters used to identify regions of interest and determine volumes. The areas corresponding to each region were identified on the brain slice photographs, which were randomly overlaid with a grid of 3848 points (each separated by 4 mm). The total number of points falling on each region of interest was counted. Volumes were calculated by multiplying the sum of the points falling on a given structure by the volume represented by each point (volume/point = 16 mm\(^2\) \( \times \) mean slice thickness; average of 50 mm\(^2\)). The raters were initially trained to identify the same regions of interest in three brains with \(<5\%\) variation. The raters were considered competent where \(<5\%\) variation was obtained in 10 repeated measures of the regions of interest in three cases. Two independent raters traced the regions of interest in three cases with a correlation between counts
of 0.978. The only departure from this method was the volumetric measurement of the mammillary bodies which was traced onto digital images of the brain slice photographs to measure the cross-sectional area. In repeated measurements on eight cases the main rater had <2% variation. Two independent raters traced the regions of interest in eight cases with a correlation between measures of 0.988. Volumes of the mammillary bodies were calculated using Cavalieri’s principle (area of region of interest × mean slice thickness).

Quantitation of hippocampal inclusions
Sections of the hippocampus immunohistochemically stained with TDP-43 (rabbit anti-human TDP-43 diluted 1:800; ProteinTech) and tau (mouse anti-human tau diluted 1:1000; Thermo Scientific) antibodies were evaluated for each case. Systematic sampling of the same anatomical regions of both the CA1 region and dentate gyrus were used. For the CA1, the proportion of neurons containing immunohistochemically-identified inclusions were evaluated using standard inclusion and exclusion borders within one 400 × 400 μm field located laterally in the mid-CA1 region at the superficial ventricular surface, as previously published (Kersaitis et al., 2004). For the dentate gyrus, the proportion of neurons containing immunohistochemically-identified inclusions were evaluated using standard inclusion and exclusion borders within two 200 × 200 μm fields located midway along the lateral aspects of the cup-shaped gyrus. A further 200 × 200 μm field containing the highest observable density of immunohistochemically-identified inclusions was also evaluated. There was no difference between two raters in the density of inclusions counted in all cases (paired t-test P = 0.24; R = 0.079, P < 0.0001). The density measures were standardized to numbers/mm².

Statistical analysis
Data were analysed using SPSS19.0 (IBM Corp.). Parametric demographic (age, education, disease duration), clinical (Clinical Dementia Rating) and general cognitive (Addenbrooke’s Cognitive Examination-Revised) and post-mortem region of interest volumes and hippocampal lesion densities were compared across groups via one-way ANOVAs followed by Tukey post hoc tests. A priori, variables were plotted and checked for normality of distribution by Kolmogorov–Smirnov tests. Variables revealing non-normal distributions were log transformed and the appropriate log values were used in the analyses. Variables showing non-parametric distribution after log transformation were analysed via Chi-square, Kruskal–Wallis and Mann–Whitney U tests. Spearman rank correlations were used to confirm any relationships between regional volumes, hippocampal lesion densities and measures of disease severity (disease stage, duration and Clinical Dementia Rating score) in the post-mortem cohort.

Results
Demographics and cognitive tests
Demographics and general cognitive scores for the clinical cohort are shown in Table 1. Alzheimer’s disease and behavioural variant FTD participants in the clinical cohort did not differ in terms of age or sex distribution (all P-values > 0.1), whereas the patients with behavioural variant FTD in the post-mortem cohort died at a younger age (P < 0.01) and less advanced pathological disease stage (P < 0.001) than the patients with Alzheimer’s disease. Importantly, patients with behavioural variant FTD and Alzheimer’s disease did not differ in disease duration in either cohort (P > 0.1) and had a similar post-mortem delay in the post-mortem cohort. On cognitive testing, the clinical patient groups differed significantly on the general cognitive measure (Addenbrooke’s Cognitive Examination) from controls (P < 0.001) but not from each other (P > 0.1) and the post-mortem dementia groups also did not differ on their clinical dementia ratings. Similarly, on all memory measures, except the Rey Auditory Verbal Learning Test recognition score, the clinical behavioural variant FTD and Alzheimer’s disease groups scored significantly lower than controls (P < 0.05), but did not differ from each other (P > 0.1). A split by immunohistochemical inclusions in the post-mortem cohort revealed that cases with behavioural variant FTD with both types of TDP pathology, but particularly cases with TDP type A, were younger (P < 0.05), had significantly shorter disease durations (P < 0.05) and were less advanced in disease stage (P < 0.01) than those with both types of tau pathology. Age was used as a covariate in further analyses.

