Selective Frontoinsular von Economo Neuron and Fork Cell Loss in Early Behavioral Variant Frontotemporal Dementia

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Behavioral variant frontotemporal dementia (bvFTD) erodes complex social-emotional functions as the anterior cingulate cortex (ACC) and frontoinsula (FI) degenerate, but the early vulnerable neuron within these regions has remained uncertain. Previously, we demonstrated selective loss of ACC von Economo neurons (VENs) in bvFTD. Unlike ACC, FI contains a second conspicuous layer 5 neuronal morphotype, the fork cell, which has not been previously examined. Here, we investigated the selectivity, disease-specificity, laterality, timing, and symptom relevance of frontoinsular VEN and fork cell loss in bvFTD. Blinded, unbiased, systematic sampling was used to quantify bilateral FI VENs, fork cells, and neighboring neurons in 7 neurologically unaffected controls (NC), 5 patients with Alzheimer’s disease (AD), and 9 patients with bvFTD, including 3 who died of comorbid motor neuron disease during very mild bvFTD. bvFTD showed selective FI VEN and fork cell loss compared with NC and AD, whereas in AD no significant VEN or fork cell loss was detected. Although VEN and fork cell losses in bvFTD were often asymmetric, no group-level hemispheric laterality effects were identified. Right-sided VEN and fork cell losses, however, correlated with each other and with anatomical, functional, and behavioral severity. This work identifies region-specific neuronal targets in early bvFTD.

Keywords: Alzheimer’s disease, behavioral variant frontotemporal dementia, fork cell, frontoinsula, von Economo neuron

Introduction

Frontotemporal dementia (FTD) describes a prevalent group of early age-of-onset dementia syndromes associated with underlying frontotemporal lobar degeneration (FTLD) pathology (Neary et al. 1998). The behavioral variant FTD (bvFTD) is the most common clinical subtype and begins with subtle loss of recently evolved, late-developing, right-lateralized social-emotional functions, such as empathy, self-conscious emotions, and emotional aspects of morality, with relative sparing of frontal cognitive functions (Lough et al. 2006; Rankin et al. 2006; Sturm et al. 2006; Mendez and Shapira 2009). In parallel, patients show prominent anterior cingulate cortex (ACC) and frontoinsula (FI) degeneration, often worse in the nondominant hemisphere (Rosen et al. 2002; Schroeter et al. 2008 Boccardi et al. 2005). Comorbid motor neuron disease (MND) emerges in 20–30% of patients and truncates an already rapid and fatal disease course (Lomen-Hoerth et al. 2002; Roberson et al. 2005).

Neuropathological and imaging studies of early bvFTD suggest that the disease begins in ACC and FI before spreading throughout a circumscribed network of anterior brain regions (Broe et al. 2003; Seeley et al. 2008). It remains unresolved, however, whether bvFTD features early differential vulnerability of a network-specific neuronal subtype, as seen in other neurodegenerative diseases (Hyman et al. 1984; Graveland et al. 1985). For numerous reasons, von Economo neurons (VENs) provide a compelling vulnerable neuron candidate. VENs are large, bipolar, often nonphosphorylated neurofilament protein-rich layer 5b projection neurons (Nimchinsky et al. 1995) first noted in passing by Betz (1874, 1881) and Ramón y Cajal (1900, 1904). These neurons were later described in detail and localized to ACC and FI by von Economo (von Economo and Koskinas 1925; von Economo 1926), who predicted that these cells support phylogenetically new functions in humans, perhaps by creating internal representations of the autonomic nervous system. Subsequent comparative studies showed that VENs exist only among great apes and humans within the primate lineage (Rose 1928; Nimchinsky et al. 1999; Allman et al. 2010) but can also be found within the ACC, FI, and frontal pole of cetaceans (Hof and Van der Gucht 2007; Butti et al. 2009) and elephants (Hakeem et al. 2009). VENs prove 30% more abundant in the right hemisphere when absolute numbers are estimated, suggesting a potential role for these cells in social-emotional network function (Allman et al. 2005, 2010).

