

Neurofibrillary tangle pathology and Braak staging in chronic epilepsy in relation to traumatic brain injury and hippocampal sclerosis: a post-mortem study

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The long-term pathological effects of chronic epilepsy on normal brain ageing are unknown. Previous clinical and epidemiological studies show progressive cognitive decline in subsets of patients and an increased prevalence of Alzheimer's disease in epilepsy. In a post-mortem series of 138 patients with long-term, mainly drug-resistant epilepsy, we carried out Braak staging for Alzheimer's disease neurofibrillary pathology using tau protein immunohistochemistry. The stages were compared with clinicopathological factors, including seizure history and presence of old traumatic brain injury. Overall, 31% of cases were Braak Stage 0, 36% Stage I/II, 31% Stage III/IV and 2% Stage V/VI. The mean age at death was 56.5 years and correlated with Braak stage ($P < 0.001$). Analysis of Braak stages within age groups showed a significant increase in mid-Braak stages (III/IV), in middle age (40–65 years) compared with data from an ageing non-epilepsy series ($P < 0.01$). There was no clear relationship between seizure type (generalized or complex partial), seizure frequency, age of onset and duration of epilepsy with Braak stage although higher Braak stages were noted with focal more than with generalized epilepsy syndromes ($P < 0.01$). In 30% of patients, there was pathological evidence of traumatic brain injury that was significantly associated with higher Braak stages ($P < 0.001$). Cerebrovascular disease present in 40.3% and cortical malformations in 11.3% were not significantly associated with Braak stage. Astrocytic-tau protein correlated with the presence of both traumatic brain injury ($P < 0.01$) and high Braak stage ($P < 0.001$). Hippocampal sclerosis, identified in 40% (bilateral in 48%), was not associated with higher Braak stages, but asymmetrical patterns of tau protein accumulation within the sclerotic hippocampus were noted. In over half of patients with cognitive decline, the Braak stage was low indicating causes other than Alzheimer's disease pathology. In summary, there is

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evidence of accelerated brain ageing in severe chronic epilepsy although progression to high Braak stages was infrequent. Traumatic brain injury, but not seizures, was associated with tau protein accumulation in this series. It is likely that Alzheimer's disease pathology is not the sole explanation for cognitive decline associated with epilepsy.

Keywords: Braak stage; epilepsy; head trauma; hippocampal sclerosis

Abbreviations: AT8 = anti-phosphorylated tau antibody; GFAP = glial fibrillary acidic protein

Introduction

Epilepsy is a common disorder that can be complicated by neuro-behavioural co-morbidities including cognitive impairment (Hermann *et al.*, 2008a). Around a third of patients have recurring seizures are not controlled by conventional medical (French, 2007; Kwan *et al.*, 2010) or even appropriate surgical treatments (McIntosh *et al.*, 2001; Spencer and Huh, 2008; Thom *et al.*, 2010b). The long-term effect of chronic epilepsy on the adult and ageing brain is an important but neglected area (Hermann and Seidenberg, 2007; Hermann *et al.*, 2008b). There is evidence that early onset of epilepsy may result in impairment of intellectual ability and specific cognitive functions, including memory (Hermann and Seidenberg, 2007; Helmstaedter and Elger, 2009). Progressive cognitive deterioration over time has also been shown, particularly in people with temporal lobe epilepsy (Oyegbile *et al.*, 2004; Hermann *et al.*, 2006, 2008a; Marques *et al.*, 2007). Epidemiological studies of co-morbidities have demonstrated an increased prevalence of dementia and Alzheimer's disease with chronic epilepsy (Gaitatzis *et al.*, 2004a; Tellez-Zenteno *et al.*, 2005; Hermann *et al.*, 2008a) and an increased relative risk for dementia in epilepsy compared with the general population (Breteler *et al.*, 1995). Furthermore, patients with chronic epilepsy are more exposed to lifestyle and risk factors that promote cerebrovascular disease, vascular dementia and Alzheimer's disease (Hermann *et al.*, 2008b).

In addition, neuroimaging data show widespread cortical changes in chronic epilepsy, including grey matter volume reduction and cortical thinning, particularly studied in association with temporal lobe epilepsy (Cormack *et al.*, 2005; Lin *et al.*, 2007; McDonald *et al.*, 2008; Riederer *et al.*, 2008; Bernhardt *et al.*, 2009; Keller *et al.*, 2009). These abnormalities may progress over time (Bernhardt, 2009) and an association of these changes with cognitive impairment has been shown (Baxendale *et al.*, 1998; Hermann *et al.*, 2006; Keller *et al.*, 2009). Despite these converging epidemiological, psychometric and neuroimaging data, there is no neuropathological study exploring brain ageing in chronic epilepsy, in particular the cellular mechanisms that underpin any age-accelerated cerebral atrophy.