Voxel-based morphometry and diffusion tensor imaging analyses in the clinical cohort
Figures 2 and 3 show that both clinical patient groups (behavioural variant FTD, Alzheimer’s disease) had substantial atrophy of the bilateral hippocampus, fornix and cingulate cortex in comparison with controls, while only behavioural variant FTD had atrophy of the left anterior thalamus compared with controls. Analysis of mammillary body volumes revealed no significant results between patients and controls.

A direct contrast of the patient groups revealed no additional hippocampal atrophy for the patients with behavioural variant FTD in comparison with Alzheimer’s disease, but more fornix degeneration (Fig. 2), left anterior thalamus and bilateral anterior cingulate atrophy (Fig. 3) in behavioural variant FTD than in Alzheimer’s disease. The reverse contrast (Alzheimer’s disease > behavioural variant FTD) showed greater right posterior hippocampal atrophy in Alzheimer’s disease than in behavioural variant FTD (Fig. 2).

We median split the patients with behavioural variant FTD of the clinical cohort into high (bvFTD + ; n = 7) versus low (bvFTD –; n = 8) memory impairment based on their performance on the Rey Auditory Verbal Learning Test A6 score, which a previous study revealed as one of the most sensitive measures to detect memory deficits in behavioural variant FTD (Hornberger et al., 2010). We conducted an a priori power analysis to calculate the minimum sample size to detect group difference based on the Rey Auditory Verbal Learning Test A6 memory score. The power calculation, based on the previous findings in independent and much bigger patient and control cohorts (behavioural variant FTD: n = 50; Alzheimer’s disease: n = 64; controls: n = 64) (Hornberger et al., 2010), revealed an effect size of d = 2.56 for the Rey Auditory Verbal Learning Test A6. Based on this effect size, power calculations revealed that with an α-level of 0.05 the estimated minimal group size to detect significant differences across groups is n = 5, which we exceeded in this analysis. Importantly, the behavioural
variant FTD subgroups (bvFTD+, bvFTD−) did not differ on age or disease severity (P > 0.1). As shown in Fig. 4, bvFTD+ patients showed greater atrophy in left posterior hippocampus and left anterior thalamus compared with the bvFTD− group. The opposite contrasts revealed no significant results. More importantly, bvFTD+ patients also differed from patients with Alzheimer’s disease by showing additional atrophy of the left anterior thalamus and bilateral anterior cingulate cortex (Fig. 4). By contrast, patients with Alzheimer’s disease showed more atrophy than bvFTD+ patients in the bilateral posterior cingulate cortex.

### Post-mortem volumetric analyses and hippocampal lesion densities

Significant between-group differences in region of interest volumes were present (P < 0.001). These differences were of similar magnitude in both hemispheres (P > 0.1) and no clinical group by hemisphere interaction was present (P > 0.1). Compared with the controls, all regions were atrophic in the cases with behavioural variant FTD (average percentage of atrophy in comparison with controls; hippocampus: 45%; mammillary bodies: 33%; anterior thalamus: 39% and cingulate cortex: 40%) (Table 2). Compared with controls, cases with Alzheimer’s disease had significant atrophy of the hippocampus (average 33% atrophy) and cingulate cortex (average 20% atrophy) (Table 2). Overall, the degree of atrophy in the hippocampus and cingulate cortex was significantly worse in behavioural variant FTD compared with Alzheimer’s disease (Table 2), although the cases with corticobasal degeneration had similar hippocampal atrophy to Alzheimer’s disease (Table 2).

Within the behavioural variant FTD cohort, those with FTLD-TDP type A pathology had significantly greater mammillary body atrophy (P < 0.01) and those with FTLD-tau Pick’s disease had significantly greater anterior cingulate atrophy (P < 0.01) with no significant differences between behavioural variant FTD subgroups in the other regions (Table 2).