The human FI (Fig. 1) is further distinguished by a second large layer 5 neuron, the fork cell, which features a VEN-related but distinct morphology, having a single large basal dendrite but 2 large, divergent apical dendrites (Fig. 2). These neurons were also depicted by Ramón y Cajal (1900) and von Economo and Koskinas (1925) but received more focused attention from Ngowyang (1932), who noted the particular abundance of these neurons in FI and referred to them as “Gabelzellen” (fork cells). Later, VEN and fork cell–like morphotypes were noted to coexist in Ammon’s horn (De Crinis 1933; Ngowyang 1936). Whether VENs and fork cells share a similar phylogeny remains uncertain; Ngowyang observed scattered fork cells in the anterior insula of the chimpanzee and orangutan, far fewer in monkeys, and none throughout the cat brain (Ngowyang 1932, 1936). Details regarding the early history of VEN and fork cell investigations are provided in an accompanying paper (Seeley et al. 2011).

Early focal ACC–FI degeneration in bvFTD led us to hypothesize that VENs might represent an early neuronal target in this disorder. Modest initial support for this hypothesis came from a small study of left ACC (Seeley et al. 2008). For numerous reasons, von Economo neurons (VENs) provide a compelling vulnerable neuron candidate. VENs are large, bipolar, often nonphosphorylated neurofilament protein-rich layer 5b projection neurons (Nimchinsky et al. 1995) first noted in passing by Betz (1874, 1881) and Ramón y Cajal (1900, 1904). These neurons were later described in detail and localized to ACC and FI by von Economo (von Economo and Koskinas 1925; von Economo 1926), who predicted that these cells support phylogenetically new functions in humans, perhaps by creating internal representations of the autonomic nervous system. Subsequent comparative studies showed that VENs exist only among great apes and humans within the primate lineage (Rose 1928; Nimchinsky et al. 1999; Allman et al. 2010) but can also be found within the ACC, FI, and frontal pole of cetaceans (Hof and Van der Gucht 2007; Butti et al. 2009) and elephants (Hakeem et al. 2009). VENs prove 30% more abundant in the right hemisphere when absolute numbers are estimated, suggesting a potential role for these cells in social-emotional network function (Allman et al. 2005, 2010).

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VEN and Fork Cell Loss in bvFTD

Materials and Methods

Subjects, Specimens, and Neuropathological Assessment
Archival brain tissues were obtained from the Institute for Brain Aging and Dementia Tissue Resource at the University of California, Irvine (UCI) and the Department of Pathology at the University of California, San Francisco (UCSF). In addition, we studied a consecutive series of 7 patients with bvFTD and 5 with AD, autopsied within the UCSF Alzheimer’s Disease Research Center (ADRC) Neuropathology Core, for whom bilateral FI was available. Subject demographic and neuropathological data are provided in Table 1 and Supplementary Table S1. Neurologically unaffected control subjects, though not assessed cognitively before death, had no known neurological or psychiatric illness or major structural pathology at autopsy. Lack of antemortem cognitive data for controls was tolerated due to the need for minimally dissected tissue from bilateral FI, infrequently available through ADRC brain banks due to diagnostic sampling and the practice of freezing one side whole hemisphere for research studies. Patients with bvFTD and AD had undergone a clinical, functional, neuropsychological, and neuroimaging assessment at UCI or UCSF to facilitate clinical diagnoses, which were rendered according to published research criteria for bvFTD (Neary et al. 1998) and probable AD (McKhann et al. 1984). Pathological diagnoses among the bvFTD subjects (Supplementary Table S1) followed current FTLD consensus criteria (Mackenzie et al. 2010), and transactive response DNA-binding protein of 43 kDa (TDP-43) subtypes were designated according to the Sampathu et al scheme (Sampathu et al. 2006). Patients with AD met National Institute of Aging-Reagan pathological criteria for high likelihood AD (1997). Two bvFTD and 4 AD subjects were included in a previous study of left ACC VEN loss (Seeley et al. 2006).

