Beyond the temporal pole: limbic memory circuit in the semantic variant of primary progressive aphasia

Rachel H. Tan,1,2 Stephanie Wong,1 Jillian J. Kril,3 Olivier Piguet,1,2,4 Michael Hornberger,1,2,4 John R. Hodges1,2,4 and Glenda M. Halliday 1,2

Despite accruing evidence for relative preservation of episodic memory in the semantic variant of primary progressive aphasia (previously semantic dementia), the neural basis for this remains unclear, particularly in light of their well-established hippocampal involvement. We recently investigated the Papez network of memory structures across pathological subtypes of behavioural variant frontotemporal dementia and demonstrated severe degeneration of all relay nodes, with the anterior thalamus in particular emerging as crucial for intact episodic memory. The present study investigated the status of key components of Papez circuit (hippocampus, mammillary bodies, anterior thalamus, cingulate cortex) and anterior temporal cortex using volumetric and quantitative cell counting methods in pathologically-confirmed cases with semantic variant of primary progressive aphasia (n = 8; 61–83 years; three males), behavioural variant frontotemporal dementia with TDP pathology (n = 9; 53–82 years; six males) and healthy controls (n = 8, 50–86 years; four males). Behavioural variant frontotemporal dementia cases with TDP pathology were selected because of the association between the semantic variant of primary progressive aphasia and TDP pathology. Our findings revealed that the semantic variant of primary progressive aphasia and behavioural variant frontotemporal dementia show similar degrees of anterior thalamic atrophy. The mammillary bodies and hippocampal body and tail were preserved in the semantic variant of primary progressive aphasia but were significantly atrophic in behavioural variant frontotemporal dementia. Importantly, atrophy in the anterior thalamus and mild progressive atrophy in the body of the hippocampus emerged as the main memory circuit regions correlated with increasing dementia severity in the semantic variant of primary progressive aphasia. Quantitation of neuronal populations in the cingulate cortices confirmed the selective loss of anterior cingulate von Economo neurons in behavioural variant frontotemporal dementia. We also show that by end-stage these neurons selectively degenerate in the semantic variant of primary progressive aphasia with preservation of neurons in the posterior cingulate cortex. Overall, our findings demonstrate for the first time, severe atrophic in behavioural variant frontotemporal dementia, we suggest here that the neural preservation of crucial memory relays (hippocampal→mammillary bodies and posterior cingulate→hippocampus) likely reflects the conservation of specific episodic memory components observed in most patients with semantic variant of primary progressive aphasia.
Introduction

The semantic variant of primary progressive aphasia (PPA), also known as semantic dementia, is one of the three main clinical variants within the spectrum of frontotemporal dementia (FTD) syndromes (Gorno-Tempini et al., 2011). This progressive neurodegenerative disorder is characterized pathologically by asymmetric atrophy of the anterior temporal lobes, and clinically by anoma, loss of receptive vocabulary, and erosion of general semantic memory (Hodges and Patterson, 2007). Investigations of episodic memory in semantic variant PPA have yielded a complex picture (reviewed in Hornberger and Piguet, 2012). Studies of autobiographical memory have generally shown preservation of recent personal memories in contrast to more distant life events, although this has not been a universal finding (Graham and Hodges, 1997; Moss et al., 2003; Ivanou et al., 2006; McKinnon et al., 2006; Matuszewski et al., 2009; Maguire et al., 2010; Irish et al., 2011, 2012a). Anterograde recall of personally relevant memories can be strikingly normal in patients with advanced disease (Adlam et al., 2009), even in the face of impairment in traditional neuropsychological tasks, and patients remain well orientated with preservation of topographical memory (Pengas et al., 2012).

Semantic and episodic memory have traditionally been viewed as functionally distinct memory systems. The neural substrate of semantic memory is attributed to the antero-inferior temporal lobe, whereas for episodic memory the medial temporal lobe is considered most relevant (Chan et al., 2001; Davies et al., 2005; Rosen et al., 2005; Williams et al., 2005; Desgranges et al., 2007; Adlam et al., 2009; Mion et al., 2010), although it is increasingly recognized that relays within the Papez memory circuit rather than solely the medial temporal lobe account for deficits in episodic memory (Pleizier et al., 2012; de Souza et al., 2013). It should be noted that there are few quantitative studies assessing neural integrity histologically throughout memory circuits due to the scale of such a task, and in particular none in semantic variant PPA. The core Papez network of memory structures includes the hippocampus, mammillary bodies, anterior thalamus and cingulate cortices, and was recently investigated in the behavioural variant of FTD where the integrity of the anterior thalamus and fornix emerged as crucial for episodic memory performance in this syndrome (Hornberger et al., 2012). Embedded within the anterior cingulate cortex are neurons considered to be selectively targeted in behavioural variant FTD that can only be studied histologically (Seeley et al., 2006; Seeley, 2008; Kim et al., 2012; Santillo et al., 2013). These neurons, known as von Economo neurons, have only recently evolved in highly social species with large brains and are most numerous in humans. Their function remains speculative but they are thought to work like ‘air traffic controllers’ to allow rapid communication across networks (Seeley, 2008; Allman et al., 2011). These neurons have not been studied in semantic variant PPA. What is known about memory circuits in semantic variant PPA is that, in spite of intact anterograde episodic memory at onset, there is severe atrophy largely restricted to the anterior medial temporal lobe (Chan et al., 2001; Rosen et al., 2002; Du et al., 2007), and that sparing of episodic memory in semantic variant PPA is likely to reflect preservation of other components of the Papez circuit (Nestor et al., 2006).