Significant numbers of neurons had tau-immunoreactive inclusions in the CA1 region of the hippocampus in Alzheimer’s disease and the behavioural variant FTD-tau subgroups (Supplementary Fig. 1A and B). In contrast, relatively few neuronal inclusions were observed in the CA1 hippocampal region in the behavioural variant FTD.
variant FTD-TDP subgroups (Supplementary Table 2). Higher densities of neuronal inclusions were observed in the dentate gyrus compared with the CA1 region of the hippocampus in all the types of behavioural variant FTD cases (Supplementary Fig. 1C–F). Cases with Pick’s disease had the highest densities of inclusions in the CA1 and dentate gyrus of the hippocampus, while cases with Alzheimer’s disease had the lowest densities of neuronal inclusions in the dentate gyrus (Supplementary Table 2).

Spearman rank correlations were used to determine any relationships between the severity of inclusion pathology and the degree of hippocampal atrophy using the whole cohort (including controls), Alzheimer’s disease and behavioural variant FTD cases only, or behavioural variant FTD cases only. In all instances, there were no correlations between the degree of hippocampal atrophy and the severity of CA1 inclusion pathology. There were correlations between increasing hippocampal atrophy and increasing densities of inclusions in dentate neurons using the entire cohort (Rho > 0.45, \( P < 0.0001 \)) or when only Alzheimer’s disease and behavioural variant FTD cases were assessed (Rho > 0.25, \( P < 0.03 \)), but not when behavioural variant FTD cases were assessed alone (\( P > 0.2 \)).

Spearman rank correlations were used to identify any relationships between post-mortem regional atrophy or the density of regional hippocampal inclusion pathology and disease stage, disease duration and last Clinical Dementia Rating score in the Alzheimer’s disease and behavioural variant FTD cases. Atrophy of the hippocampus and cingulate cortices were more pronounced and the severity of dentate inclusion pathology increased with increasing disease duration (\( P < 0.05 \)) and Clinical Dementia Rating score (\( P < 0.01 \), indicating ongoing degeneration and inclusion pathology over the disease course. To determine the strengths of the associations for disease duration and last Clinical Dementia Rating score, partial correlations were performed controlling for each of these variables. Increasing atrophy of the hippocampus and anterior cingulate cortex occurred with greater disease durations, controlling for Clinical Dementia Rating score (\( P < 0.05 \)), while increasing posterior cingulate cortex atrophy and higher maximal densities of dentate gyrus inclusions occurred with increasing Clinical Dementia Rating scores, controlling for disease duration (\( P < 0.01 \)). Atrophy of the hippocampus and posterior cingulate cortex was also related to pathological stage (\( P < 0.05 \)), confirming a relationship between hippocampal

**Figure 3** VBM analyses showing grey matter atrophy for the anterior thalamus and cingulate cortex. Clusters are overlaid on the MNI standard brain (\( t > 2.41 \)). Coloured voxels show regions that were significant in the analyses for \( P < 0.001 \) FDR corrected and a cluster threshold of 20 contiguous voxels. AD = Alzheimer’s disease; bvFTD = behavioural variant FTD.
pathology and atrophy and indicating a similar relationship with the posterior cingulate cortex.

**Discussion**

This study is the first to report degenerative changes affecting all relay nodes of the Papez circuit in behavioural variant FTD. Patients with behavioural variant FTD with high memory loss had more severe neural degeneration of the fornix, anterior thalamus and anterior cingulate cortex than patients with Alzheimer’s disease, while patients with Alzheimer’s disease had more significant atrophy of the posterior cingulate cortex. Hippocampal atrophy was similar for both the behavioural variant FTD and Alzheimer’s disease cohorts, as predicted from previous studies (Broe et al., 2003; van de Pol et al., 2006; Rabinovici et al., 2007); however, over time, atrophy of the hippocampus and posterior cingulate cortex become more severe in behavioural variant FTD compared with pathologically-confirmed cases of Alzheimer’s disease. This novel finding demonstrates that hippocampal and cingulate pathology begin early in behavioural variant FTD and progresses more rapidly than in Alzheimer’s disease. Hippocampal atrophy in behavioural variant FTD was driven by TDP pathology, consistent with previous findings showing that patients with behavioural variant FTD with TDP pathology have more significant atrophy of the posterior cingulate cortex (Piguet et al., 2011). Importantly, greater subcortical atrophy in behavioural variant FTD can result in severe memory disturbance at presentation (Graham et al., 2005). This raises the possibility that more severe episodic memory in combination with hippocampal atrophy may be a potential biomarker for FTLD-TDP in vivo (Josephs et al., 2011).

