Hyperphosphorylated tau in refractory epilepsy patients correlates with cognitive decline: a study of temporal lobe resections

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Running title: Tau pathology in Temporal Lobe Epilepsy

Abstract

Temporal lobe epilepsy, the most prevalent form of chronic focal epilepsy, is associated with a high prevalence of cognitive impairment but the responsible underlying pathological mechanisms are unknown. Tau, the microtubule associated protein, is a hallmark of several neurodegenerative diseases including Alzheimer's disease and chronic traumatic encephalopathy. We hypothesized that hyperphosphorylated tau pathology is associated with cognitive decline in temporal lobe epilepsy and explored this through clinico-pathological study. We first performed pathological examination on tissue from 33 patients who had undergone temporal lobe resection between ages 50 and 65 years to treat drug-refractory temporal lobe epilepsy. We identified hyperphosphorylated tau protein using AT8 immunohistochemistry and compared this distribution to Braak patterns of Alzheimer's disease and patterns of chronic traumatic encephalopathy. We quantified tau pathology using a modified tau score created specifically for analysis of temporal lobectomy tissue and the Braak staging, which was limited without extra-temporal brain areas available. Next, we correlated tau pathology with pre- and post-operative cognitive test scores and clinical risk factors including age at time of surgery, duration of epilepsy, history of secondary generalised seizures, history of head injury, handedness and side of surgery. Thirty-one of 33 cases (94%) showed hyperphosphorylated tau pathology in the form of neuropil threads and neurofibrillary tangles and pre-tangles. Braak stage analysis showed 12% of our epilepsy cohort had a Braak staging III-IV compared to an age-matched non-epilepsy control group from the literature (8%). We identified a mixture of tau pathology patterns characteristic of Alzheimer's disease and chronic traumatic encephalopathy. We also found unusual patterns of sub-pial tau deposition, sparing of the hippocampus and co-localisation with mossy fibre

sprouting, a feature of temporal lobe epilepsy. We demonstrated that the more extensive the tau pathology, the greater the decline in verbal learning (Spearman correlation, r= −0.63), recall (r= −0.44) and graded naming test scores (r= −0.50) over one year post-temporal lobe resection ($P < 0.05$). This relationship with tau burden was also present when examining decline in verbal learning from three months to one year post-resection (r= −0.54). We found an association between modified tau score and history of secondary generalised seizures (likelihood-ratio χ^2, $P < 0.05$) however there was no clear relationship between tau pathology and other clinical risk factors assessed. Our findings suggest an epilepsy-related tauopathy in temporal lobe epilepsy which contributes to accelerated cognitive decline and has diagnostic and treatment implications.

Keywords: Temporal lobe epilepsy, neurofibrillary tangles, tau, Alzheimer's disease, dementia

Abbreviations: HS=hippocampal sclerosis, CTE = chronic traumatic encephalopathy, NFTs = neurofibrillary tangles

Introduction

Patients with temporal lobe epilepsy may suffer from cognitive decline and have an increased prevalence of developing dementias including Alzheimer's disease (Høgh *et al.*, 2002).The mechanisms contributing to cognitive decline in temporal lobe epilepsy are unknown, with few effective management options (Hermann *et al.*, 2006).

Cross-sectional neuropsychological studies have shown that chronic partial epilepsy patients treated with anti-epileptic drugs have lower cognitive scores, including verbal memory tests, compared to age-matched controls: one study shows older temporal lobe epilepsy patients, aged 80 years and above, performed the worst even when compared to control individuals diagnosed with mild cognitive impairment (Griffith *et al.*, 2006, 2007). An increasing number of older drug-refractory temporal lobe epilepsy patients are being offered temporal lobe resection as treatment and age above 50 years at time of surgery has been identified as a major risk factor for subsequent memory decline (Thompson *et al.*, 2015). Other clinical risk factors associated with cognitive decline include duration of epilepsy, seizure type, head injuries and cognitive reserve (Black *et al.*, 2010; Helmstaedter and Elger, 2009; Oyegbile *et al.*, 2004).

Evidence suggests an association between epilepsy and dementia as epidemiological data show increased prevalence of dementia and Alzheimer's disease in individuals with chronic epilepsy (Gaitatzis *et al.*, 2004; Tellez-Zenteno *et al.*, 2005). Transgenic mice models of familial Alzheimer’s disease have been shown to suffer from recurrent seizures (Palop *et al.*, 2007) while serial neuroimaging in chronic epilepsy, particularly temporal lobe epilepsy, shows progressive grey matter volume loss and cortical thinning which are associated with

cognitive impairment (Liu et al., 2003; Bernhardt *et al.*, 2009; Cormack *et al.*, 2005; Lin *et al.*, 2007). Despite accumulating epidemiological, radiological and animal model evidence associating epilepsy with dementia, there are few studies of the pathological changes that may underlie cognitive decline in epilepsy.

Tau, a microtubule associated protein, is a natively unfolded protein in human brains and has several roles including microtubule assembly and stabilisation (Goedert and Spillantini, 2006; Ittner *et al.*, 2010). A defining pathological feature of several neurodegenerative diseases, including Alzheimer's disease and chronic traumatic encephalopathy (CTE) is aggregated, hyperphosphorylated tau. Hyperphosphorylated tau is a key component of neurofibrillary tangles (NFTs) which are central to the diagnosis and staging of such diseases (Braak *et al*., 2011; McKee *et al*., 2013). Pathological tau is also associated with early cognitive decline in other diseases such as Parkinson's disease, motor neuron disease and, recently, Huntington's disease (Vuono *et al.*, 2015; Wolfe, 2012).

In a recent human post-mortem analysis of 138 patients with chronic, refractory epilepsy, we reported neurofibrillary tangle (hyperphosphorylated tau) pathology with age-accelerated changes within the mid-Braak stages (III/IV) compared to an age-matched non-epilepsy series (Thom *et al.*, 2011). Interestingly, increasing Braak stages did not clearly correlate with cognitive decline measured prior to death. Traumatic brain injury, indicated primarily by histological findings of frontotemporal contusions, correlated significantly with hyperphosphorylated tau burden and suggested an underlying CTE pathological process.