Brains were fixed whole by immersion in 10% neutral buffered formalin or cut fresh into 1-cm thick coronal slabs and fixed in 4% paraformaldehyde, depending on the site and date of procurement. Initial fixation lasted no less than 1 week for brains fixed whole (room temperature), whereas brains were fixed in slabs for 48–72 h (at 4 °C). After fixation, standard dementia diagnostic procedures were performed as previously described (Seeley et al. 2006; Tartaglia et al. 2010).

Subjects or their surrogate decision makers provided informed consent to undergo autopsy according to the Declaration of Helsinki, and all study procedures were approved by the institutional review boards at the participating sites.

Disease Stage and Behavioral Symptom Assessment
For each patient with bvFTD, anatomical disease stage was assessed with a validated FTD rating scale (stages 0–4: 0 = no detectable atrophy and 4 = most severe atrophy) (Broe et al. 2003), following described procedures (Seeley et al. 2006). Ratings indicated that our subjects represented a range of bvFTD disease stages (Supplementary Table S1). Because this scale was developed for bvFTD, patients with AD, who show a different anatomical pattern, were not staged according to the Broe et al. (2003) scheme. All patients with AD showed advanced
neurofibrillary changes consistent with Braak stage VI (Braak H and Braak E 1991). Symptom duration was estimated from clinical records for all patients. Clinical severity was assessed using the Clinical Dementia Rating (CDR) scale total and sum of boxes scores (CDR-SB), and behavioral symptom severity was assessed with the Neuropsychiatric Inventory (NPI). Although designed as an AD functional assessment scale, the CDR has been shown to separate patients with bvFTD (or bvFTD-MND) into anatomically distinct subgroups (Seeley et al. 2008), supporting its use in this context. For NPI analyses, we extracted the frequency x severity scores for the 4 subscales that best define the bvFTD syndrome: apathy, disinhibition, aberrant motor, and eating (Liu et al. 2004) and the NPI Total score. Global cognition was assessed with the Mini-Mental State Examination (MMSE) because this was the summary measure most often available for our subjects. Inevitable challenges arise when correlating neuropathological and behavioral variables, the latter acquired at variable and often long intervals before death. We took 2 steps to address these issues. First, rather than using the most recent NPI before death, we used each subject’s highest score on record for each NPI subscale and the total. This approach proves important for bvFTD, in which progressive apathy and immobility often attenuate the florid behavioral phenotype in the months or even years before death. Taking the highest NPI scores allowed us to capture the peak behavioral syndrome in patients who later became akinetic and mute. CDR scores obtained proximal to death were accepted because this measure continues to increase with disease progression. Second, we controlled for the interval between CDR or NPI and death in all relevant analyses.

Tissue Processing and Region of Interest Identification
The human FL lies between the orbitofrontal cortex and the anterior insula. We dissected 4- to 10-mm thick FL blocks from coronal slabs cut at the level of the temporal pole. Anatomical dissection landmarks and sampling procedures are detailed in Supplementary Methods. Tissue blocks were cryoprotected in graded sucrose solutions, frozen, cut on a sliding microtome, Nissl-stained with cresyl violet, and mounted on glass slides coded to obscure hemisphere and diagnosis from raters. FL layer 5 regions of interest (ROIs) were traced using Stereoinvestigator software (MBF Bioscience) by one of 2 examiners (E.J.K. or M.S.) at a microscope–computer interface attached to a motorized stage and reviewed by an expert (W.W.S.), following previous methods (Seeley et al. 2006). We chose layer 5 as our ROI for neuron counting because VENs and fork cells are located primarily in layer 5 (Nimchinsky et al. 1995). Although VENs can at times be found in layer 6, we excluded this layer to avoid smaller spindle-shaped cells or “fusiform” neurons found there more commonly (Nimchinsky et al. 1999). Fork cells often occupy a more superficial position in layer 5, spanning layers 5a and 5b (Fig. 1C).