While degeneration of the fornix is a feature of Alzheimer’s disease (Copenhaver et al., 2006; Mielke et al., 2009), the status of the fornix has not been previously studied in behavioural variant FTD, despite knowledge of hippocampal involvement. A recent DTI study in healthy participants confirmed that white matter integrity of the fornix is closely associated with memory recollection (Rudebeck et al., 2009). Our findings show a greater degree of degeneration in this vital white matter tract in behavioural variant FTD than in Alzheimer’s disease, consistent with recent in vivo DTI studies showing substantial white matter degeneration in behavioural variant FTD (e.g. Zhang et al., 2009; Whitwell et al., 2010). However, the difference in damage to the fornix is somewhat puzzling considering that hippocampal atrophy was similar in both behavioural variant FTD and Alzheimer’s disease. Although fornix integrity can be reliably measured via DTI (Wakana et al., 2007; Hua et al., 2008; Mori et al., 2008), anatomical analyses of the composition of the fornix have recently shown that this fibre tract does not just contain efferent hippocampal fibres to the mammillary bodies and anterior thalamus, but also contains efferent anterior thalamic fibres back to the hippocampus (Aggleton et al., 2010, 2011). As we found atrophy of the anterior thalamus in both the clinical and post-mortem FTD cohorts but not in Alzheimer’s disease, these data suggest that cases with behavioural variant FTD have disconnection of both pathways travelling within the fornix, while Alzheimer’s disease without substantive anterior thalamic involvement have hippocampal efferent fibres primarily affected. Thalamic atrophy has been reported recently in FTD (Cardenas et al., 2007; Chow et al., 2008; Garibotto et al., 2011), particularly in cases that have subsequently been shown to have TDP pathology at post-mortem (Rohrer et al., 2010). Our data confirm this concept, but show that both FTLD-TDP and FTLD-tau post-mortem cases have thalamic and hippocampal atrophy, although those with TDP pathology have greater hippocampal loss. This finding suggests that the thalamus is affected early in both pathological forms of behavioural variant FTD, contributing to the greater damage to the
Table 2  Mean regional volumes (standard error) for the post-mortem control group, with covariate-adjusted percentages of regional control volume (standard error) found in Alzheimer’s disease, behavioural variant FTD and behavioural variant FTD subgroups

<table>
<thead>
<tr>
<th>Region</th>
<th>Controls (mm³)</th>
<th>Alzheimer’s disease (% control volume)</th>
<th>Behavioural variant FTD (% control volume)</th>
<th>Behavioural variant FTD-TDP (% control volume)</th>
<th>Behavioural variant FTD-tau (% control volume)</th>
<th>FTD-TDP A (% control volume)</th>
<th>FTD-TDP C (% control volume)</th>
<th>FTD-tau (% control volume)</th>
<th>FTD-tau CBD (% control volume)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hippocampus</td>
<td>4025 (110)</td>
<td>67 (3)a</td>
<td>55 (3)ab</td>
<td>50 (3)abc</td>
<td>65 (3)abc</td>
<td>49 (5)ab</td>
<td>52 (6)ab</td>
<td>53 (4)ab</td>
<td>67 (6)a</td>
</tr>
<tr>
<td>Mammillary bodies</td>
<td>75 (3)</td>
<td>100 (5)</td>
<td>67 (5)ab</td>
<td>60 (7)abc</td>
<td>82 (7)ab</td>
<td>46 (11)ab</td>
<td>63 (13)ab</td>
<td>63 (8)ab</td>
<td>86 (13)ab</td>
</tr>
<tr>
<td>Anterior thalamus</td>
<td>1802 (68)</td>
<td>88 (5)</td>
<td>61 (5)ab</td>
<td>57 (5)ab</td>
<td>62 (5)ab</td>
<td>69 (10)*</td>
<td>65 (12)*</td>
<td>50 (8)ab</td>
<td>66 (12)*</td>
</tr>
<tr>
<td>Cingulate</td>
<td>11000 (223)</td>
<td>80 (2)a</td>
<td>60 (2)ab</td>
<td>59 (3)ab</td>
<td>63 (2)ab</td>
<td>65 (5)ab</td>
<td>59 (6)ab</td>
<td>56 (4)ab</td>
<td>63 (6)ab</td>
</tr>
<tr>
<td>Anterior cingulate</td>
<td>6107 (152)</td>
<td>85 (3)ab</td>
<td>59 (3)ab</td>
<td>63 (3)abc</td>
<td>55 (5)ab</td>
<td>62 (7)ab</td>
<td>71 (8)ab</td>
<td>44 (5)ab</td>
<td>66 (8)ab</td>
</tr>
<tr>
<td>Posterior cingulate</td>
<td>4893 (118)</td>
<td>78 (3)ab</td>
<td>62 (3)ab</td>
<td>58 (4)ab</td>
<td>68 (4)ab</td>
<td>68 (6)a</td>
<td>56 (7)ab</td>
<td>63 (5)ab</td>
<td>62 (7)ab</td>
</tr>
</tbody>
</table>