Post-mortem analysis of brain tissue usually occurs some years after the last cognitive assessment. By contrast, temporal lobe resections performed for refractory epilepsy allows contemporaneous histological examination and clinical assessments. Examination of

temporal lobe resection tissue in 47 temporal lobe epilepsy patients revealed granule cell layer dispersion correlating inversely with verbal memory scores (Kandratavicius *et al.*, 2013). Microtubule-associated proteins, MAP2 and tau, were present but with variable correlation to clinical outcome and cognitive decline with below average verbal memory scores described with increased tau expression in the CA2 region. Sheng *et al.* (1994) identified increased immunoreactive amyloid-β precursor protein in temporal lobe tissue from eight temporal lobe epilepsy patients while a larger study of 101 temporal lobe specimens compared with 406 post-mortem controls showed an age-accelerated presence of senile amyloid plaques in 10% of epilepsy patients (Mackenzie and Miller, 1994).

In this study, we aimed to assess the extent, nature and significance of tau pathology in temporal lobectomy subjects aged between 50-65 years of age at time of surgery. We also compared and contrasted our findings with those reported for Alzheimer's disease and CTE. We then aimed to investigate the relationship between tau burden and post-operative decline in cognitive test scores and to examine the effect of potential clinical risk factors in this interaction. We hypothesized that hyperphosphorylated tau pathology in the resected temporal lobe, reflective of tau pathology in the entire brain, is associated with cognitive decline in our cohort.

Materials and Methods

Case selection

Post-surgical cases (*n*=33) were selected from the archives of the Department of Neuropathology, National Hospital for Neurology and Neurosurgery, London, from 1995-2014. We included all temporal lobe epilepsy patients who had undergone anterior temporal lobe resection, aged between 50 and 65 years at the time of surgery, with pathological diagnosis of hippocampal sclerosis. All cases had a primary clinical diagnosis of temporal lobe epilepsy with history of chronic, drug-refractory disease. Cases excluded were of non-hippocampal sclerosis pathology, those who developed epilepsy during a course of another neurodegenerative disease, including Alzheimer's disease, and if consent for research was not recorded. The tissues were consented for use in research and the study was approved by the local ethics committee.

Clinical Data

Demographic data and the clinical history of each case were retrieved from clinical notes. Recorded information included the age at onset of epilepsy, duration of epilepsy, side of surgery and handedness. Information on seizure type and frequency, occurrence of status epilepticus and history of head injury was also noted when available. Any reports of cognitive decline post-operatively or development of dementia were also noted.

Cognitive test data

Temporal lobe epilepsy patients who undergo temporal lobe resections usually undergo cognitive testing during the pre-operative pathway and at three and twelve months post-

operatively. Pre-operative cognitive data was available for 27 patients (82%). A measurement for 'change in cognition over time' was derived from paired pre-operative to one year post-operative test scores, and three month post-operative to one year post-operative test scores that were available for 21 patients (63%).

Intellectual level was measured using the Wechsler Adult Intelligence Scale (WAIS). All patients with pre- and post-operative data had an IQ of >69.

Memory and other cognitive measures were performed using the List Learning and Design Learning subtests from the Adult Memory and Information Processing (AMIPB) and its successor the BIRT memory and information processing battery (BIMPB) provided measures of verbal learning and recall and visual learning and recall. These measures have been shown to be sensitive to temporal lobe pathology and have been described previously (Thompson *et al.*, 2015). The Graded Naming Test and tests of phonemic and semantic fluency provided measures of word-retrieval proficiency (Thompson *et al.,* 2015). Test scores were converted into *z*-scores based on age-related norms. A negative z-score indicated a decline in memory.

Immunohistochemistry

Resected tissue was archived as formalin-fixed, paraffin embedded tissue blocks containing temporal lobe and hippocampus for all cases and additional blocks of pes hippocampus, para-hippocampal gyrus and amygdala were available for thirteen more recent temporal lobe resections. Blocks were selected for immunohistochemical analysis according to anatomical position, best preserved tissue, and representative pathology (from review of archived stained sections previously prepared; Supplementary Table 1).

AT8 (hyperphosphorylated tau) and βA4 (amyloid-β) immunohistochemistry was performed on 5-µm thick paraffin-embedded sections using the Bond Max automated immunostainer (Leica, Microsystems, Milton Keynes, UK) following established laboratory protocols with primary antibody anti-AT8 (1:1200, Innogenetics, AutogenBioclear, Wiltshire, UK) or anti-βA4 (1:100, DAKO, Cambridgeshire, UK). Positive (confirmed cases with Alzheimer's Disease) and negative controls were included for each staining run. Sections from selected cases were also stained with both anti-RD3 and anti-RD4 specific for 3-repeat (1:3000) and 4-repeat tau isoforms (1:4000; gift from Rohan de Silva) using automated immunostainer (A.Menarini Diagnostics Ltd, Berkshire, UK). All sections were viewed using a light microscope (Olympus BX40). Selected slides were digitised using a whole slide scanner (Leica SCN400, Leica Microsystems, Milton Keynes, UK). Images were acquired using a digital camera (Nikon Eclipse 80i) or as a snapshot from digitised slides.