Neuron Quantification
Neuron counting was performed with systematic unbiased procedures as previously described (Seeley et al. 2006) and detailed in Supplementary Methods. Counting runs employed ×600 magnification, a 150 × 120 μm counting frame, and an 18 μm dissector height with 2 μm guard zones at top and bottom. Neuron classification was based on morphology. Inclusion rules for VENs and layer 5 neighboring neurons (L5NNs) followed previous guidelines (Seeley et al. 2006). Fork cells were identified by their striking and unique morphology (Fig. 2), which features a large dominant basal dendrite and 2 large apical dendrites that course away from the soma at angles that diverge from the perpendicular axis, forming a fork- or Y-shaped neuron. Rare FL layer 5 neurons feature a morphology similar to fork cells, but with 3 apical dendrites (forming a trident-shaped neuron), but these neurons were not included in the study. Counting parameters for each subject are detailed in Supplementary Table S2.

A single blindered rater (E.J.K.) performed neuron counting after training to predefined competency criteria (Seeley et al. 2006). Blocks were counted in random order, and intrarater reliability was assessed every N minus 20th section, where N equals the total number of sections counted to that point of the experiment, yielding a total of 13 sections recounted for reliability analysis. Intraass correlation coefficients were 0.995 (95% confidence interval [CI], 0.985-0.999) for VEN counts, 0.985 (95% CI, 0.951-0.995) for fork cell counts, and 0.984 (95% CI, 0.949-0.995) for L5NN counts.

Data Reduction and Statistical Analyses
The major outcome measures for the study were VENs/Total neurons, Fork cells/Total neurons, and VENs + Fork cells/Total neurons from each hemisphere. Total neurons were defined as the sum of VENs, fork cells, and L5NNs. Analytical approaches are detailed in the Supplementary Methods. Briefly, repeated measures analysis of variance was used to assess group and hemisphere effects on the major outcome measures, as well as interactions between group and hemisphere. Pearson or Spearman correlations were employed, as appropriate, to assess relationships between pathological and clinical variables, correcting for relevant confounders and multiple comparisons (see Supplementary Methods).

Results
bvFTD Is Associated with Selective VEN and Fork Cell Loss in Bilateral FL
Our first goal was to determine whether bvFTD is associated with selective FL VEN and fork cell losses. Following previous

Figure 2. VEN and fork cell morphology. (A, B) Low magnification views of the thin belly of the FI from the median control subject (Fig. 1) reveal layer 5 VENs (pink arrowheads) and fork cells (blue arrowheads) in clusters. (C, D) Fork cells are identified by a characteristic pair of large apical dendrites that typically form a 35-70 degree angle relative the cell’s vertical axis. In (D), 2 fork cells are seen in close proximity. (E) Canonical fork cell and VEN exemplars. Cresyl violet. Scale bars = 100 mM (A), 50 μm (B), 20 μm (C, D), and 15 μm (E).
approaches (Seeley et al. 2006), we applied unbiased systematic neuron counting procedures to estimate the local densities of VENs, fork cells, and total layer 5 neurons in right and left FI (Fig. 3A–C). We found a significant effect of diagnosis on VEN (F 2,18 = 11.667, P = 0.001) and fork cell (F 2,18 = 10.038, P = 0.001) densities, with bvFTD showing 53% VEN and 68% fork cell reductions compared with controls. Groups showed no significant differences in total layer 5 neuron density (F 2,18 = 1.957, P = 0.170), with bvFTD showing a slight absolute increase. This observation most likely reflects FI volume loss out of proportion to neuronal loss, a frequent neurodegenerative disease–related phenomenon, which inflates apparent local neuron density and makes it critical to evaluate cell-specific losses after accounting for total neuron density.