Existing pathological evidence of neurodegenerative processes in epilepsy is available from studies of young adult patients who have undergone surgical treatment. Excessive deposition of amyloid- β protein was reported in patients with epilepsy aged 36–61 years compared with controls (Mackenzie and Miller, 1994; Gouras *et al.*, 1997). Neurofibrillary tangle formation was noted in a lobectomy specimen resected at the age of 27 years from a

patient with epilepsy and history of head injuries, in the absence of amyloid- β deposition (Geddes *et al.*, 1999). In epilepsy-associated pathologies, age-related neurofibrillary tangle formation has been reported in focal cortical dysplasia (Sen *et al.*, 2007a, 2008) and deregulation of the cdk5 pathway shown in hippocampal sclerosis (Sen *et al.*, 2006, 2007b). These observations could indicate a vulnerability to abnormal tau phosphorylation in focal epilepsy.

The aim of this study was to review a post-mortem epilepsy series to explore the effects of decades of seizures on brain ageing, in particular for Alzheimer's disease neurofibrillary pathology, and to identify any clinicopathological risk factors.

Materials and methods

Case selection

Post-mortem cases ($n = 138$) were included from the archives in the Department of Neuropathology, National Hospital for Neurology and Neurosurgery, London, collected over the period 1988–2009. We included all cases where there was a documented history of chronic, partially-responsive or drug-refractory epilepsy where tissue blocks were available. Epilepsy was the primary neurological diagnosis in this cohort and patients where seizures had developed during the course of a neurodegenerative illness, such as Alzheimer's disease, were not included. The majority (103 cases) had been residents at the National Society for Epilepsy (NSE) at the Chalfont Centre, with detailed medical records of their epilepsy through their lifetime. Furthermore, 49 of the 138 cases (35.5%) were classified as sudden unexplained death in epilepsy with known drug-resistant epilepsy. As such, this cohort represents patients with severe epilepsy. All post-mortem tissue had been retained for use in research with next of kin consent and in keeping with the code of the Human Tissue Authority (2006). The Joint Research Ethics Committee of the Institute of Neurology and National Hospital for Neurology and Neurosurgery approved this study.

Clinical data

The epilepsy history was reviewed in each case and the epilepsy syndrome (based on seizure semiology, electroencephalographic, neuroimaging and in some, genetic data) was recorded (Commission on Classification and Terminology of the International League Against Epilepsy, 1989; Engel, 2006). The duration of epilepsy (age of onset of habitual seizures to most recent recorded seizure), type and frequency of seizures were noted. Any significant head injuries were noted (both clinically documented episodes or based on evidence derived from MRI) and whether head injury pre-dated or followed

onset of epilepsy. Documented learning disability earlier in life or progressive cognitive decline over time during adulthood, were recorded. Evidence for progressive cognitive decline was obtained from repeat neuropsychological assessments and—where available—cognitive status examinations, most usually the Mini-Mental State Examination. All patients identified had spent at least their latter years in a residential setting and for most there was sufficient documentation from life planning reviews to derive information about their care needs and cognitive status. Using this information, the Clinical Dementia Rating Scale was applied by an experienced neuropsychologist (P.T.) with ratings made in six domains (memory, orientation, judgement and problem solving, community affairs, home and hobbies and personal care) from mild to severe dementia (Morris, 1993). A low rating was made only if they were limited in performing activities due to mental decline and not just due to physical frailty.

Braak staging

Formalin-fixed, paraffin-embedded archival tissue blocks of all cases, including both the right and left mesial temporal lobe and hippocampus, were identified. The hippocampal blocks were selected from the level of the lateral geniculate nucleus or red nucleus. Following assessment of the temporal lobe block in each case, sections from prefrontal cortex, temporal neocortex (superior and middle temporal gyrus) bilaterally and occipital (calcarine/striate) cortex unilaterally were also included in cases noted to reach Braak stage III in the mesial temporal lobe section. Immunohistochemistry for phosphorylated tau protein using monoclonal antibody AT8 was carried out on 8 µm formalin sections (1:1200, Innogenetics, Autogen Bioclear). Positive (cases with Alzheimer's disease) and negative controls were used in each staining run.

Following analysis of the AT8 staining, Gallyas silver staining from temporal and occipital sections was carried out on selected cases from each Braak stage to confirm reliability of AT8 immunostaining and staging (data not shown). Immunohistochemistry for amyloid-βA4 peptide (1:100, Dako) was also carried on either the left or right temporal lobe block from each case and in both sides with unilateral hippocampal sclerosis. In addition, in four selected cases, double immunofluorescence labelling for AT8 and glial fibrillary acidic protein (GFAP) on 8-µm sections (AT8 diluted 1:1200 and polyclonal GFAP 1:500, Dako) was carried out, detected with anti-Rabbit Alexa Fluor 594 and Fluorescein with Tyramide Signal Amplification (PerkinElmer Life and Analytical Sciences), counterstained with DAPI (4',6-diamidino-2-phenylindole dihydrochloride, Vector Labs). Sections were viewed with a Leica SP2 laser confocal microscope.