Against the backdrop of a growing emphasis to study whole neural networks as opposed to specific brain regions, the present study set out to quantify the severity of neurodegeneration in the limbic memory (Papez) circuit in pathologically-confirmed semantic variant PPA to determine the integrity of this system in this FTD syndrome. We conducted post-mortem volumetric analysis of grey matter regions of interest in the Papez circuit (hippocampus, mammillary bodies, anterior thalamus, cingulate cortex) and anterior temporal cortex in pathologically-confirmed semantic variant PPA cases with TDP pathology and compared these to behavioural variant FTD cases with TDP pathology. Despite the strong association between semantic variant PPA and TDP pathology (Josephs et al., 2011), this is the first study to specifically assess and contrast regional brain volumes across a known neural circuit in semantic variant PPA and behavioural variant FTD cases with this type of pathology. Our reasoning was that regions within the Papez circuit that are relatively preserved in end-stage semantic variant PPA may have particular relevance to differences in episodic memory between these and other dementia syndromes. To confirm that neuronal integrity was significantly compromised, neuronal loss was determined where there could be uncertainty in the interpretation of the volumetric results (anterior and posterior cingulate compared with anterior temporal cortices). In this way any degeneration of small, select neuronal populations, or preservation of neuronal populations that may have changed morphology (shrinkage) or only lost incoming synaptic relays (neuronal preservation in a region with atrophy), could be identified with certainty in this study, information that is not available using other research methods.

Materials and methods

Case selection

Eight cases that fulfilled clinical criteria for semantic variant PPA (Neary et al., 1998; Hodges and Patterson, 2007; Gorno-Tempini et al., 2011) and frontotemporal lobar degeneration with TDP pathology (Cairns et al., 2007; Mackenzie et al., 2010), nine cases with a clinical diagnosis of behavioural variant FTD (Neary et al., 1998; Rascovsky et al., 2011) and frontotemporal lobar degeneration with TDP pathology (Cairns et al., 2007; Mackenzie et al., 2010), as well as eight controls without dementia or significant neuropathological abnormalities were selected from a neuropathological series of cases collected through ethically-approved regional brain donor programmes in Sydney, Australia and Cambridge, England. The neuropathological series from which the present semantic variant PPA cases were selected comprised only TDP type C cases. Apart from TDP pathology, behavioural variant FTD cases and

Keywords: frontotemporal dementia; primary progressive aphasia; semantic dementia; Papez circuit

Abbreviations: FTD = frontotemporal dementia; PPA = primary progressive aphasia; TDP = TAR DNA binding protein
controls were selected to match the semantic variant PPA cases as closely as possible for age and gender. The tissues were collected by the Sydney and Cambridge Brain Banks with consent from the families for tissue donation at death and institutional ethics approvals. Patients were diagnosed in life by experienced clinicians using standard clinical diagnostic criteria (Neary et al., 1998; McKhann et al., 2001; Gorno-Tempini et al., 2011; Rascovsky et al., 2011) following a medical interview, cognitive testing and an informant history. Standardized tests were used to longitudinally follow patients with their last assessments performed within 14 months of death. All Cambridge cases had been seen by the same experienced clinician (J.R.H.) who used the language assessment as described in Cognitive Assessment for Clinicians, later formalized as the Progressive Aphasia Language Scale (PALS). The semantic variant PPA cohort comprised eight cases with TDP type C pathology and the behavioural variant FTD comprised three with TDP type A, three with TDP type B and three with TDP type C pathology (Supplementary material) (Mackenzie et al., 2011). Predominant atrophy only in the temporal lobe was not observed clinically in behavioural variant FTD cases with neuroimaging (Supplementary Fig. 1) and by end-stage, severe atrophy was observed in both the frontal and temporal lobe of these patients. This research project was approved by the Human Research Ethics Committee of the University of New South Wales.

Case details are shown in Table 1. The post-mortem interval for all cases was <28 h with the exception of two cases where it was 44 h (range; 2–44 h, mean ± standard deviation: for control = 16.8 ± 12.2, for semantic variant PPA = 7.2 ± 3.4, and for behavioural variant FTD = 13.6 ± 14.6, \( F = 3.018, P = 0.059 \)). All dementia cases had Clinical Dementia Rating (Morrison, 1997) scores between 1 and 3 whereas controls had scores of <0.5. Some of the control subjects and cases with behavioural variant FTD included in this study have been reported in previous publications (Halliday et al., 2003; Kril et al., 2005; Davies et al., 2009; Hornberger et al., 2012; Tan et al., 2013b).

### Brain preparation and volumetric methods

The volumetric methods used in this study have been published in detail elsewhere (Halliday et al., 2003; Hornberger et al., 2012).