Immunofluorescence

Sequential double labelled immunofluorescent studies were performed manually over three days based on previously published protocols to identify specific cell populations which co-localise with pathological tau (Thom *et al.*, 2011). In brief, after sections were microwaved at 800W for 12 minutes in the antigen unmasking solutions (H3300, Vector Labs, Peterborough, UK), blocking solution containing 2.5% normal horse serum (Vector labs, Peterborough, UK was applied. Sections were incubated in anti-AT8 primary antibody solution overnight at 4^{o}C (1:1200). The next day, monoclonal-specific horseradish peroxidase-conjugated secondary antibodies (Vector Labs, Peterborough, UK) were added for 30 minutes before fluorescein-conjugated antibodies diluted in tyramide-amplifying buffer (1:800; Perkin Elmer, Coventry, UK) was applied for five minutes. Sections were immersed in 0.9% hydrogen peroxidase

solution for 10 minutes, and then the second primary antibodies were applied as described in Supplementary Table 2. The next day, sections were incubated in solutions consisting of species-specific secondary antibodies conjugated to either Alexa Flour 546 (Lifesciences, UK) for two hours at room temperature, or HRP and then Cy3 diluted in tyramide-amplifying buffer (1:800; Perkin Elmer, Coventry, UK) for 30 and five minutes, respectively. All sections were coverslipped in Vectashield mounting media with 4', 6-diamidino-2-phenylindole (DAPI; Vector Labs, Peterborough, UK), and washes were performed using phosphate buffer saline (Oxoid Limited, Hampshire, UK). Negative controls with omission of all or one primary or secondary antibodies were included in each run, and no false positive labelling was observed in negative controls.

Analysis of tau and amyloid pathology

We assessed tau burden semi-quantitatively using two methods. Firstly, the presence and location of AT-8 immuno-labelling (hyperphosphorylated tau) was assessed using a 'limited Braak staging' according to the standards published by Braak *et al.,* (2006). Resected temporal lobe tissue only allows for Braak staging from 0-IV (since stages V-VI require examination of extra-temporal regions which were not removed during surgery). Secondly, we created a 'modified tau score' specifically for assessing AT-8 labelling (tau burden) within resected temporal lobe tissue. The modified tau score ranges from 0-6 and follows early Braak stages (0-IV) but allows greater sensitivity with a wider scoring range and did not follow the anatomical progression of the Braak staging. Table 1 gives a detailed breakdown of the modified tau scores and how they compare with Braak stages. In terms of tau burden, modified tau score 0 (no AT-8labelling) is equivalent to Braak stage 0 while modified tau score 6 (greatest tau load) is equivalent to Braak stage IV (representative images for each modified

tau score are found in Supplementary Fig 2). We noted other AT-8 labelling characteristics including the cell type and location, in particular glial or neuronal and perivascular or sulcal. We also noted distributions of AT-8 labelling that were consistent with CTE based on standards published by McKee *et al.* (2015).

Amyloid-β immunohistochemistry (βA-4 labelling) was assessed with plaque scores of: sparse, moderate or frequent, as outlined in the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) (Alafuzoff *et al.,* 2008). Additional pathological features including type of hippocampal sclerosis, mossy fibre sprouting, brain trauma, and cerebrovascular disease were also noted.

Experimental design

Pathology analysis and scoring was performed by two independent assessors (XT and MT). Cases with different scores were reassessed together and a final score was agreed upon. Neuropsychometry analysis was performed independently by PT and comparison with pathology examination was done only when all scores were finalised.

Statistical analysis

Statistical analysis and graphical representation was performed using SPSS for windows (IBM Corporation, version 20) or Excel 2010 (Microsoft Office). Student *t*-testing was used compare Braak staging between the study cohort and age-matched population controls from a post-mortem series (Braak *et al.* 2013). Spearman correlation analysis was carried out with modified tau scores in relationship with age at time of surgery and onset of epilepsy as well as change in neuropsychometry scores from pre-operative to 12 months post-operative and three months post-operative to 12 months post-operative. Statistical analysis between

modified tau scores with ANOVA testing, Pearson Chi^2 testing or likelihood-ratio Chi^2 testing were employed for other clinical characteristics including duration of epilepsy, side of resection, history of head injury, history of secondary generalised seizures and handedness of each patient. Multi-linear regression was also carried out between modified tau score and clinical characteristics. Measure of Agreement (Cohen's κ coefficient) was used to assess inter-observer agreement of modified tau scoring. For all statistical methods, a *P*-value of < 0.05 was considered significant.

Results

Hyperphosphorylated tau (AT-8 labelling) in resected temporal lobe tissue

Using immunohistochemistry for AT-8 labelling, we identified hyperphosphorylated tau pathology in the form of neuropil threads, neurofibrillary tangles and pre-tangles within temporal lobe tissue (Fig. 1A, B). We applied the six point modified tau score and limited Braak staging for tau semi-quantification and showed 31 of 33 cases (93.9%) had evidence of AT-8 labelling (Fig. 2A). Twelve cases (36%) scored a modified tau score 3, the most frequent distribution observed. Two cases (6%) scored 0 (virtually no AT-8 labelling), five cases (15%) scored 1, seven cases (21%) scored 2, two cases (6%) scored 4, four cases (12%) score 5 and one case (3%) scored 6 (Fig. 2). Agreement was achieved within a one point margin for modified tau scoring in 91% of the cases with a moderate level of agreement between observers (Cohen's coefficient κ= 0.54, $P < 0.005$).

Comparing patients aged 50-60 years at the time of the surgery (26 cases) with age-matched population controls from a post-mortem series of 330 patients in the literature (Braak *et al.*, 2011), we found a higher proportion of patients with Braak stages III-IV (12% vs. 8% but the

differences were not significant). As noted above, comparisons of Braak stage V-VI could not be performed as our cohort lacked extra-temporal tissue samples.

Distribution of hyperphosphorylated tau within the temporal lobe relative to Alzheimer's disease and chronic traumatic encephalopathy patterns

Having found high tau phosphorylation burden within the resected temporal lobe tissue of some epilepsy cases, we next compared the distribution and patterns of tau accumulation to known tauopathies Alzheimer's disease and CTE.