Braak staging was carried out according to the published standards ((Braak *et al.*, 2006) (Supplementary Table 1 and Fig. 1). Staging was carried out by three assessors independently (M.T, M.N., R.P.), in accordance with recent guidelines (Alafuzoff *et al.*, 2008). In all cases, there was agreement between two of three assessors for Braak stage and in 50% there was agreement by three assessors (97% of these disagreements were by one stage only and 75% of these for Stages 0–III). Amyloid-β immunohistochemistry was assessed semi-quantitatively as absent, moderately frequent or frequent amyloid plaques (Supplementary Fig. 1). In cases with low Braak stage where clinical cognitive decline was confirmed, addition immunohistochemistry for alpha-synuclein (Leica/Novocastra, clone KM5, dilution 1:50, formic acid pretreatment), ubiquitin (Dako; dilution 1:1200) and TDP-43 (Abnova, clone 2E2-D3, dilution 1:30000) was carried out on a selected hippocampal block.

Other pathology features

The presence of hippocampal sclerosis was evaluated including the pattern of sclerosis (classical, atypical) and whether bilateral or unilateral. In asymmetrical bilateral cases with hippocampal sclerosis, if a classical pattern was present on one side this was recorded as a classical hippocampal sclerosis diagnosis. The qualitative assessment of hippocampal sclerosis at post-mortem has been previously validated in a stereological study (Thom *et al.*, 2005). Dynorphin immunohistochemistry for confirmation of epilepsy-specific mossy fibre sprouting was carried out in cases with hippocampal sclerosis (Thom *et al.*, 2009). Pathological evidence of old traumatic brain injury, for example old cortical contusions or subdural haematoma, was recorded based on macroscopic and histological sections. The presence of cerebrovascular disease (degenerative small vessel white matter disease, regional cortical infarction, spontaneous cerebral haemorrhage or lacuna infarcts) was noted. The presence of atheroma in the main cerebral vessels alone was not regarded as cerebrovascular disease.

Analysis

Statistical analysis of associations between Braak stage, clinical and pathological features was carried out using SPSS (version 16) using Pearson's correlation, ANOVA, paired *t*-tests and multifactor linear regression analysis; $P < 0.01$ were considered statistically significant.

Results

Braak staging in epilepsy cohort

Thirty-one per cent of cases were Braak Stage 0 (virtually no AT8 labelling), 15% Stage I, 21% Stage II, 13% Stage III, 18% Stage IV and 2% Stage V; no Braak stage VI cases were identified. Overall 63% of cases were male, but there was no significant influence of sex on Braak stage. The mean age at death was 56.5 years (range 15–96). There was a significant positive correlation between Braak stage and age at death ($P < 0.0001$) (Table 1). Comparing the age distribution in our series with a previous published large post-mortem series of 2661 cases of a general autopsy practice (Braak and Braak, 1997), a relative over-representation of young to middle age adults was noted (age range 30–50) (Fig. 1A). This likely reflects higher age-corrected mortality rates in patients with severe epilepsy, for example from premature unexpected deaths (Gaitatzis *et al.*, 2004b). When age-related Braak scores in the epilepsy series were compared with this control ageing population (Braak and Braak, 1997), the frequency of lower Braak stages in the youngest adults was similar. For example in the 30–35 years of age group, 79% in the epilepsy series compared with 83% of cases in the normal ageing series were Braak Stage 0 (Fig. 1B and C). However in the middle-aged group (40–65 years), increased representation of low (I/II) and mid (III/IV) Braak stages were apparent in the epilepsy series with significant increases noted for Braak Stages (III/IV) ($P < 0.01$). For example, 1.8% of patients aged 50–60 years were Braak Stage III/IV in the normal ageing series compared with 37.5% in the epilepsy series (Fig. 1B and C).

Table 1 Braak staging in relation to epilepsy history

Clinical feature	Number of cases in which data available	Mean (range) or frequency (%)	Mean (range) or frequency (%) in different Braak stages					Significant association with Braak Stage	
			0	I	II	III	IV		V
Age of onset of seizures years (range)	113	10.23 (0.3–78)	9 (0.3–29)	10.7 (0.5–58)	11.3 (1–60)	6.5 (0.5–77)	13.8 (0.3–78)	6.1 (1.5–13)	NS ($P = 0.52$)
Mean duration of epilepsy years (range)	106	42.3 (1–86)	26.4 (1.5–64)	42.1 (2–71)	50.8 (1–79)	52 (8–86)	48.8 (1–76)	55.3 (44–62)	$P = 0.518^a$
Mean age at death (range) years	138	56.5 (15–96)	35.1 (15–67)	58.7 (29–85)	67.6 (40–86)	68.6 (38–90)	68.4 (33–96)	70 (61–55.3)	$P < 0.0001^a$
Seizure types									
GS (including secondary generalized)	123	96.7	93.9	100	100	88.2	92	100	NS ($P = 0.7$)
Complex partial seizures	99	77.8	76.2	61.5	88.9	78.6	76.2	66.7	NS ($P = 0.238$)
Maximum (GS) seizure frequency recorded	60	8.1/month (0.1–200)	12.3 (1–60)	3.1 (0.1–12)	14.5 (0.1–200)	2.4 (0.1–10)	2.2 (0.1–10)	0.5 (0.1–1)	NS ($P = 0.77$)
Total number of generalized seizures	18	488 (3–2600)	560 (400–724)	530 (35–1366)	510 (3–2600)	1250	172 (46–300)	–	NS ($P = 0.73$)
Learning difficulty diagnosed	62	59.7	58.8	88.9	66.7	20	50	50	NS ($P = 0.199$)
Progressive cognitive decline/dementia	69	65.2	42	42.9	64	67	70.5	67	$P < 0.01^b$

^a Statistical analysis between Braak stages with ANOVA (multiple linear regression analysis was also employed for these parameters to factor in the contribution of age of death on Braak stage).