Briefly, following 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, anterior thalamus, cingulate and as a positive control, the anterior temporal cortex) were quantified in the left and right hemispheres of each brain. Gyral boundaries and anatomical structures most consistently associated with cytoarchitectonic boundaries used to identify regions have been previously published in detail (Halliday et al., 2003; Hornberger et al., 2012). In addition, assessment of hippocampal subregions was performed as recently described (Greene et al., 2012), that is, the head of the hippocampus was divided from the body of the hippocampus at the level of the uncal apex, while the tail was divided from the hippocampal body in the coronal slice where the fimbria of the fornix was first evident. For the hippocampus, anterior thalamus, cingulate and anterior temporal cortex, their volumes were determined by a point counting procedure with two raters used to identify regions of interest and determine volumes (Fig. 1). 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 and volumes calculated by multiplying the sum of the points falling on a given structure by the volume represented by each point (volume/point = 16 mm\(^2\) × mean slice thickness; average of 50 mm\(^3\)). The raters were initially trained to identify the same regions of interest in three brains and considered competent when <5% variation in volumes was obtained in 10 repeated measures (correlation between two independent raters was 0.978). For the mammillary bodies, the structure was traced on digital photographs of the brain slices and the cross-sectional area measured in all slices. In repeated

### Table 1 Demographic, clinical and post-mortem characteristics in semantic variant PPA, behavioural variant FTD and control groups (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Semantic variant PPA</th>
<th>Behavioural variant FTD</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (% male)</td>
<td>8 (50)</td>
<td>8 (38)</td>
<td>9 (67)</td>
</tr>
<tr>
<td>Mean age at death (y)</td>
<td>69 ± 12</td>
<td>70 ± 6</td>
<td>66 ± 9</td>
</tr>
<tr>
<td>Post-mortem delay (h)</td>
<td>16.8 ± 12.2</td>
<td>7.2 ± 3.4</td>
<td>13.6 ± 14.6</td>
</tr>
<tr>
<td>FTD stage (0-4)</td>
<td>N/A</td>
<td>2.4 ± 0.7</td>
<td>2.6 ± 0.5</td>
</tr>
<tr>
<td>Last CDR (0-3)</td>
<td>0</td>
<td>2.5 ± 0.9</td>
<td>2.1 ± 0.9</td>
</tr>
<tr>
<td>Disease duration (y)</td>
<td>8.3 ± 4.2</td>
<td>4.7 ± 4.3</td>
<td></td>
</tr>
<tr>
<td>Duration of behavioural, language and memory impairment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Language deficits: time from diagnosis to onset (y)</td>
<td>N/A</td>
<td>0 ± 0</td>
<td>0.13 ± 0.35</td>
</tr>
<tr>
<td>Behavioural deficits: time from diagnosis to onset (y)</td>
<td>N/A</td>
<td>1.6 ± 1.5</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Memory impairment: time from diagnosis to onset (y)</td>
<td>N/A</td>
<td>4.4 ± 1.9*</td>
<td>0.3 ± 0.7</td>
</tr>
<tr>
<td>Language deficits: onset to death (y)</td>
<td>N/A</td>
<td>8.3 ± 4.2*</td>
<td>3.0 ± 1.8</td>
</tr>
<tr>
<td>Behavioural deficits: onset to death (y)</td>
<td>N/A</td>
<td>7.0 ± 4.5</td>
<td>4.6 ± 4.4</td>
</tr>
<tr>
<td>Memory deficits: onset to death (y)</td>
<td>N/A</td>
<td>4.1 ± 1.3</td>
<td>4.7 ± 4.0</td>
</tr>
</tbody>
</table>

CDR = clinical dementia rating; N/A = not applicable. *P < 0.05 compared with behavioural variant FTD.
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 \( \times \) mean slice thickness).

**Quantitation of neuron numbers**

We recently published a detailed histological evaluation of Papez circuit in behavioural variant FTD cases (Hornberger et al., 2012). As such, regions with a similar extent of atrophy in semantic variant PPA were considered to have a similar degree of neuronal loss, and regions that differed between semantic variant PPA and behavioural variant FTD (anterior temporal and anterior cingulate cortex), or regions with atrophy but questionable neuronal loss (posterior cingulate cortex; Tan et al., 2013b), were quantified further using stereological principles. von Economo neurons were only quantified in the anterior cingulate cortex where they concentrate (Seeley, 2008; Allman et al., 2011). Given that we and others have shown that hippocampal and mammillary atrophy is directly related to neuron number (Harding et al., 2000; Kril et al., 2004), neuronal density was not assessed in these structures.