*P < 0.001 disease groups different from controls.

†P < 0.001 behavioural variant FTD different from Alzheimer’s disease.

‡P < 0.01 behavioural variant FTD–TDP different from behavioural variant FTD-tau.

§P < 0.01 different from all other subgroups.

CBD = Corticobasal degeneration; PiD = Pick’s disease.

fornix, and that over time progressive degeneration to the hippocampus is more TDP-43 dependent in behavioural variant FTD.

Interestingly, in the clinical cohort we did not observe mammillary body atrophy in either disease group, or thalamic atrophy in the Alzheimer’s disease group. In addition, substantive mammillary body atrophy was not observed in pathologically-confirmed cases with Alzheimer’s disease (Table 2). This result was unexpected given the substantial fornix degeneration observed using DTI in both behavioural variant FTD and Alzheimer’s disease, and the atrophy of these structures shown in previous in vivo studies in Alzheimer’s disease (Callen et al., 2001; Copenhaver et al., 2006).

Methodological differences are likely to contribute to our findings, including the possibility that amnesic FTD cases have been included in some previous Alzheimer’s disease studies, particularly considering our post-mortem confirmed volumetric results. Previous studies of mammillary body and thalamic atrophy in Alzheimer’s disease (Callen et al., 2001; Copenhaver et al., 2006) were conducted using region of interest manual tracing on scans and did not employ the automated VBM imaging methods used in the present analysis. VBM uses spatial smoothing to make valid cross-subject and group comparisons, and the current data were smoothed with a Gaussian kernel of 3 mm, which reduces the spatial resolution substantially for smaller regions such as the mammillary bodies. This, in association with the timing of evaluations (thalamic atrophy has been linked to disease progression in Alzheimer’s disease) (Chetelat et al., 2005), may explain the differences in results obtained between studies. Importantly, the same method of region of interest manual tracing of brain slices was performed in our post-mortem cohort, showing substantial atrophy of the mammillary bodies and anterior thalamus only in behavioural variant FTD, with no substantive differences between pathological subtypes (TDP versus tau). In contrast, the patients with Alzheimer’s disease showed mammillary body volumes similar to age-matched controls with a minor reduction in the thalamus (~10%) that was not statistically significant (Table 2). No studies of mammillary body atrophy post-mortem in Alzheimer’s disease have been published since consensus was achieved for differentiating common dementia syndromes (McKhann et al., 2001; McKeith et al., 2005), and post-mortem studies of thalamic atrophy in Alzheimer’s disease have not been conducted to date. In earlier post-mortem studies, high variability in mammillary body measurements were found (Hooper and Vogel, 1976; Wilkinson and Davies, 1978), with both age and dementia accompanied by a loss of mammillary body volume without neuronal loss (Wilkinson and Davies, 1978) or neuronal atrophy (Ishunina et al., 2003). Only 60% of earlier Alzheimer’s disease cases reported had senile plaques or neurofibrillary tangles in the mammillary bodies (McDuff and Sumi, 1985; Grossi et al., 1989). These data and the data presented in this study suggest that in Alzheimer’s disease the mammillary bodies (and by analogy the anterior thalamus) have a variable loss of hippocampal afferents and variable neuronal pathology in the absence of significant neuronal loss.