^b Statistical analysis between groups includes all cases in each group.

GS = generalized seizures; NS = not significant.

Braak staging in relation to seizure history, epilepsy syndrome and cognitive decline

Generalized seizures (whether primary or secondary generalized) were reported as occurring in 96.7% patients and complex partial seizures in 77.8%; there was no association between these seizure types and Braak stages (Table 1). Other seizures, including myoclonic (10.4%), simple partial seizures (20%) and status epilepticus (31%) were less frequently recorded overall and not statistically analysed between Braak groups.

The mean age of onset of epilepsy in this series was 10.2 years (range 3 months to 78 years) and was not significantly different between Braak stages (Table 1). The mean duration of epilepsy was 42.3 years (range 1–86 years). Longer duration of epilepsy was significantly associated with higher Braak stage ($P < 0.001$). However, using multiple linear regression analysis, duration of epilepsy was not associated with Braak stage when the age of patient was considered ($P = 0.52$). Seizure events (total number of seizures or frequency) are a further measure of lifetime seizure burden. Data regarding maximum seizure frequency was available in 60 patients with maximum recorded generalized seizure frequency of 200/month (mean 8.12/month). In addition, accurate estimates of the total number of generalized seizures were recorded in 18 patients with a mean of 488 (range 3–2600). There was, however, no correlation between seizure frequency or number and Braak stage. Sudden unexplained deaths in epilepsy were significantly more frequent in lower Braak stages ($P < 0.001$); however, sudden unexplained death in epilepsy was significantly associated with younger age at death (mean age at death 40 years; range 15–79 years).

An epilepsy syndrome diagnosis was possible in over half of cases, either during the patients' lifetime or retrospectively confirmed following review of the clinical notes and investigations, including genetic studies in some cases (Table 2). There was a significant difference in the Braak stages between epilepsy syndrome diagnoses, with partial epilepsies (including temporal lobe epilepsy) more often associated with higher Braak stages than genetic or idiopathic generalized epilepsies.

In 69 patients, there were records of psychometric tests carried out in adult life and cognitive decline was confirmed in 45 (65%) with severe dementia in 29, moderate dementia in 14 and mild dementia in two (Table 1). In 11/69 patients, learning disability was noted on the first evaluation, in addition to progressive cognitive decline. Over the entire series, there was no association between diagnosis of learning disability and Braak stage ($P = 0.19$) (Table 1). Seventy-seven per cent of patients with Braak score of 3 or more showed progressive cognitive decline. Of note, 24 patients with cognitive decline had low Braak scores (0–II); these included four cases with traumatic brain injury, nine with cerebrovascular disease, three with Dravet syndrome, one with progressive myoclonic epilepsy, five with bilateral hippocampal sclerosis and one patient with hippocampal Lewy bodies on alpha-synuclein staining.