Temporal neocortex and pole

We characterised tau distribution in 24 cases in who sufficient AT-8 was present and found a mixture of pathological patterns (see supplemental table 3). Ten cases had a 'Braak-like' pattern of tau pathology with greater AT-8 labelling within the transentorhinal region and entorhinal region compared to temporal neocortex. In eight cases, we identified 'CTE-like' tau patterns, based on the identification of one or more of the following: axon labelling in white matter, cortex, layer I or hippocampus in single long or groups of axon (Fig. 1C, 3N); patches of cortical AT-8 labelling restricted to a single region as the middle temporal gyrus or temporal pole (Fig. 1d), greater tau labelling in neocortical samples compared to entorhinal cortex or more prominent tau accumulation in superficial (layers I-III) than deeper cortex (layers IV-VI) (supplemental table 3) (McKee *et al.*, 2013). None of the cases showed the typical sulcal or well-defined perivascular neuronal tau pathology or obvious astrocytic or sub-pial tangles, considered pathognomonic of CTE.

We also identified unusual patterns of AT-8 labelling, not recognised as typical for either Alzheimer's disease or CTE. A characteristic 'sub-pial' band of AT-8 labelling was observed in 18 of the cases (Fig. 1E) with an axonal-like pattern. This band was seen in cases with both 'Braak-like' and 'CTE-like' patterns of tau pathology as well as other cases where it was the only positive AT-8 labelling observed. In six of these cases there was also an impression of intense labelling of Cajal-Retzius cells in layer I (Fig. 1E) but an impression of sub-pial astroglial labelling, as reported in CTE and epilepsy post-mortem studies (McKee *et al.*, 2013; Thom *et al.*, 2011), was lacking. Double labelling with AT8 and reelin (Cajal Retzius cells) or delta-GFAP and nestin (subpial astroglial markers) did not confirm definite overlap between cell bodies or processes in this compartment (Fig. 1F, G). Prominent labelling of small neurones at the interface of layer I/II was noted in some cases (Fig. 1H); as we have previously identified populations of immature DCX-positive neurones in the superficial cortical layers of the mesial temporal lobe in temporal lobe epilepsy/HS , which have also been reported in other species (Liu *et al.*, 2008; Srikandarajah *et al.*, 2009; Xiong *et al.*, 2008; Zhang *et al.*, 2009) we explored if these subsets were vulnerable to tau accumulation, but this was not supported by double labelling (Fig. 1H inset).

In five cases cortical granular-aggregates of tau formed small 'burst-like pattern' of tau-positive grains. These were noted in the neocortex of cases of low tau score (Fig. 1I-N) in some cases present primarily in superficial layers. These granular-aggregates did not have the typical morphology of neuritic or astroglial plaques, in that they lacked a central core or nucleus, were not specifically related to neurones (Fig. 1J) but were more frequently noted close to small capillaries (Fig. 1K), supported with double labelling for neuronal, glial and vascular markers (Fig. 1L-N). Furthermore in all these five cases no plaques were seen with

beta-amyloid. The precise cellular compartmentalisation of these tau granular-aggregates remains uncertain but they may represent a unique finding in epilepsy not previously reported in CTE or Alzheimer's disease.

Hippocampus, pes hippocampus and amygdala

All patients in this group had HS and in many mossy fibre sprouting was confirmed in the routine diagnostic work-up by Timms stain or immunohistochemistry (for dynorphin or ZnT3) as evidence of epilepsy-associated hippocampal network reorganisation (Thom, 2014). Relative sparing of AT-8 labelling within the hippocampal subfields, particularly CA1 was a striking finding in tau-positive cases in as previously reported in post-mortem series of HS in epilepsy (Thom *et al.*, 2011). Only nine of the 33 cases had AT8 labelling within hippocampal regions. Of these nine cases, AT-8 labelling was more prominent in the subiculum while CA1, CA2, CA3 and CA4 showed proportionally much lower levels of AT-8, with labelling in only four cases (Fig. 3A). This is out of step for the normal sequence of AT8 accumulation in Alzheimer's disease, where CA1 is typically involved earlier than the subiculum (Braak *et al.*, 2011). In addition early involvement of granule cells was noted in seven cases (see supplemental table 3) which are typically involved late in Alzheimer's disease. In one case prominent labelling by AT8 of radial processes through the molecular layer of the dentate gyrus, reminiscent of the pattern of mossy fibre sprouting (Fig. 3B-E) was seen with compact aggregates in the CA4 region (Fig. 3C). There was evidence AT8 localised with ZnT3 aggregates (Fig. 3G) and around neurofilament-positive neurones in CA4 (Fig. 3F), supporting tau aggregation in the mossy fibre axons and terminals. Additional prominent staining was noted in the hippocampal body included labelling of axons in the alveus (Fig. 3 l-M), horizontal neurones in CA1 and axons in parahippocampal gyrus white matter.

In the pes hippocampal specimens, similar tau patterns were noted. In one case with more severe granule cell dispersion (Fig. 3H) and mossy fibre sprouting (Fig. 3I) in the pes compared to the hippocampal body, there was a striking increase in the AT8 labelling in the pes granule cell layer (Fig. 3J) compared to other subfields and the hippocampal body (Fig. 3K). Other observations were axonal-like labelling along the subpial surface of the pes (Fig. 3N,O) and the peri-ventricular white matter (Fig. 3P).The amygdala region was not available in all cases and fragmented making anatomical orientation difficult; occasional findings were AT-8 labelling of axonal fibres and bundles (Fig. 3Q). Supplementary Table 3 provides a summary of our classification of tau patterns.

Biochemical characterisation of tau in epilepsy cohort

We further characterised tau composition by immunostaining for TDP-43 inclusions, 3R- and 4R- isoforms in four cases with more abundant AT8 labelling. Neurones and tangles were both 3R and 4R positive (Fig. 3R, S) indicating mixed 3R:4R tau isoform accumulation and was negative for TDP-43 immunoreactive inclusions. The absence of immunoreactive TDP-43 inclusions contrasts with previous reports of Alzheimer's disease (Amador-Ortiz *et al.*, 2007) but is consistent with epilepsy hippocampal sclerosis (Lee *et al.*, 2008).

Absence of β-amyloid positive plaques in majority of cases

β-amyloid positive plaques were absent in 28 cases (85%) with a 'sparse' plaque score identified in three cases (10%), 'moderate' plaque score in one case (3.0%) and 'frequent' plaque score in one case (3%).