Standardized tissue samples of the anterior cingulate cortex (just posterior to the genu of the corpus callosum), posterior cingulate cortex (just anterior to the splenium of the corpus callosum), and anterior temporal cortex (anterior to the coronal slice containing the insula) were sampled and embedded in paraffin wax using routine procedures. Ten-micrometre serial sections were cut from each block and stained with Cresyl violet (0.5%) for quantitation of cortical neuronal populations, as previously described (Kril et al., 1997; Tan et al., 2013b). Briefly, two strips of cortex, 500-μm wide through the entire cortical thickness from the pial surface to white matter were sampled in each cortical section and neurons assessed, including von Economo neurons (Fig. 2) identified as bipolar elongated neurons with prominent dendrites emerging from apical and basal poles (Nimchinsky et al., 1995, 1999). All

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**Figure 1** Photographs of coronal brain slices showing the regions of interest (outlined) identified in the Papez memory network (Vogt et al., 2006; Aggleton et al., 2010; Shah et al., 2012) in which volumetric analysis was performed. The connections between the regions of interest are also shown (white arrows). The level at which these brain sections are taken is listed in the ‘Materials and methods’ section.
Figure 2 Photomicrographs of TDP pathology in sections from the anterior cingulate cortex in cases with behavioural variant FTD (bvFTD) and with semantic variant PPA (SD). Behavioural variant FTD cases with TDP subtypes A have upper layer neuronal intracytoplasmic inclusions (arrows in A) and short neurites (arrowheads in A), subtype B have neuronal inclusions (arrows in B) throughout the cortex, and subtype C have long neurites (white arrowheads in C). All cases with semantic variant PPA showed TDP subtype C pathology (white arrowheads in D). Haematoxylin and eosin stained section showing von Economo neurons (E: arrows) and pyramidal neurons (E: star) in layer V of the anterior cingulate cortex in an elderly control. High resolution photomicrograph of a Cresyl violet stained section showing a von Economo neuron (F: arrow) and pyramidal neuron (F: star) in a case with semantic variant PPA. Photomicrographs of cresyl violet stained sections showing the significant neuronal loss in the anterior temporal cortex in semantic variant PPA (H) compared with elderly controls (G) and behavioural variant FTD cases (I).
neurons were counted at ×200 magnification using a 10 × 10 eyepiece graticule (500 μm × 500 μm) with standard inclusion (lower and left) and exclusion (upper and right) borders in contiguous, non-overlapping fields. Von Economo neurons were counted after their morphological identification in layer V of sub-area 24b of the anterior cingulate cortex (Fig. 2). Our previous detailed analysis of serial cortical sections has shown this sampling scheme to be most efficient and effective at stereologically estimating cortical neuronal densities (Kril et al., 1997; Tan et al., 2013b). The neuronal density measures were standardized to numbers/mm³. Quantitation of neuronal populations were performed by two raters blind to case details with an inter-rater variance of 2.4% and intra-rater variability of 2.2%, and no significant differences between raters.

Statistical analysis

Data were analysed using SPSS19.0 (IBM Corp.). Demographic (age, sex, post-mortem delay) and clinical (disease duration, disease stage, last Clinical Dementia Rating score) variables were compared across groups via one-way ANOVA followed by Bonferroni post hoc tests. The severity of regional atrophy in Papez circuit identified post-mortem was assessed by hemisphere using multivariate ANOVA covarying for age followed by Bonferroni post hoc tests. Where no significant hemisphere by group differences were identified, group differences are reported combining both hemisphere measures. The severity of neuronal loss in atrophic regions was assessed using multivariate ANOVA covarying for age followed by Bonferroni post hoc tests. Spearman rank correlations were used to identify any associations between regional volumes, neuronal loss, lesion densities and measures of disease severity (disease duration and Clinical Dementia Rating score).

Results

Demographics

The semantic variant PPA and behavioural variant FTD cohorts did not differ from control cases in age, sex or post-mortem delay (all P-values >0.1). Multivariate analysis demonstrated an interaction between neuronal loss and age (P < 0.05), therefore age was included as a covariate in all analyses of neurodegeneration. There was no significant difference between patient groups in their average Clinical Dementia Rating scores or disease stage at death. Disease duration was significantly longer in cases with semantic variant PPA compared with those with behavioural variant FTD (P = 0.009).

Clinical data

A review of the clinical records showed that in patients with semantic variant PPA behavioural deficits (disinhibition, apathy, loss of empathy, stereotyped behaviour, diet, executive deficits) were present on average 1.6 years from onset of language-related symptoms. Episodic memory deficits were clinically absent at presentation, and typically developed 4.4 years from onset (Table 1). In the behavioural variant FTD group, language-related symptoms (word finding difficulties, naming, sentence word comprehension, surface dyslexia) were seen usually 0.1 years after the onset of behavioural changes. Memory impairment also occurred much sooner than in semantic variant PPA cases and was seen after 0.3 years on average (Table 1). No significant differences in the duration of behavioural deficits were present across patient groups at end stage. The duration of language deficits was longer in semantic variant PPA compared with behavioural variant FTD (P = 0.016).