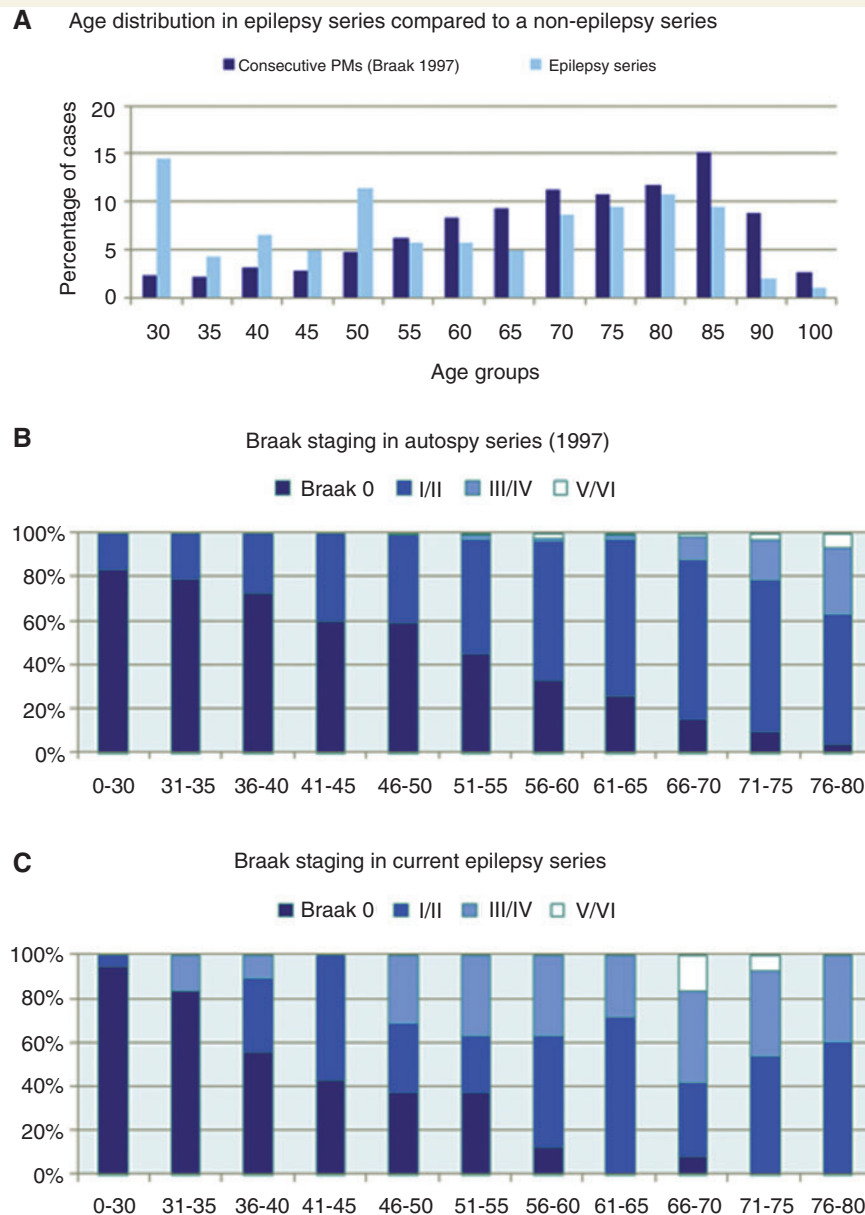


Figure 1 (A) Distribution of age at death in current epilepsy series: 138 cases compared with 2661 consecutive adult post-mortems (PMs) in a series published by Braak and Braak (1997) highlighting a greater representation of younger adult deaths in epilepsy series. (B) Braak stages in age categories from 0 to 80 years at 5-year intervals taken from the same series of Braak and Braak (1997) demonstrating progressive increase in higher Braak stages with normal ageing. (C) Comparative age categories in current epilepsy series with evidence for increased representation of mid-Braak stages in middle-aged adults (35–65 age range). Paired *t*-tests between normal ageing and epilepsy series confirmed significant differences for Braak stage III/IV ($P = 0.001$) compared with other stages; 0 ($P = 0.014$), I/II ($P = 0.062$) and V/VI ($P = 0.63$).

Braak staging in relation to trauma, cerebrovascular disease and malformations of cortical development

The presence of old traumatic brain injury, most frequently frontotemporal contusions, was identified in 30% of patients on macroscopic and histological examination of the brain (Table 3). There was a correlation between pathological evidence of

traumatic brain injury and clinical documentation of head injury, which was recorded in 44% of patients in this series ($P < 0.01$; Pearson's correlation). Of note, only three patients in this series were categorized as having post-traumatic epilepsy (Table 2). In the majority of patients, head injury was incurred as result of falls and accidents following onset of seizures. There was a significant correlation between the pathological identification of traumatic brain injury and higher Braak stage ($P < 0.01$) (Fig. 2). The presence of cerebrovascular disease was also significantly associated

Table 2 Epilepsy syndrome and Braak staging in 138 cases

Epilepsy syndrome classification and aetiology	Syndrome	Number of cases (of 138)	Braak stages (% cases)					
			0	I	II	III	IV	V
Partial/focal/structural	Partial epilepsy	66	17.7	12.9	29	16.1	24.2	0
	Temporal lobe epilepsy	16	43.8	0	37.5	0	6.2	12.5
	Post-traumatic epilepsy ^a	3	33.3	0	0	33.3	33.3	0
Genetic/idiopathic	Idiopathic generalized	7	28.6	42.9	0	14.3	14.3	0
	Dravet syndrome	3	33	67	0	0	0	0
	Progressive myoclonic epilepsy	1	0	100	0	0	0	0
Unknown	Syndrome classification not possible	46	43.5	17.4	10.9	10.9	15.2	2.2

a Post-traumatic epilepsy has been included as a separate category for the purposes of this study. There was a significant difference between Braak staging and epilepsy syndrome (ANOVA) ($P < 0.01$).

with higher Braak stages ($P < 0.001$). There was a significant association between the post-mortem diagnosis of cerebrovascular disease and older age at death ($P < 0.01$), but not between traumatic brain injury and age of death ($P = 0.56$). Furthermore, using multiple linear regression analysis to factor for the patients' age at death, traumatic brain injury remained significantly associated with Braak stage ($P < 0.0001$), whereas the effect of cerebrovascular disease became less significant ($P = 0.068$). Cortical malformation was identified in 11% of cases, including focal cortical dysplasia type IIB, tuberous sclerosis, polymicrogyria, and subependymal nodular or lamina heterotopia. Neurofibrillary tangles were occasionally observed in neurons within the malformations in some cases, particularly focal cortical dysplasia, as previously reported (Sen *et al.*, 2007a), but diagnosis of malformations of cortical development was not associated with higher overall Braak stage (Table 3).