Table 1

<table>
<thead>
<tr>
<th>Subject characteristics</th>
<th>Controls</th>
<th>bvFTD</th>
<th>AD</th>
<th>P value*</th>
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<tr>
<td>Gender (M:F)</td>
<td>4:3</td>
<td>5:4</td>
<td>2:3</td>
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<td>Age at death (years)</td>
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<td>58.7 (7.3)</td>
<td>64.2 (6.8)</td>
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<tr>
<td>Education (years)</td>
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<tr>
<td>Symptom duration (years)</td>
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<td>5.8 (3.8)</td>
<td>9.4 (1.3)</td>
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<tr>
<td>Last CDR total</td>
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<td>2.4 (0.9)</td>
<td>1.5 (0.6)</td>
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<tr>
<td>Last CDR-SB</td>
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<td>11.0 (5.2)</td>
<td>9.0 (4.2)</td>
<td>0.53</td>
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<tr>
<td>Last MMSE</td>
<td>n/a</td>
<td>15.8 (11.9)</td>
<td>13.0 (10.2)</td>
<td>0.68</td>
</tr>
<tr>
<td>PMI (hr)</td>
<td>22.1 (18.9)</td>
<td>11.6 (8.5)</td>
<td>16.7 (10.8)</td>
<td>0.29</td>
</tr>
<tr>
<td>Neuropathological diagnoses</td>
<td>7 NSPA</td>
<td>7 FTLD-TDP b, 1 Pick's, 1 FTLD-FUS</td>
<td>4 AD, 1 AD/DLB c</td>
<td></td>
</tr>
</tbody>
</table>

Values listed as mean (standard deviation). DLB, Dementia with Lewy bodies; FTLD-TDP, FTLD with fused in sarcoma immunoreactive inclusions; FTLD-FUS, FTLD with ubiquitin/TDP-43-positive, tau-negative inclusions; n/a, not available; NSPA, No specific pathological abnormality; PMI, Postmortem interval.

*P values are those generated by the F or chi-square tests (as appropriate) for group differences between each variable.

bOf the patients with FTLD-TDP, 5 had pathological evidence of MND and Type 2 (4) or type unclassifiable (1) histopathology, 1 showed Type 2 (without MND), and one showed Type 1 (see Supplementary Table S1 for additional details).

cDLB was diffuse neocortical type.

Figure 3. Selective VEN and fork cell loss in bvFTD. The densities of VENs (A) and fork cells (B) showed statistically significant reductions in bvFTD, whereas there were no significant differences in layer 5 total neuron densities among the 3 groups (C). VENs/Total neurons (D), Fork cells/Total neurons (E), and VENs + Fork cells/Total neurons (F) showed statistically significant reductions in bvFTD compared with NC and AD, whereas no significant differences were detected between AD and NC. Error bars represent standard error of the mean. *P < 0.05, **P < 0.005. (G) Single subject data for VENs + Fork cells/Total neurons illustrate the separation of bvFTD from the other groups. Patients with bvFTD-MND (dashed lines) showed milder but definite VEN and fork cell loss compared with controls. AD; bvFTD; FC, fork cell; MND; NC, neurologically unaffected controls.
Therefore, we corrected for total layer 5 neuron number and found a significant effect of diagnosis on VENs/Total neurons (Fig. 3D) \( F_{2,18} = 13.82, P = 0.0002 \), with bvFTD showing significant reductions compared with controls (56%, \( P = 0.003 \)) and AD (36%, \( P = 0.020 \)). No significant difference was detected between AD and controls (\( P = 0.072 \)). Fork cells/Total neurons also showed a significant diagnosis effect (Fig. 3E; \( F_{2,18} = 7.95, P = 0.0045 \)), with reductions in bvFTD compared with controls (71%, \( P = 0.016 \)) and AD (69%, \( P = 0.014 \)). Fork cells/Total neurons did not differ between AD and controls (\( P = 0.099 \)).

Finally, a combined index of VENs + Fork cells/Total neurons revealed a significant main effect of diagnosis (Fig. 3F; \( F_{2,18} = 15.28, P = 0.0001 \)), with bvFTD showing a significant reduction compared with controls (60%, \( P = 0.002 \)) and AD (48%, \( P = 0.003 \)) but no difference between AD and controls (\( P = 0.251 \)).