Post-mortem volumetric analyses

Significant differences in the volumes of the regions of interest in the Papez circuit were identified between groups (P < 0.001) and these differences were similar across both hemispheres (P = 0.955) with no clinical group by hemisphere interaction present (P > 0.1 for all). In comparison to controls, cases with semantic variant PPA had marked atrophy in the anterior temporal cortex (average 68% atrophy) and head of the hippocampus (average 57% atrophy), as well as significant atrophy in the anterior thalamus (average 27% atrophy), anterior cingulate (average 21% atrophy) and posterior cingulate (average 30% atrophy) cortices (Table 2). Compared with controls, cases with behavioural variant FTD with TDP pathology had significant and similar atrophy across all regions analysed (average atrophy for hippocampus: 46%, mammillary bodies: 41%; anterior thalamus: 34%, anterior cingulate cortex: 36%, posterior cingulate cortex: 36%, and anterior temporal cortex: 46%) (Table 2). While the severity of post-mortem atrophy in all regions of the Papez circuit was greater in behavioural variant FTD compared to semantic variant PPA, the only region in which post-mortem atrophy was significantly worse in behavioural variant FTD was the anterior cingulate cortex (average 15% more atrophy). The only region in which post-mortem atrophy was significantly worse in semantic variant PPA was the anterior temporal cortex, where semantic variant PPA cases had on average 24% greater atrophy than behavioural variant FTD cases.

Neuronal loss

Significant between-group differences in regional neuronal densities were identified (P < 0.001). A significant loss in density of von Economo neurons in the anterior cingulate cortex was found in behavioural variant FTD [mean ± standard error (SE): 206 667 ± 59 703 neurons/mm³], behavioural variant FTD reduced to 15 ± 11% of mean control values, range of maximum von Economo neurons counted per ×200 field: 0–3; P < 0.005] and semantic variant PPA (reduced to 37 ± 6.5% of mean control values, range of maximum von Economo neurons counted per ×200 field: 1–2; P = 0.056) compared with controls (range of maximum von Economo neurons counted per ×200 field: 1–12) (Table 3). The density of von Economo neurons was not significantly different between behavioural variant FTD and semantic variant PPA (Table 3). Overall neuronal populations other than von Economo neurons were also not significantly different across groups in both the anterior cingulate cortex (mean ± SE: 9067 986 ± 779 997 neurons/mm³, semantic variant PPA 86 ± 17% of mean control values,
Table 2 Mean regional volumes (± SE) for the control group, with percentages of regional control volume (± SE) in semantic variant PPA and behavioural variant FTD groups

<table>
<thead>
<tr>
<th>Region</th>
<th>Control volume ± SE (mm³)</th>
<th>Semantic variant PPA % control mean ± SE</th>
<th>Behavioural variant FTD % control mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hippocampus</td>
<td>4199 ± 184</td>
<td>65 ± 4*</td>
<td>54 ± 4*</td>
</tr>
<tr>
<td>Head</td>
<td>2162.3 ± 135</td>
<td>43 ± 9*</td>
<td>54 ± 5*</td>
</tr>
<tr>
<td>Body</td>
<td>1330.16 ± 76</td>
<td>85 ± 5</td>
<td>50 ± 4*</td>
</tr>
<tr>
<td>Tail</td>
<td>706.18 ± 49</td>
<td>83 ± 7</td>
<td>57 ± 4*</td>
</tr>
<tr>
<td>Mammillary bodies</td>
<td>80 ± 3</td>
<td>81 ± 7</td>
<td>59 ± 6*</td>
</tr>
<tr>
<td>Anterior thalamus</td>
<td>1782 ± 88</td>
<td>73 ± 8*</td>
<td>66 ± 7*</td>
</tr>
<tr>
<td>Anterior cingulate</td>
<td>6027 ± 141</td>
<td>79 ± 4*</td>
<td>64 ± 4*</td>
</tr>
<tr>
<td>Posterior cingulate</td>
<td>4961 ± 148</td>
<td>70 ± 4*</td>
<td>64 ± 4*</td>
</tr>
<tr>
<td>Anterior temporal</td>
<td>10 772 ± 408</td>
<td>32 ± 4.5*</td>
<td>56 ± 4*</td>
</tr>
</tbody>
</table>

Patient data are expressed as a percentage of control mean for each sex. Values significantly different from controls are marked.

*P < 0.05 after correction for multiple comparisons disease groups different from controls.

Table 3 Mean neuron numbers (± SE) for the control group, with percentages of regional neuron numbers (± SE) in semantic variant PPA and behavioural variant FTD groups

<table>
<thead>
<tr>
<th>Region</th>
<th>Mean control neuron density (mm³) ± SE</th>
<th>Semantic variant PPA % control mean ± SE</th>
<th>Behavioural variant FTD % control mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior cingulate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All neurons</td>
<td>9 067 986 ± 779 597</td>
<td>86 ± 17</td>
<td>104 ± 27</td>
</tr>
<tr>
<td>Von Economo neurons</td>
<td>206 667 ± 59 703</td>
<td>37 ± 6.5***</td>
<td>15 ± 11**</td>
</tr>
<tr>
<td>Posterior cingulate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All neurons</td>
<td>10 002 288 ± 776 728</td>
<td>82 ± 30</td>
<td>102 ± 12</td>
</tr>
<tr>
<td>Anterior temporal</td>
<td>10 660 000 ± 1 541 057</td>
<td>28 ± 3.2**</td>
<td>84 ± 4.9</td>
</tr>
</tbody>
</table>