The case with frequent plaques had a modified tau score of 6 and the moderate plaque case had a modified tau score of 3. The three cases with occasional plaques had a modified tau scores of 1-2. There was no statistically significant correlation between β-amyloid plaque appearance and the modified tau score ($P > 0.05$)

Modified tau score in relation to age and cognitive testing

A weak positive correlation was seen between modified tau score and age at time of surgery and between modified tau score and age at onset of epilepsy of the study cohort (Spearman correlation r=0.39 and r=0.21 respectively, Fig. 4). No statistically significant correlations were observed between the modified tau score and pre-operative neuropsychometric scores (Correlation coefficient range $-0.25 < r < 0.13$).

To further explore cognitive phenotype, we determined post-operative cognitive decline by comparing neuropsychometric scores at different time points. Neuropsychometric data were condensed into memory learning and recall components (verbal and visual) and naming ability. We first compared the scores at one year post-operation with pre-operative scores. We observed a significant decline in verbal learning and recall at one year (mean *z*-score drop −0.67 and −0.58), compared to slight improvement on the visual memory indices at one year ($P < 0.01$) (Table 2).

When examining change in cognitive test scores over one year (pre-temporal lobe resection to one year post-operation), modified tau scores had a strong negative correlation with change in verbal learning (Spearman correlation r= −0.63, $*P < 0.05$), a moderate negative correlation with change in verbal recall (Spearman correlation r= −0.44, $*P < 0.05$) and a moderate negative correlation with graded naming test scores (Spearman correlation r=

−0.50, *P < 0.05) (Fig. 5). No statistically significant associations were observed between the modified tau score and post-operative changes in visual memory (P > 0.05) (Table 2).

We also examined the change in cognitive test scores from three months post-temporal lobe resection to one year post-operation. Modified tau scores had a moderate negative correlation with change in verbal learning during that period (Spearman correlation r= −0.54, *p<0.05). The correlations with verbal recall (r= −0.06), visual memory (r= −0.11) and visual recall (r= −0.12) were not significant.

Formal long term neuropsychometric follow-up was not performed for all cases of this study. We can report that the case with the highest tau burden of the study was diagnosed with Alzheimer's dementia nine years after temporal lobe resection. This patient had no evidence of dementia at pre-operative assessment and their temporal lobe resection tissue showed tau pathology with distributions typical to the cohort including hippocampal sparing.

Cognitive decline in relation to secondary generalised seizures and other clinical factors of epilepsy

History of secondary generalised seizures was identified in 19 of 26 cases (73%). There was a significant association between modified tau score and history of secondary generalised seizures (likelihood-ratio χ^2, P < 0.05) (Supplementary table 4). Other clinical factors including age at time of surgery (mean 53.6 years), age at onset of epilepsy (mean 14.7 years), duration of epilepsy (mean 39.5 years), side of temporal lobe resection (59.1% of cases were right sided temporal resection), history of head injury and handedness (66.7% of cases were right handed) did not have a clear relationship to modified tau score (P > 0.05). There was a greater decline in verbal memory scores over one year with left sided temporal

lobe resection (mean z-score drop in verbal learning, −0.97 vs. −0.49, $P > 0.05$) compared to right sided resections but this was not statistically significant. Subsequent stepwise multiple regression analysis did not identify further clinical indicators as a significant independent variable associated with memory decline (verbal or visual domains) or graded naming ability.

Discussion

We confirm and extend previous findings that tau pathology is present in temporal lobe epilepsy patients and establish a key involvement by showing a strong correlation between tau pathology and post-operative cognitive decline in a cohort of older patients with epilepsy.

Tau hyperphosphorylation is an important post-translational pathological step causing an inability to interact with microtubules (Bramblett *et al.*, 1993) with imbalances of different tau isoforms contributing to variation across neurodegenerative tauopathies (Spillantini and Goedert, 2013). Epilepsy is not classically thought of as a neurodegenerative disease, however, we identified mixed characteristics of tau pathology similar to both Alzheimer's disease and CTE, two neurodegenerative diseases previously linked to epilepsy (Amatniek *et al.*, 2006; McKee *et al.*, 2015).

Double labelling identified neuronal tau, mainly within axons, and no tau within glial cell populations which is consistent with Alzheimer's disease. The absence of consistent amyloid-β in this study however does not accord with typical Alzheimer's disease and a similar absence of amyloid-β was noted in the post-mortem study by Thom et al. (2011). Based on these findings, we propose the underlying tau-related neurodegeneration in temporal lobe epilepsy patients should not be classified as a typical Alzheimer's disease process and other 'tau-predominant pathologies' should be considered.

We identified several cases with CTE-like patterns of tau distribution, but the lack of perivascular and sulcal tau foci, a prominent feature of early CTE, argues against classical CTE pathology in this epilepsy cohort. CTE tau pathology may reflect the angle of acceleration of head trauma, for example boxers experience angular acceleration injuries from trauma

originating below the chin causing a predisposition to basal ganglia pathology with more prominent parkinsonism while American football players sustain linear acceleration head injuries from in-game tackles leading to pathology that predominates fronto-temporal regions (Gavett *et al.*, 2011). During seizures, we hypothesize two potential head injury mechanisms: falls as a result of seizures leading to direct head trauma and small repetitive trauma of the cerebrum against the skull vault during head jerking movements of secondarily generalised seizures. The latter mechanism may explain the prominent sub-pial location of tau pathology observed in this study. We argue that tau pathology in our study is not classical, but shares some overlap with CTE which may be due to the nature of the repetitive head injury and intrinsic factors of epilepsy.