Although VEN and fork cell counts were often asymmetric within individuals (Fig. 3G), hemisphere had no effect on the number of VENs, fork cells, or VENs + Fork cells per total neurons at the group level nor were there significant interactions between hemisphere and diagnosis. Patients with bvFTD–MND died during early disease stages (Supplementary Table S1) and showed milder VEN and fork cell losses, but the distribution of VENs + Fork cells/Total neurons in bvFTD–MND did not overlap with that of controls (Fig. 3G). Overall, these findings indicate early, selective, bilateral, and disease-specific VEN and fork cell loss in bvFTD.

**Right FI VENs and Fork Cells Exhibit Correlated Degeneration in bvFTD**

Although bvFTD showed equally severe left and right VEN and fork cell loss at the group level (Fig. 3), we questioned whether these losses were more strongly correlated within each cell type (between the hemispheres) or within each hemisphere (across the 2 cell types). Despite positive correlations among all neuron pairs examined (Fig. 4, Supplementary Table S3), only the right VEN and right fork cell losses showed a significant correlation (Pearson \( r = 0.85, P = 0.004 \); Fig. 4D) in bvFTD. Although preliminary, this observation suggests that right hemisphere VENs and fork cells, in particular, die in response to a shared vulnerability mechanism or that degeneration of one cell type begets degeneration of the other.

**Right Hemisphere VEN and Fork Cell Losses Correlate with bvFTD Anatomical, Functional, and Behavioral Deficits**

bvFTD can be staged postmortem according to a validated atrophy severity scale (Broe et al. 2003). We applied this system to assess how VEN and fork cell losses relate to bvFTD atrophy severity. At an uncorrected significance threshold (\( P < 0.05 \)), atrophy severity correlated with nearly all neuronal losses measured, in part because neuron counts strongly correlated with each other (Fig. 4, Supplementary Table S3). After multiple comparisons correction, only right VEN (Spearman rho = −0.89, \( P = 0.002 \)) and right VEN + Fork cell (Spearman rho = −0.89, \( P = 0.002 \)) losses showed significant correlations with atrophy severity (Supplementary Table S4), consistent with a close relationship between bvFTD anatomical progression and right-lateralized VEN and fork cell degeneration. For illustrative purposes, averaged VEN and fork cell measures from the 2 hemispheres are shown in Figure 5A.

In bvFTD, we further explored whether VEN and fork cell losses correlated with functional and cognitive impairments (CDR–SB, \( n = 7 \); MMSE, \( n = 8 \)). At an uncorrected significance threshold, the CDR–SB correlated only with right VEN loss (Pearson \( r = -0.85, P = 0.016 \); Fig. 5B). This correlation persisted after correcting for the time from CDR to death (Pearson \( r = -0.85, P = 0.031 \)) but not after multiple comparisons correction. MMSE score, a measure of global cognitive function, showed no correlation with VEN or fork cell losses (\( P > 0.131 \), Supplementary Table S4).

Finally, we sought to determine whether VEN and fork cell losses correlate with characteristic bvFTD symptoms. We hypothesized that such relationships would exist independent of diagnosis, reasoning that the relative integrity of the neural system, not the underlying histopathology, determines the clinical profile (Weintraub and Mesulam 2009). Therefore, we examined correlations between VEN and fork cell losses and the NPI across all patients (\( n = 11 \), including 7 bvFTD and 4 AD). Patients’ worst NPI Total scores correlated inversely with VENs + Fork cells/Total neurons remaining on the left (Pearson \( r = -0.64, P = 0.034 \)) and right (Pearson \( r = -0.82, P = 0.002 \), Fig. 5C, Supplementary Table S5), but only the right-sided correlation remained significant after correcting for multiple comparisons and controlling for diagnosis and time from worst NPI Total score to death (Pearson \( r = -0.78, P = 0.013 \)). Finally, worst apathy and disinhibition, but not aberrant motor or eating behavior,
correlated inversely with VENs + Fork cells/Total neurons remaining, at an uncorrected significance level (Supplementary Table S5). Only the right-sided correlation with disinhibition survived the multiple comparisons correction (Pearson r = -0.80, P = 0.003, Fig. 5D), and this correlation also persisted after controlling for diagnosis and time from worst NPI disinhibition score to death (Pearson r = -0.80, P = 0.010).