**P < 0.005 compared to controls.

***P = 0.056 compared to controls.

*P < 0.05 compared to behavioural variant FTD.

behavioural variant FTD 104 ± 27% (mean control values) and posterior cingulate cortex (mean ± SE: 10 002 288 ± 776 728 neurons/mm³), semantic variant PPA 82 ± 30% of mean control values, behavioural variant FTD 102 ± 12% of mean control values (Table 3). Neuronal data corrected for volume confirmed no significant loss in overall neuronal numbers in both the anterior cingulate cortex (mean ± SE: 55 716 628 ± 5 635 515 neurons, ANOVA P > 0.05) and posterior cingulate cortex (mean ± SE of 45 656 251 ± 4 935 339 neurons, ANOVA P > 0.05). In the anterior temporal cortex, severe neuronal degeneration was seen in the semantic variant PPA cohort (mean ± SE: 10 660 000 ± 1 541 057 neurons/mm³), semantic variant PPA reduced to 28 ± 3.2% of mean control values, P < 0.005) but not in the behavioural variant FTD cohort (84 ± 4.9% of mean control values, P > 0.05) (Table 3).

Correlations
Spearman rank correlations were used to identify any relationships between regional atrophy, neuronal density, Clinical Dementia Rating score and disease duration within each patient group. Separate analyses of the left and right brain regions in the semantic variant PPA group revealed that higher Clinical Dementia Rating scores were associated with more severe atrophy in the left anterior temporal cortex (P < 0.05) and left anterior thalamus (P < 0.05), and that atrophy in the left anterior temporal cortex (P = 0.001) and left hippocampal body (P = 0.008) was more pronounced with increasing disease duration. Analysing the average volumes for each region across hemispheres for the semantic variant PPA group confirmed that higher Clinical Dementia Rating scores were most related to more severe atrophy in the anterior thalamus (P = 0.002) and hippocampal body (P = 0.02), and that longer disease duration related to the more severe atrophy in the hippocampal body (P = 0.05). For the behavioural variant FTD group, higher Clinical Dementia Rating scores were associated with more severe atrophy in the hippocampal head (P = 0.013), hippocampal body (P < 0.005), anterior cingulate cortex (P = 0.009), posterior cingulate cortex (P = 0.014) and anterior temporal cortex (P < 0.001). Increasing disease duration was associated only with atrophy of the posterior cingulate cortex (P = 0.017). There were no associations between the severity of neuronal loss and degree of TDP pathology.
Discussion

The present study reports, for the first time, degenerative changes in all relay nodes of the limbic memory (Papez) circuit in semantic variant PPA patients coming to post-mortem, with the exception of the mammillary bodies and the body and tail of the hippocampus. Preservation of these crucial regions in the Papez circuit may have particular relevance in the context of the relative sparing of episodic memory in semantic variant PPA. In comparison to behavioural variant FTD cases with TDP pathology, semantic variant PPA had a similar extent of degeneration in the anterior thalamus and posterior cingulate cortex. Importantly, the anterior temporal lobe, which is the predilection site of pathology in semantic variant PPA (Nestor et al., 2006; Desgranges et al., 2007; Davies et al., 2009; Mion et al., 2010), had significantly greater atrophy at post-mortem in semantic variant PPA than behavioural variant FTD, as expected. Greater involvement of the left hemisphere is common in semantic variant PPA at initial presentation (Mummery et al., 2000; Chan et al., 2001; Galton et al., 2001; Rosen et al., 2002) and our data show worsening atrophy over the course of the disease. As the disease progresses, both hemispheres become affected to a similar degree, in keeping with the behavioural changes commonly observed in patients who initially present with right, rather than left, hemisphere involvement (Perry et al., 2001; Gorno-Tempini et al., 2004; Hodges and Patterson, 2007). The anterior cingulate cortex was the other region that differentiates the two groups, being more atrophic in behavioural variant FTD than in semantic variant PPA. Interestingly, quantitation of neuronal populations in this region revealed the selective loss of von Economo neurons in both semantic variant PPA and behavioural variant FTD, although this loss was more marked in the behavioural variant FTD cohort.