Another hypothesis is that epileptic ictal and interictal activity may be involved in the formation of tau pathology that is related to this specific cohort of chronic temporal lobe epilepsy patients. We identified unusual patterns of tau pathology that include the frequent finding of a sub-pial band of tau pathology in almost all cases (in both Braak-like and CTE-like cases), relative sparing of tau pathology within the hippocampus but involvement of granule cells and mossy fibre pathways. In some cases, the sub-pial band was the only positive tau labelling and may represent the earliest site of deposition of hyperphosphorylated tau. These patterns do not conform with the stereotypical accumulation of tau (or its propagation) in the mesial temporal lobe as described in the Braak staging of Alzheimer's disease (Braak & Del Tredici, 2015; Braak *et al.*, 2006). In temporal lobe epilepsy/HS (as in this study cohort) there is early onset of seizures with neuronal loss and resultant alterations of circuitry and hippocampal networks (Haneef *et al.*, 2014). With the current favoured 'prion-like' hypothesis of neuron-to neuron dissemination of tau along long axonal pathways, this could be one

explanation for the observed differences (Supplementary Fig. 1). There is also experimental evidence that tau is released by synaptic activity (Lewis and Dickson, 2015; Pooler *et al.*, 2013) and it is plausible that epileptic activity in these subjects may have influenced tau accumulation patterns, particularly in the excitable granule cell layer. The Alzheimer's disease literature suggests that tau accumulation primarily effects phylogenetically recent and less mature neurones (Braak & Del Tredici, 2015); in this temporal lobe epilepsy series, however, we did not confirm evidence for involvement of DCX-positive or reelin-positive cortical neurones. The relative lack of hippocampal involvement also distinguishes HS/ temporal lobe epilepsy cases from PART (primary age related tauopathies) which are confined to the hippocampus (Duyckaerts *et al.*, 2015). Other unique findings in our series included cortical granular aggregates and subpial axonal bands. Possible explanations are that these features in temporal lobe epilepsy represent early patterns of tau-accumulation with aging or related to trauma. However what seems more likely is that this represents an epilepsy-specific early 'axonal-tauopathy', as a consequence of seizures, ictal/inter-ictal activity, neuronal loss and the re-organised temporal lobe axonal networks (Supplementary Fig. 1); this 'model' invites further investigation to explore mechanisms of tau acquisition.

Interestingly, our study did not find a correlation between tau pathology burden and pre-operative cognitive test scores. Pre-operative scores represent a 'snapshot' of cognition affected by several factors including co-morbidities, educational level and AED medication, which is likely not to be sufficiently sensitive to be compared with tau burden. Longitudinal data afforded by the post-operative change in cognitive scores reduces contaminating influence of confounding factors such as AED medication, which is usually unaltered over the first post-operative year. Tau burden correlated with a drop in cognitive test scores,

particularly verbal learning, from pre-temporal lobe resection to one year post-operation. We also examined cognitive changes from three months post-temporal lobe resection to one year post-operation, in order to mitigate the direct effects of the surgical resection, and showed similar correlation with verbal learning scores but poorer correlation with the other memory domains. This difference may be explained by a 'cognitive effect' of the actual surgery which is excluded in the nine month analysis or may reflect the shorter follow-up period being less sensitive in detecting cognitive changes. Longer term follow-up of cognitive decline in this cohort would be important to explore this.

Despite the atypical patterns of temporal-lobe hyperphosphorylated tau in our temporal lobe epilepsy cohort and differences between cognitive decline when including surgery in the analysis, there is nevertheless evidence for clinical impact. This impact is demonstrated by the correlation between overall tau pathology burden and cognitive decline, particularly verbal learning, post-temporal lobe resection. Our study supports a neurodegenerative role for tau and advances the findings of a previous post-mortem epilepsy study which did not find a linear correlation with higher Braak stages and pre-mortem cognitive scores but reported over 70% of Braak stage III or greater patients had progressive cognitive decline leading up to death (Thom *et al.*, 2011).

Age is an important factor for tau deposition (Braak *et al.*, 2013), and thus we studied a cohort of between 50 and 65 years at time of surgery, which is much younger than the expected typical age at onset in sporadic Alzheimer's disease. Post-mortem temporal lobe epilepsy study found consistent tau pathology in quantities higher than expected for their age (Thom *et al*., 2011). We observed tau pathology in almost all cases with a weak positive correlation between age at time of surgery and tau burden suggesting age is an important, but not the

only, factor in tau accumulation. History of secondary generalised seizures was the only clinical factor which showed statistically significant association with tau burden with and this would support the hypothesis of abnormal ictal activity contributing to an epilepsy-associated tauopathy. We feel, however, that a correlation between frequency of generalised seizures and tau burden would offer a more robust analysis and better reflect the literature on poor cognitive outcomes in epilepsy (Elger *et al.*, 2004). We did not identify a clear relationship between other clinical characteristics analysed including age at onset of epilepsy and duration of epilepsy with hyperphosphorylated tau burden. We observed a greater mean drop in verbal memory with left sided temporal lobe resections which, despite not being statistically significant, is consistent with the literature (Thompson *et al*., 2015). The lack of clear relationship with left sided resection and verbal memory decline may be explained by atypical language dominance and cross-dominance that is documented to occur is more frequent in epilepsy patients (Dijkstra & Ferrier, 2013; Springer *et al.*, 1999) or due to the low number of left sided resections in this study. Reliable clinical risk factors influencing hyperphosphorylated tau deposition would be required to develop preventative strategies.

The diagnosis of Alzheimer's disease nine years post-temporal lobe resection in the patient with the highest tau burden illustrates the possible long term effect of tau pathology on cognitive decline. Despite the heavy pathological tau burden at time of surgery, this patient had preserved mini-mental state examination (MMSE) for six years post-operation before an appreciable decline and a (Pittsburg-compound B) PiB-PET scan which showed amyloid deposition within average age-controlled range during that time. Long term follow-up of all cases would be needed to fully investigate the effect of tau pathology.