**Discussion**

bvFTD leads to progressive rostral forebrain degeneration that begins in ACC and FI (Broe et al. 2003; Seeley et al. 2008). Within the human FI, VENs and fork cells cluster in layer 5 and represent the region’s most salient and distinctive cytoarchitectural feature (von Economo and Koskinas 1925; von Economo 1926; Delong 1932, 1936). We quantified bilateral FI VENs and fork cells and found that these neurons exhibit striking differential vulnerability in bvFTD when compared with neighboring layer 5 neurons. While substantiating a previous smaller study of ACC VEN loss in bvFTD (Seeley et al. 2006), the present work provides several key conceptual advances. First, early-stage bvFTD proves difficult to study postmortem because, in contrast to other age-related neurodegenerative disorders (Braak H and Braak E 1991; Braak et al. 2003), bvFTD is rarely encountered among individuals who die of unrelated causes. Here, by including patients who died of comorbid MND, we constructed a bvFTD sample in which 5 of 9 patients showed little or no gross cerebral atrophy (Broe stage 0 or 1). Our findings show that FI VEN counts begin to fall even during these early stages, when social-emotional deficits have just begun to emerge. Second, this study rediscovers the fork cells, which have received almost no scholarly attention since their early descriptions (Cajal 1900; von Economo and Koskinas 1925; De Crinis 1933; Ngowyang 1932, 1936), and suggests that these neurons represent a VEN companion population within FI. In bvFTD, FI VENs and fork cells appear to degenerate in synchrony, particularly in the right hemisphere; yet in AD, these neurons survive even into the most advanced stages. Finally, we forged the first preliminary link between a specific neuronal population and bvFTD deficits by correlating right-sided VEN and fork cell loss with anatomical, functional, and behavioral symptom severity. Overall, the findings support an emerging model in which VENs and fork cells provide key early cellular targets, which, once injured, begin progressive, network-based ACC–FI degeneration that undermines social-emotional processing (Seeley 2008; Seeley et al. 2009; Zhou et al. 2010).

**Timing and Pathogenesis of VEN and Fork Cell Loss in bvFTD**

Limited information is available regarding early cell-specific pathology in bvFTD, and no quantitative neuroanatomical study has focused on the frontoinsular cortex despite its early (Seeley et al. 2008), consistent (Schroeter et al. 2008), and stage-related (Zhou et al. 2010) involvement in bvFTD imaging analyses. Classical papers of Brun and coworkers emphasized superficial laminar degeneration, with microvacuolation, gliosis, and synaptic loss preceding neuronal dropout (Brun 1987). In more advanced stages, frontal and temporal neuron loss becomes severe, with greater deep layer involvement (Kersaitis et al. 2004). One study (Kersaitis et al. 2006) assessed frontal (area 6 and 9) and temporal (area 21/22 or 20) layer 3 neuron densities in early (Stages 1–2) and late (Stages 3–4) FTLD-MND and FTLD with ubiquitin immunoreactive inclusions (before TDP-43 and fused in sarcoma (FUS) inclusions had been identified). Across all patients in their sample, layer 3 neuronal density reductions averaged just over 30% compared with the 53% VEN and 68% fork cell density reductions identified in the present earlier-stage cohort. Measures that correct for total neuron loss provide better estimates of selective vulnerability; here, such measures revealed even more significant VEN and fork cell losses than detected using density measures alone (Fig. 3). Future studies could explore whether FI VENs, fork cells, and superficial layer neurons die together in bvFTD, as part of an initial local circuit degeneration.
misfolded tau, TDP-43, and FUS converge on VENs and fork cells by disrupting some cellular process critical for these neurons’ survival. Alternatively, each FTLD subtype may target VENs and fork cells through independent, molecule-specific toxicity or loss-of-function mechanisms. Aberrant TDP-43, for example, may injure neurons through toxic TDP-43 aggregates or through loss of nuclear TDP-43 function (Iguchi et al. 2009; Zhang et al. 2009). Similar considerations apply to FUS, also a nuclear RNA-binding protein, which travels into mature healthy dendrites and associates with mRNAs locally translated within spines to promote activity-dependent actin cytoskeletal reorganization (Fujii and Takumi 2005).