The anterior cingulate cortex is one of the earliest sites targeted in behavioural variant FTD (Seeley et al., 2006; Brambati et al., 2007) and cases with behavioural variant FTD with tau pathology have significantly greater degeneration in this region compared to those with TDP pathology (Santillo et al., 2013; Tan et al., 2013b). In the present study, the severity of atrophy in the anterior cingulate cortex was similar in both TDP-depositing behavioural variant FTD and semantic variant PPA cases. von Economo neurons are located almost exclusively in the anterior cingulate cortex and are large, bipolar, projection neurons (Nimchinsky et al., 1995; Fajardo et al., 2008) that are selectively targeted early on in behavioural variant FTD (Seeley et al., 2006; Kim et al., 2012). The loss of these neurons has been associated with the behavioural deficits characteristic of behavioural variant FTD (Seeley et al., 2006; Kim et al., 2012). Despite the substantial overlap in behavioural abnormalities found in patients with behavioural variant FTD and semantic variant PPA (Snowden et al., 2001; Hodges and Patterson, 2007), no study to date has assessed von Economo neurons in the anterior cingulate cortex in semantic variant PPA. We replicate here previous accounts of selective and significant von Economo neuron loss in behavioural variant FTD (Seeley et al., 2006; Kim et al., 2012) and show that this loss occurs even in TDP-depositing cases with semantic variant PPA with less overall degeneration in the anterior cingulate cortex, albeit to a more variable extent. In fact, we show that overall total neuronal numbers in the anterior cingulate cortex were spared in both TDP-depositing behavioural variant FTD and semantic variant PPA, although they are significantly reduced in tau-depositing cases of behavioural variant FTD (Tan et al., 2013b). This suggests that the anterior cingulate atrophy in these TDP-depositing cases is likely to be due to a loss of synaptic inputs to this region and/or cell shrinkage rather than substantial global neuronal loss. With respect to the Papez circuit, there is strong input to the anterior cingulate cortex from anterior thalamus (Jang and Yeo, 2013), with other strong inputs from the amygdala and orbitofrontal cortex as well as from the perirhinal region of the anterior temporal lobe (Lavenex et al., 2002), the region most affected in semantic variant PPA. Of course the selective but variable loss of von Economo neurons in semantic variant PPA requires further functional analysis, but is in line with the behavioural deficits recorded in these patients at end-stage. Future studies should investigate the relationship between such behavioural changes in semantic variant PPA and the anterior cingulate cortex.

Increasing evidence over the past decade has led to a critical reappraisal of episodic memory in FTD syndromes (Graham et al., 2005; Hornberger and Piguet, 2012). We have shown previously for behavioural variant FTD (Hornberger et al., 2012) and now for semantic variant PPA, atrophy of many, but critically not all, of the important memory nodes in the Papez circuit (Fig. 1). Unilateral anterior hippocampal atrophy occurs early in cases with semantic variant PPA (Galton et al., 2001) but despite progression more posteriorly and bilaterally within the hippocampus, anterograde episodic memory remains relatively intact (Nestor et al., 2006; Adlam et al., 2009). The relative sparing of memory in these patients is suggested to be due to the relative intactness of the posterior hippocampus in semantic variant PPA (Chan et al., 2001). In our study of pathologically-confirmed patients with semantic variant PPA, we demonstrate relative selective atrophy of the head of the hippocampus (57% volume reduction) whereas the body and tail were relatively preserved (Table 2). Worsening atrophy in the body of the hippocampus was found with increasing dementia severity and duration in semantic variant PPA, corroborating neuroimaging evidence showing that episodic memory is contingent upon the posterior two-thirds of the hippocampus (Moser and Moser, 1998; Greicius et al., 2003).

The major memory output from the hippocampus is to the mammillary bodies and anterior thalamus, with the mammillary bodies also relaying hippocampal information to the anterior thalamus (Fig. 1). With the exception of a small number of neurons that project to both the mammillary bodies and anterior thalamus, these two pathways within the Papez circuit are distinct (Aggleton et al., 2010) and pivotal to human episodic memory (Aggleton et al., 2005). Shrinkage of the mammillary bodies has been observed in response to fornix damage (Loftus et al., 2000). We demonstrate here that the mammillary bodies are preserved even in end-stage semantic variant PPA, implying that the afferents from the hippocampus to this region are intact. This suggests that the atrophy observed in the head of the hippocampus in semantic variant PPA is likely to be because of degeneration of alternate structures rather than the hippocampal-mammillary
connections in Papez circuit. Primate studies show dense connections between the hippocampal head and the amygdala and anterior temporal cortices, whereas connections to the mammillary bodies originate more from the more posterior regions of the hippocampus (Moser and Moser, 1998; Greicius et al., 2003; Fanselow and Dong, 2010; Shamy et al., 2010; Fudge et al., 2012). In patients with behavioural variant FTD with impaired episodic memory, early atrophy of the fornix and anterior thalamus is found with subsequent shrinkage of the mammillary bodies (Hornberger et al., 2012), consistent with neuropsychological investigations showing a strong positive correlation between mammillary body volume and patients' performance on tasks of episodic memory recall (Tsivilis et al., 2008; Vann et al., 2008; Rudebeck et al., 2009). The lack of mammillary body atrophy in semantic variant PPA may explain the previous conflicting episodic memory findings, particularly with regards to the preservation of personally relevant episodic memory in many patients with semantic variant PPA (Adlam et al., 2009) despite severe atrophy in the hippocampus (Nestor et al., 2006). Prospective studies are needed to elucidate the interplay between the hippocampus and mammillary bodies to understand the generation of amnestic symptoms in neurodegenerative patients.