Braak staging and hippocampal sclerosis

Hippocampal sclerosis was identified in 40% of the cases with a classical pattern (neuronal loss in CA4 and CA1) in 28.9% and an atypical pattern (neuronal loss restricted to either CA1 or the CA4 region) in 11.1%. In 19.3% of patients (48% of cases with hippocampal sclerosis), hippocampal damage was bilateral. The validity of qualitative assessment of hippocampal sclerosis in post-mortem tissues has been previously verified in a stereological study (Thom *et al.*, 2005). Dynorphin immunohistochemistry also confirmed epilepsy-specific patterns of mossy fibre sprouting in association with hippocampal sclerosis, as previously reported (Houser *et al.*, 1990; Thom *et al.*, 2005). There was no correlation overall between the presence, pattern or bilaterality of hippocampal sclerosis and Braak stage in this series (Table 3). Bilateral cases with hippocampal sclerosis did not demonstrate TDP-43 positive inclusions in contrast to previous reports of hippocampal sclerosis associated with Alzheimer's disease and dementia (Amador-Ortiz *et al.*, 2007) but in keeping with previous studies of TDP-43 in hippocampal sclerosis associated with epilepsy (Lee *et al.*, 2008).

Seven cases had Braak Stage III or more and unilateral classical hippocampal sclerosis; in six a marked asymmetry of AT8 accumulation within the hippocampal subfields was observed

(Figs 3 and 4). On the sclerotic side, significant diminution of AT8 immunoreactivity was noted in CA1 region compared with the non-sclerotic side (Figs 3A–B and 4). However, progressive accumulation of AT8 in the subiculum, molecular layer of the dentate gyrus, CA2 and lastly CA3/4 with advancing Braak stages on the sclerotic side, paralleled corresponding subfield accumulation on the non-sclerotic side, although a reduced load (Figs 3F–J and 4). Furthermore, AT8 accumulation in hippocampal projection cortex (frontal, temporal poles and deep entorhinal cortex layers) appeared symmetrical between hemispheres in these unilateral classical cases with hippocampal sclerosis (Fig. 3E and G).

Tau protein in astrocytic cells and distribution in temporal neocortex

Variable gliosis was noted in the neocortex in all cases with epilepsy, particularly in the subpial region (Chaslins' gliosis) and layer I. AT8-positive astrocytes (as confirmed with double labelling for GFAP in selected cases) were noted in 35% of all cases, located in layer I (in 52%) and/or periventricular region (in 68%) (Fig. 5A–C). They were present in all age categories, the youngest 15 years, but overall AT8-positive astrocytes correlated significantly with advancing age of patient ($P < 0.001$) as well as Braak stage ($P < 0.001$). Their morphology included the typical thorn-shaped astrocytes as previously reported in ageing and Alzheimer's disease (Schultz *et al.*, 2004). The presence of AT8-positive astrocytes was significantly associated with the presence of traumatic brain injury ($P < 0.01$) with 53.7% of patients with traumatic brain injury having AT8-positive astrocytes compared with 27% with no evidence of traumatic brain injury. In addition, preferential distribution of tau in cortical sulci was noted in 5.8%, around cortical penetrating vessels in 6.5% (Fig. 5D and E) and the white matter in 8% of cases.

Amyloid- β positive plaques

There was an absence of amyloid- β positive plaques in 66% of epilepsy cases with occasional, moderate and frequent plaques noted in 14%, 12% and 8% of cases, respectively. The presence and frequency of amyloid- β immunostaining was associated with higher Braak stages ($P < 0.0001$) but not with pathological

Table 3 Braak staging in relation to neuropathological features

Pathology feature	Number in series in which data available	Frequency present in series (%)	Frequency present in different Braak stages (%)						Significant differences between Braak stages
			0	I	II	III	IV	V	
	All series								
Pathological evidence of traumatic brain injury	137	30	14.0	23.8	25	47.1	52	66.7	$P < 0.0001^a$
History of head injury in clinical record	118	44.9	38.7	27.8	44.4	43.8	65.2	66.7	NS ($P = 0.22$)
Pathological evidence of cerebrovascular disease	134	40.3	17.1	23.8	53.6	52.9	75	0	$P = 0.068^a$
Malformation of cortical development ^a	133	11.3	12.5	0	13.8	6.2	20	0	NS ($P = 0.67$)
Hippocampal sclerosis patterns									
Classical	135	28.9	26.2	25	34.5	29.4	25	66.7	NS ($P = 0.82$)
Atypical		11.1	9.5	10	6.9	11.8	20.8	0	NS ($P = 0.8$)
No hippocampal sclerosis		60.0	64.3	65	58.6	58.8	54.2	33.3	NS ($P = 0.8$)
Bilateral		19.3	19	10	17.2	23.5	29.2	0	NS ($P = 0.49$)
Brain weight (g) (range)	128	1243	1322	1278	1225	1210	1178	1113	NS ($P = 0.068$)
		(788–1993)	(900–1760)	(1043–1550)	(878–1590)	(860–1993)	(788–1590)	(1031–1165)	