Direct comparison of the dementia groups showed more fornix, anterior thalamus and anterior cingulate atrophy in behavioural variant FTD than in Alzheimer’s disease, while the degree of atrophy in the posterior cingulate cortex was not significantly different when the whole behavioural variant FTD group was included. The anterior thalamus is the major relay to the anterior cingulate cortex in the Papez circuit, and as discussed above, also sends fibres through the fornix to the hippocampus, linking degeneration in these three sites. Our in vivo findings of differential damage in these sites were further corroborated by our post-mortem findings, which again showed greater thalamic and cingulate atrophy in behavioural variant FTD. However, splitting the clinical behavioural variant FTD cohort into those with more or less memory impairment revealed that patients with Alzheimer’s disease had significantly greater atrophy of the posterior cingulate cortex than patients with behavioural variant FTD with memory impairment, while memory impaired patients with behavioural variant FTD had greater damage in the fornix, anterior thalamus and anterior cingulate gyrus (similar to differences observed in the entire cohort). Previous studies have shown a selective involvement of the posterior cingulate in Alzheimer’s disease compared with behavioural variant FTD (Killiany et al., 2000; Frisoni et al., 2002), consistent with these findings, while studies recently challenging
this concept (Barnes et al., 2007) may have studied a behavioural variant FTD cohort at later disease stages. Importantly, behavioural variant FTD with limited memory impairment had less hippocampal and anterior thalamic atrophy than those with significant memory impairment, suggesting that significant damage to these regions is most important for memory impairment in behavioural variant FTD. Overall, our in vivo and post-mortem results indicate that anterior cingulate atrophy is a strong predictor of behavioural variant FTD, and that greater hippocampal and thalamic atrophy occurs in cases with behavioural variant FTD with significant memory impairment.

Taken together, our findings demonstrate that a greater number of sub-regions in the Papez circuit are affected in clinical and pathological behavioural variant FTD compared with Alzheimer's disease. Nevertheless, there are several limitations to our approach. In particular, the clinical and pathological samples were from different patient cohorts and thus our findings need to be replicated in a clinical sample that has been followed-up until post-mortem. Similarly, our group sizes were small and therefore replication of our results in a bigger sample would further strengthen our findings. Perhaps not surprisingly, our clinical findings further corroborate the notion in the literature that patients with behavioural variant FTD can show episodic memory deficits of a similar severity to Alzheimer's disease, and we suggest that diagnostic exclusion of behavioural variant FTD on grounds of memory performance alone should be considered carefully as poor memory does not preclude a FTLD diagnosis. Our data show that damage to the Papez circuit and memory impairment are related, as predicted, and that it is the severity of damage to the anterior thalamus in behavioural variant FTD that differentiates memory impaired behavioural variant FTD patients from Alzheimer's disease and other patients with behavioural variant FTD. Importantly, future research should focus on identifying specific 'thalamic' memory deficits in these cohorts.

Acknowledgements

The authors would like to thank the participants and their families, especially those who donated their brain tissue after death. Data for the clinical study was obtained from FRONTIER clinic at Neuroscience Research Australia, which is funded by an Australian Research Council Centre of Excellence grant and National Health and Medical Research Council of Australia grants. Data for the post-mortem study was obtained from the Sydney Brain Bank, which is supported by Neuroscience Research Australia, the University of New South Wales and the National Health and Medical Research Council of Australia (NHMRC). The authors report no conflicts of interest or disclosures. We wish to thank the staff of FRONTIER and the Sydney Brain Bank for their assistance, Shelley Forrest for performing the inter-rater histological quantitation and Heidi Cartwright for assistance with the figure work.

Funding

This work was supported by the Australian Research Council [DP110104202 to M.H., FF0776229 to J.R.H.]; and the National Health and Medical Research Council of Australia [APP1022684 to O.P., 630434 to G.H.].

Supplementary material

Supplementary material is available at Brain online.

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