Strikingly, atrophy of the left anterior thalamus and left temporal lobe most related to memory deficits, with increasing atrophy of the anterior thalamus and the emergence of mild atrophy in the body of the hippocampus strongly correlating with increasing dementia severity in semantic variant PPA. Although the Clinical Dementia Rating score used for this analysis is not specifically designed for patients with frontotemporal dementia, it is an assessment weighted for episodic memory deficits (Morris, 1997). The anterior thalamus appears to be unaffected in early stage semantic variant PPA (Galton et al., 2001; Diehl-Schmid et al., 2006; Rohrer et al., 2010), consistent with an absence of early memory deficits. Thalamic atrophy has been reported 1 year from disease onset (Brambati et al., 2009) corroborating the thalamic changes we have observed at post-mortem. As discussed above, early atrophy of the anterior thalamus is associated with impaired episodic memory in patients with behavioural variant FTD, but in such cases increasing mammillary body atrophy also occurred (Hornberger et al., 2012). In contrast, in semantic variant PPA atrophy of the anterior thalamus and mild, progressive atrophy of the hippocampal body occurred independently of mammillary body atrophy, suggesting that memory deficits are likely to be more dependent on the direct pathways between these structures (Fig. 1). Of course, the anterior temporal cortex also has major projections to the midline anterior thalamic nuclei as well as to hippocampus and anterior cingulate cortex (Kealy and Commins, 2011), and the substantial loss of afferents from the anterior temporal cortex may explain the degree of atrophy in these closely associated regions.

The posterior cingulate cortex, which contains the retrosplenial cortex, constitutes the remaining major Papez region that directly influences the hippocampal memory network (Fig. 1). Although we have previously shown atrophy without neuronal loss in the posterior cingulate cortex in pathologically-confirmed behavioural variant FTD, atrophy does not occur in either the posterior cingulate or retrosplenial cortices early in the disease (Hornberger et al., 2012; Tan et al., 2013a). Consistent with this, the present study demonstrates increasing atrophy in the posterior cingulate cortex with disease duration in TDP-depositing patients with behavioural variant FTD, with the severity of degeneration at end-stage similar to that seen in the anterior cingulate cortex. Despite such atrophy, overall neuronal numbers were spared in this region in cases with behavioural variant FTD, suggesting that such atrophy reflects synaptic loss and/or cell shrinkage rather than frank neuronal degeneration. In patients with semantic variant PPA, a similar extent of posterior cingulate atrophy was observed at post-mortem, and this was also not accompanied by neuronal loss. A critical in vivo neuroimaging study that combined quantitative structural and metabolic (fluorodeoxyglucose-PET) neuroimaging showed normal volumes and activity of the posterior cingulate cortex in early semantic variant PPA (Nestor et al., 2006) suggesting that any involvement of the posterior cingulate cortex occurs relatively late in the course of the disease. The posterior cingulate cortex is one of the most consistently activated regions in functional imaging studies of autobiographical memory (Irish et al., 2012b) and semantic processing where it is thought to interface between the semantic network and episodic memory system (Binder et al., 2009; Binder and Desai, 2011). Although the posterior cingulate cortex does not receive direct projections from the anterior temporal cortex, it has strong connections from the anterior thalamus (Parvizi et al., 2006), a region previously identified as important for memory deficits in behavioural variant FTD (Hornberger et al., 2012) and that we have identified here as related to increasing dementia severity in cases with semantic variant PPA. We suggest that the loss of anterior thalamic afferents to the posterior cingulate cortex underlies the posterior cingulate atrophy observed, contributing to increasing memory and semantic retrieval deficits in these frontotemporal dementia syndromes.

By examining the integrity of the episodic memory network in semantic variant PPA, this study adds to our knowledge regarding frontotemporal dementia syndromes. Overall, our findings in semantic variant PPA demonstrate for the first time severe atrophy, although not always with neuronal loss, across all relay nodes of the Papez circuit with the exception of the mammillary bodies and hippocampal body and tail. Despite the longer disease course in semantic variant PPA compared with behavioural variant FTD, we found neural preservation in a number of crucial memory relays (hippocampal→mammillary bodies and posterior cingulate→hippocampus) that is likely to reflect the conservation of specific elements of episodic memory observed in most patients with semantic variant PPA (Adlam et al., 2009; Irish et al., 2011). Future studies should explore the integrity of memory circuits in vivo in relation to episodic memory performance.

Despite these promising findings, as for most quantitative pathological studies, group sizes remain relatively small and future replication of our results in a bigger sample will further strengthen our findings. Also, the nature of post-mortem collections of cohorts over time often means an absence of consistent measures of specific clinical features and particularly the same neuropsychological measures for episodic memory over the same timeframe. Assessment of cases with fine-grained clinical measures will be particularly important for better understanding the role of the small population of anterior cingulate-located von Economo
neurons in the symptomatology of the frontotemporal dementia syndromes. Importantly, our study has identified an association between anterior thalamic involvement and progression of disease severity in semantic variant PPA, similar to that previously shown in behavioural variant FTD (Hornberger et al., 2012). We suggest that this brain region may be particularly significant for dementia propagation and progression. As specific severity staging tools for FTD syndromes become available (Mioshi et al., 2010), future studies should examine this region in relation to the onset and progression of clinical deficits in these syndromes. In conclusion, our data show significant degeneration in the Papez circuit in patients with pathologically-confirmed semantic variant PPA and highlights the anterior thalamus in relation to disease severity in this FTD syndrome.

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Supplementary material

Supplementary material is available at Brain online.

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