^a Malformations include focal cortical dysplasia type IIB, tuberous sclerosis, polymicrogyria and nodular heterotopia. Statistical analysis using ANOVA (multiple linear regression analysis was also employed to factor in the contribution of age of death on Braak stage).

evidence of traumatic brain injury ($P = 0.99$). Beta-amyloid immunostaining in unilateral cases with hippocampal sclerosis with high Braak stages showed asymmetrical patterns in some cases, but this was not as consistent a finding as for AT8 immunohistochemistry.

Discussion

The pathological effects of decades of seizures on the brain have been little explored. In this post-mortem series of patients with mainly long histories of drug-resistant epilepsy, we have confirmed age-accelerated tau-protein accumulation. The extent of tau-pathology correlated with clinical cognitive decline although few cases with higher Braak Stages (V/VI), associated with high likelihood of dementia (Braak and Braak, 1997; Duyckaerts *et al.*, 1997; Iqbal and Grundke-Iqbal, 2008) and based on National Institute of Ageing-Reagen Institute (NIA-RI) criteria (1997), were identified in our series. The lack of Braak Stage VI cases in our post-mortem series could reflect the younger age range and higher standard mortality rates in chronic epilepsy (Gaitatzis *et al.*, 2004b), with premature death occurring before any progression to higher Braak stages.

Alzheimer's disease is a multifactorial and heterogenous condition (Iqbal *et al.*, 2010) and known risk factors for sporadic Alzheimer's disease include age, genetic factors including ApoE $\epsilon 4$ genotype, and head trauma. The influence of seizures on Alzheimer's disease pathology is unknown. Alzheimer's disease pathology in young adults has been reported in epilepsy surgical tissues (Mackenzie and Miller, 1994; Gouras *et al.*, 1997; Geddes *et al.*, 1999; Sen *et al.*, 2007a) and dysregulation of cdk5 and its activators, known to regulate tau-phosphorylation (Iqbal and Grundke-Iqbal, 2008), has been shown in hippocampal sclerosis in epilepsy (Sen *et al.*, 2006, 2007b). This raises the possibility that seizures, or their cellular effects, might influence degenerative neuronal pathways or promote a susceptibility to Alzheimer's disease pathology. In this post-mortem series, however, we failed to show a correlation between the maximum frequency of seizures, seizure number, type, age of onset or duration of epilepsy and the extent of neurofibrillary pathology. Although syndrome classification was not possible in all cases, partial and symptomatic epilepsies were more often associated with higher Braak stages than idiopathic epilepsies or those with a known genetic cause. This supports the notion that an underlying structural cortical abnormality, as the cause for epilepsy, is more relevant to promoting Alzheimer's disease pathology, but requires further investigation.

Our study supports a relationship between head injury occurring in patients with epilepsy and progressive neurofibrillary tangle pathology. Our findings invite comparison to the chronic traumatic encephalopathy occurring in sports people following repetitive closed head injuries (Corsellis and Brierley, 1959; Corsellis, 1989; McKee *et al.*, 2009). Chronic traumatic encephalopathy is characterized by accumulation of tau protein particularly in mesial temporal structures, as recently reviewed (McKee *et al.*, 2009). In chronic traumatic encephalopathy, a preferential localization of neurofibrillary tangles in a perivascular location, sulcal depths or superficial cortex is noted. Astrocytic tangles are prominent in