The importance of our findings is the demonstration of tau pathology in the brains of elderly refractory temporal lobe epilepsy patients undergoing surgery and the correlation with post-operative cognitive decline. Temporal lobectomy is not widely offered to older refractory temporal lobe epilepsy patients, and only in recent times are we increasingly focussing on 'physiological' age rather than actual age when considering candidates for surgery. Analysis of this older population with pathological correlation with 'real-time' neuropsychometry assessment for cognitive decline partly explains the low number of cases analysed but is a unique aspect of this study. Surgery in the elderly brain already compromised by tau-pathology might accelerate the decline with the little compensatory reserve capacity. Our findings suggest that epilepsy should be added to the increasing spectrum of disorders in which tau pathology may not be the characteristic feature, however is reflective of underlying neurodegeneration and cognitive decline, including Parkinson's disease, motor neuron disease and, recently, Huntington's disease (Vuono *et al.*, 2015; Wolfe, 2012).

Our study is limited in that we have examined only temporal lobe tissue which was resected during surgery and did not have the entire brain for pathological analysis. We created a new scoring system for tau pathology within the temporal lobe which was useful for the limited tissue available. For Braak staging comparison, similarly limited by available resected tissue, we used control data from large published post-mortem series from a normal ageing, non-epilepsy population (Braak *et al.*, 2011) however this may be confounded if the tau pattern in temporal lobe epilepsy does not follow the Braak distribution. We also acknowledge that we have not excluded the effect of progressive neuronal loss (resulting from seizures) as a factor of cognitive decline in our cohort. Clinical data, such as frequency of generalised seizures and head injury, were not consistently available and a prospective approach to collecting this

information may prove effective. Furthermore investigation of the effect of the *MAPT* haplotypes and ApoE status on rate of cognitive decline would be useful to explore potential genetic factors.

In summary, we show a pathological role for the involvement of tau in clinical expression of cognitive decline in temporal lobe epilepsy. We describe unusual patterns of tau pathology which may be related to CTE or represent an epilepsy-specific tauopathy. Further studies are needed to elucidate the exact mechanism underlying tau pathology which may lead to new approaches for diagnosing and treating cognitive decline in temporal lobe epilepsy.

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Figures legend

Table 1. Modified tau score description and comparison to (limited) Braak staging

Table 2. Comparing the change in neuropsychometry scores for verbal and visual memory and graded naming test between cases of different modified tau scores.

Figure 1. AT8 patterns in temporal lobe epilepsy/HS cases in the temporal neocortex.

A. Section of temporal lobe from case with maximal modified tau score of 6 showing dense accumulation of tau with AT8 immunohistochemistry in all gyri. The rectangle is shown at higher magnification in (B), highlighting neuropil threads and neuronal labelling. C. Axonal labelling with AT8 in the cortex (case 24) in inset and double labelling with neurofilament (N200) showing beaded like AT8 staining along the trajectory of radial cortical axons. D. Chronic traumatic encephalopathy (CTE-like) pattern with focal increased AT8 in threads and small neurones in the superficial cortical layers. E. Sub-pial granular band of labelling and positive neurones, reminiscent of Cajal-Retzius cells (arrow) ; double labelling with MAP2 (dendritic marker) on the right side of the panel, shows AT8 labelling in the compartment above dendritic MAP2 labelling supporting this more likely represents superficial subpial axonal projections. F. Double labelling between AT8 and nestin in layer I showed no overlap with the granular, axonal-like AT8 labelling and the nestin in the subpial glia with occasional possible co-localisation (arrow). G. Layer I in a further case with AT8 showing a mainly beaded axonal staining pattern and only rare possible expression in GFAP-delta positive subpial astroglial cell. H. Small neurones at interface of layer I and II were AT8 positive but double labelling with doublecortin (DCX) in selected cases (inset) did not shown any AT8 positivity in these immature cell types that are known to reside in this cortical layer in the temporal lobe. I. 'Granular aggregates' of tau were noted in the temporal cortex in five cases scattered in the cortex pattern but not typical of neuritic plaques of Alzheimer's disease or glial inclusions. J. Occasionally these aggregates surrounding cortical neurones but without definite labelling of the neuronal cell body. K. Granular aggregates were observed in the vicinity of small capillaries (arrow). L. The granular aggregates did not appear to co-localise with dendrites on double labelling with MAP2 (a neuronal dendritic marker). M and N. Labelling with GFAP confirmed that the AT8-granular aggregates were not in astroglia but through highlighting the glial foot processes along the vessels, indicated their proximity to vascular channels. Supplementary Figure 3 contains a panel of similar immunofluorescence images captured with a non- red/green colour spectrum. Bar is equivalent to 500 µm in A, 70 µm in I, 50 µm in B and D, 20 µm in C, E, F, G, J, K L, M and N.

Figure 2. Quantifying tau pathology in temporal lobe tissue of epilepsy patients.

Histogram showing modified tau score (described in table 1.) distribution across study cohort. 31 of 33 cases showed AT-8 labelling with modified tau score 3 being most common.

Figure 3. AT8 patterns in temporal lobe epilepsy/HS in the hippocampus, pes and amygdala

A. Hippocampus body from case with highest tau load in the cortex. The hippocampus which showed typical features of longstanding hippocampal sclerosis and gliosis with neuronal loss in CA1 and CA4. More AT8 labelling was seen in the subiculum compared to the CA1 subfield (inserts). B. In another case of HS, mossy fibre sprouting was show with dynorphin staining and granular aggregates noted with in the CA4 region and in C. A similar pattern was observed with AT8. D. Double labelling of AT8 with ZnT3 and (E) neurofilament (N200), confirmed some overlap of labelling in the molecular layer of the dentate gyrus. F. AT8-positive granules were observed in close proximity, surrounding a CA4 neurone and its processes and in G. Overlap of ZnT3 and AT8 was noted in the CA4 region corresponding to mossy fibre end terminals. H. Pes hippocampus from one case showed marked dispersion of the granule cells on NeuN compared to the body also with a greater degree of mossy fibre sprouting on dynorphin stain as shown in I. J. Abundant AT8 labelling in the granule cells and axons in the molecular layer was noted compared to very little staining in the adjacent CA1 subfield (not shown) and K. the hippocampus body of the same cases, where only rare AT8-positive threads were noted. L. An axonal like pattern of labelling was noted in the alveus beneath the ependymal of the lateral ventricle and confirmed with double labelling with N200 neurofilament marker (M) showing association of AT8-positive grains with axons. N. In the subpial surface of the pes, a band of AT8 was noted in some case (between arrows), comparable to the subpial band in the cortex; this again had an axonal appearance with localisation with non-phosphorylate neurofilament (SMI3 shown in N), but not dendritic marker (MAP2 shown in O) which labelled the processes in underlying neurones only. P. Bundles of axons were also noted in the peri-ventricular region of the pes hippocampus white matter. Q. Occasional fibre bundles were noted in the amygdala with AT8 which were weakly nestin-positive. R. Labelling of neurones with 3R and (S) 4R tau isoforms was confirmed. Supplementary Figure 3 contains a panel of similar immunofluorescence images captured with a non-red/green colour spectrum. Bar is equivalent in A to 500 μm; B, C to 200 μm; H, I and J, to 75 μm; D, E, K, N, 0, P to 50 μm; F, G, Q, R, S to 25 μm.