BvFTD and amyotrophic lateral sclerosis (ALS) (a form of MND) are linked at the molecular level by the presence of TDP-43 immunoreactive neuronal and glial inclusions in roughly half of patients with bvFTD and nearly all patients with sporadic ALS (Mackenzie et al. 2007). Despite the historical focus on FTLD superficial layer pathology, the present bvFTD findings and the ALS pathological literature (Tsuchiya et al. 2002) combine to suggest that specific TDP-43 proteinopathies also target large, region-specific cortical layer 5 projection neurons: upper motor neurons (in ALS and bvFTD–MND) and VENs and fork cells (in bvFTD–MND and bvFTD). Foundational work is needed to define the parallels between human FTLD and emerging FTLD model systems. Transgenic mice that express human mutant TDP-43 throughout the brain show striking and selective degeneration of large, nonphosphorylated neurofilament-protein-rich layer 5 neurons in cingulate, insula, and motor cortex among other regions (Wegorzewska et al. 2009). Because TDP-43, tau, and FUS are all ubiquitously expressed in brain, the most critical but elusive question remains why these proteins aggregate preferentially within specific layers and neurons. In human tissues, VENs and fork cells provide a morphologically identifiable region-specific cell population in which to explore cellular vulnerability questions.

**Relationship between VEN and Fork Cell Loss and bvFTD Symptoms**

The ACC and FI have been proposed as specialized limbic motor (ACC) and limbic sensory (FI) nodes for integrating visceral-autonomic, social–emotional, and cognitive processing streams (Heimer and Van Hoesen 2006). One model proposes the right FI, and possibly VENs, as anchoring the neural representation of human feelings in consciousness (Craig 2009). As right FI VENs and fork cells degenerate, patients with bvFTD may lose interoceptive salience cues (Seeley et al. 2007) or fail to generate time-sensitive “stop” (Aron et al. 2004) or “switch” (Sridharan et al. 2008) signals when selecting their next action. These mechanisms, alone or in combination, could result in uninhibited socially disadvantageous behavior that lacks emotional–moral and contextual sensitivity (Mendez and Shapira 2009). Regardless of the specific neural mechanism disrupted by frontoinsular damage, the relationship observed here between clinical deficits and right FI VEN and fork cell losses is consistent with a recent imaging study, in which we found that right (but not left) FI functional connectivity disruption predicted greater clinical severity in living patients with bvFTD (Zhou et al. 2010).

**Limitations**

This study’s limitations relate to sample size, tissue access, and challenges inherent to correlating neuropsychological and neuropsychological data. Nonetheless, by including bilateral FI from 9 bvFTD subjects, this study more than tripled the published number of VEN-containing regions that have been systematically quantified in bvFTD. We attempted to address pitfalls in clinicopathological correlation by adjusting for potential confounders (testing interval and clinical diagnosis) in our analyses. Employing these strategies, we identified behavioral correlations that supported our a priori hypothesis that right FI VEN and fork cell losses would relate to bvFTD severity. Even so, the behavioral correlations reported here should be interpreted with caution until confirmed in a larger sample.

**Future Directions**

VENs have been identified only in large-brained socially complex mammals (Hof and Van der Gucht 2007; Butti et al. 2009; Hakeem et al. 2009). Therefore, early VEN loss in bvFTD provides an avenue for understanding bvFTD pathogenesis and the evolution of the social brain (Seeley, Allman, et al. 2007). Comparative, developmental, and gene expression studies of FI may help to identify a molecular signature of the VENs and fork cells and determine whether these neurons coevolved and codevelop, just as they seem to codegenerate in early bvFTD. More comprehensive postmortem histopathological studies are needed to determine VEN and fork cell changes that precede neuronal death and how these changes relate to the earliest and most subtle social–emotional deficits that can be detected in bvFTD during life.

**Supplementary Material**

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

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