Figure 4. The effect of (A) age at time of surgery and (B) age of onset of epilepsy on the modified tau score in the epilepsy cohort.

Scatter plot graphs with linear correlation curve fitted. Spearman correlation shows a weak relationship between both clinical factors (r= 0.39 and r= 0.21 respectively) and are not statistically significant ($P > 0.05$). Individual points may overlap. Interrupted line indicates 95% confidence interval for the mean modified tau score for the given age.

Figure 5. Correlation between modified tau score and change in verbal memory domains and graded naming test scores.

Decline in cognitive scores from pre-operative assessment to one year post-temporal lobe resection shows a significant negative relationship with increasing tau burden for verbal memory domains and graded naming (Spearman coefficient r= −0.66, $*P < 0.01$, for verbal learning; r= −0.44, $*P < 0.05$, for verbal recall and r= −0.50, $*P < 0.05$, for graded naming test). Interrupted line indicates 95% confidence interval for the mean cognitive score for the given modified tau score.

Supplementary Figure 1. Diagramatic representation of patterns of accumulation of tau in hippocampus (A-C) and temporal neocortex (D-F) in HS/temporal lobe epilepsy cases compared to Alzheimer's disease and CTE. **A.** The normal hippocampal efferent pathways are shown, taken from Duveroy's atlas of The human hippocampus; in red (indirect) and green (direct) hippocampal pathways from the entorhinal cortex and efferent pathways (EF, yellow lines) in the alveus. **B.** Alzheimer's disease: In Braak's staging there is sequential p-tau accumulation (indicated with brown fill) in CA1 followed by the subiculum with granule cells involved late in the course of Alzheimer's disease. **C.** In temporal lobe epilepsy/HS loss of neurones in CA1 and CA4 are typically early events in childhood/young adulthood. Tau accumulation in HS is prominent in axons of these pathways affected in some cases- the sprouted mossy fibres (shown as brown lines) , axons of the granules cells and axons in the alveus. Possibilities are that these patterns (i) reflect disruption of anterograde/retrograde transneuronal axonal spread of tau in the abnormally connected epileptic hippocampus or (ii) that this is neuronal activity driven in patients with frequent seizures. **D.** Temporal neocortex: In Alzheimer's disease in stage V, large numbers of AT8 positive pyramidal cells are present in the deeper layers of the medial temporal lobe, particularly long projecting neurones in layer V and III with sparing of mid-layers (IV) resulting in a 'tram-line' staining pattern (brown fill). Tau in Alzheimer's disease primarily accumulates /propagates in long myelinated axons and later maturing neurones. **E.** Classical CTE patterns include the presence of subpial astrocytic tangles, accumulation in superficial cortical layers, around vessels, in sulci and in white matter axons. **F.** In temporal lobe epilepsy -HS with low tau burden as shown, subpial axonal and granular tau aggregates in the cortex are observed. HS = hippocampal sclerosis, CTE = chronic traumatic encephalopathy, GCL = Granule cell layer, PP=performant pathway, MFP = mossy fibre pathway, SC=Schaffer collaterals, EF = efferent pathway of outgoing axons from CA1 and subicular pyramidal neurones in the alveus to the fimbria.

Supplementary Figure 2. Modified tau score representative images

Example images of tau pathology (AT8 immunohistochemistry, brown stain) representative of each modified tau score (left hand column). Descriptions for each score are found in Table 1. Scale Bar 20 μm in all images apart from modified tau score 4, third image from the left (10 μm) and modified tau score 6, first image from the left (35 μm). NT= neuropil thread, SPB= sub-pial band, WM= white matter, NFT= neurofibrillary tangle.

Supplementary Figure 3. Immunoflourescence images taken with alternate colour spectrum

Immunofluorescence panel of images captured with a non-red/green colour spectrum more suitable for colourblinds. Similar images are found in Figure 1 and Figure 3. A. Axonal labelling with AT8 in the cortex (case 24) in inset and double labelling with neurofilament (N200) showing beaded like AT8 staining along the trajectory of radial cortical axons. B. Overlap of ZnT3 and AT8 was noted in the CA4 region corresponding to mossy fibre end terminals. C. Granular aggregates did not appear to co-localise with dendrites on double labelling with MAP2. D. Double labelling of AT8 with ZnT3. E. Labelling with GFAP confirmed that the AT8-granular aggregates were not in astroglia but through highlighting the glial foot processes along the vessels, indicated their proximity to vascular channels. F. Double labelling with N200 showing overlap within the dentate gyrus. G. AT8-positive granules were observed in close proximity, surrounding a CA4 neurone and its processes. H. Occasional fibre

bundles were noted in the amygdala with AT8 which were weakly nestin-positive. Bar is equivalent to 50 µm.

Supplementary Table 1. Individual patient data on area of temporal lobe analysed and clinical age data

Supplementary Table 2. Primary antibodies used in immunofluorescent studies

Supplementary Table 3. Classification of tau pattern in the temporal lobe

Supplementary Table 4. Clinical characteristics in relation to modified tau score

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