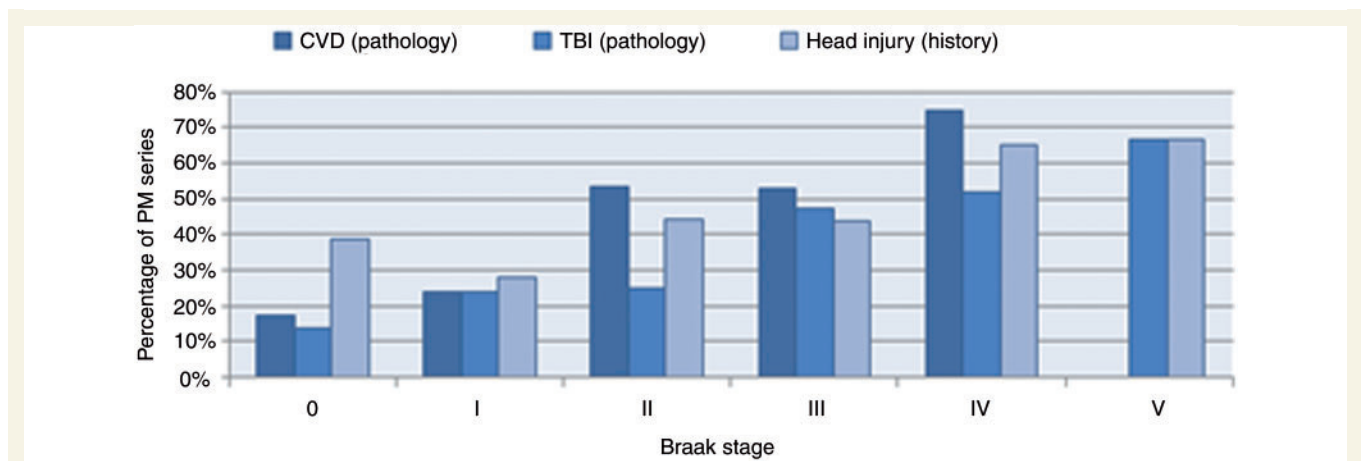


Figure 2 Bar chart representing the frequency of history of head injury, pathological confirmation of traumatic brain injury (TBI) and cerebrovascular disease (CVD) in different Braak stages. PM = post-mortem.

superficial layers, and subpial and periventricular zones, with amyloid- β deposition being a less constant feature (McKee *et al.*, 2009). In the current epilepsy series, a history of head injury was recorded in nearly half of the patients, although detailed information of the number of trauma episodes per patient was not available. In only three cases was head trauma considered to be a cause of seizures, injury pre-dating the onset of epilepsy. Pathological evidence of traumatic brain injury, mainly in the form of old frontotemporal contusions, was present in 30%. We demonstrated an association between traumatic brain injury and Braak stage. Furthermore, 66% of cases were amyloid- β negative and the presence of amyloid- β did not correlate with traumatic brain injury. In addition, astrocytic tau deposits were noted in a third of cases in our series in a periventricular or subpial location similar to reports in cases with chronic traumatic encephalopathy (McKee *et al.*, 2009) and their presence was significantly associated with traumatic brain injury. Although morphologically similar to the thorn-shaped astrocytes associated with ageing (>60 years) (Schultz *et al.*, 2004), in our epilepsy series tau-positive astrocytes were noted as early as 15 years of age. Although the precise relationship between single head injury, cognitive decline and Alzheimer's disease is ambiguous (Breteler *et al.*, 1995; Jellinger, 2004), the findings in the present series all underscore the observation that head injury, or more probably repetitive head injury, acquired as a result of drug-resistant epilepsy, is associated with the development of neurofibrillary tangle pathology. In clinical practice, our findings support recommendations that promote prevention of risk of head injury due to seizures.

Cerebrovascular disease was noted in 40% of this series. Epidemiological studies have shown that cerebrovascular disease is more common in chronic epilepsy than control groups (Gaitatzis *et al.*, 2004a; Hermann *et al.*, 2008b). Antiepileptic drugs as well as lifestyle factors in epilepsy patients may have adverse effects on cerebral vasculature (Hermann *et al.*, 2008b). We demonstrated an association between cerebrovascular disease and Braak stage, which became less significant when the patient's age of death was considered. In contrast, traumatic brain injury, which was not

significantly associated with patient's age, remained highly associated with Braak stage following multivariate statistical analysis.

The hippocampus is affected early in both Alzheimer's disease and chronic traumatic encephalopathy. Alzheimer's disease is associated with an increased incidence of unprovoked seizures (Palop and Mucke, 2009), which are reported to develop late in the course of the disease (Mendez *et al.*, 1994). In this series, we were careful not to include patients in whom a neurodegenerative illness, in particular Alzheimer's disease, was the primary diagnosis with secondary symptomatic seizures. Sclerosis of the hippocampus is one of the most common and well-characterized pathologies identified in both post-mortem (Corsellis, 1957; Margerison and Corsellis, 1966; Meencke *et al.*, 1996) and surgical series of patients with epilepsy, particularly temporal lobe epilepsy (Bruton, 1988; Blumcke, 2009). In surgical series, hippocampal sclerosis is typically observed in young adulthood, in the context of refractory seizures with sclerosis visible on MRI and confirmed in resected specimens (Wieser, 2004). The neuronal loss is centred on CA1 with more variable loss in other subfields and is accompanied by mossy fibre pathway reorganization (Houser *et al.*, 1990). Hippocampal sclerosis pathology may also arise in the elderly due to heterogeneous causes including anoxic-ischaemic injury and varied neurodegenerative conditions and is associated with slowly progressive amnesia and dementia without seizures (Probst *et al.*, 2007; Zarow *et al.*, 2008). The prevalence of hippocampal sclerosis in non-epilepsy elderly post-mortem series is ~16% (Dickson *et al.*, 1994) and is bilateral in 50% of these (Zarow *et al.*, 2008). The pattern of neuronal loss typically involves both CA1 and the subiculum.

Our post-mortem series represents patients with varied epilepsy syndromes and without systematic or serial MRI examination in the majority so that we cannot confirm the time course for the development of hippocampal sclerosis. The mean age of onset of epilepsy was 10.2 years overall (and 7 years in cases with hippocampal sclerosis). The pattern of hippocampal neuronal loss was characterized by sparing of subicular neurons with associated mossy fibre sprouting in the dentate gyrus, typical of hippocampal

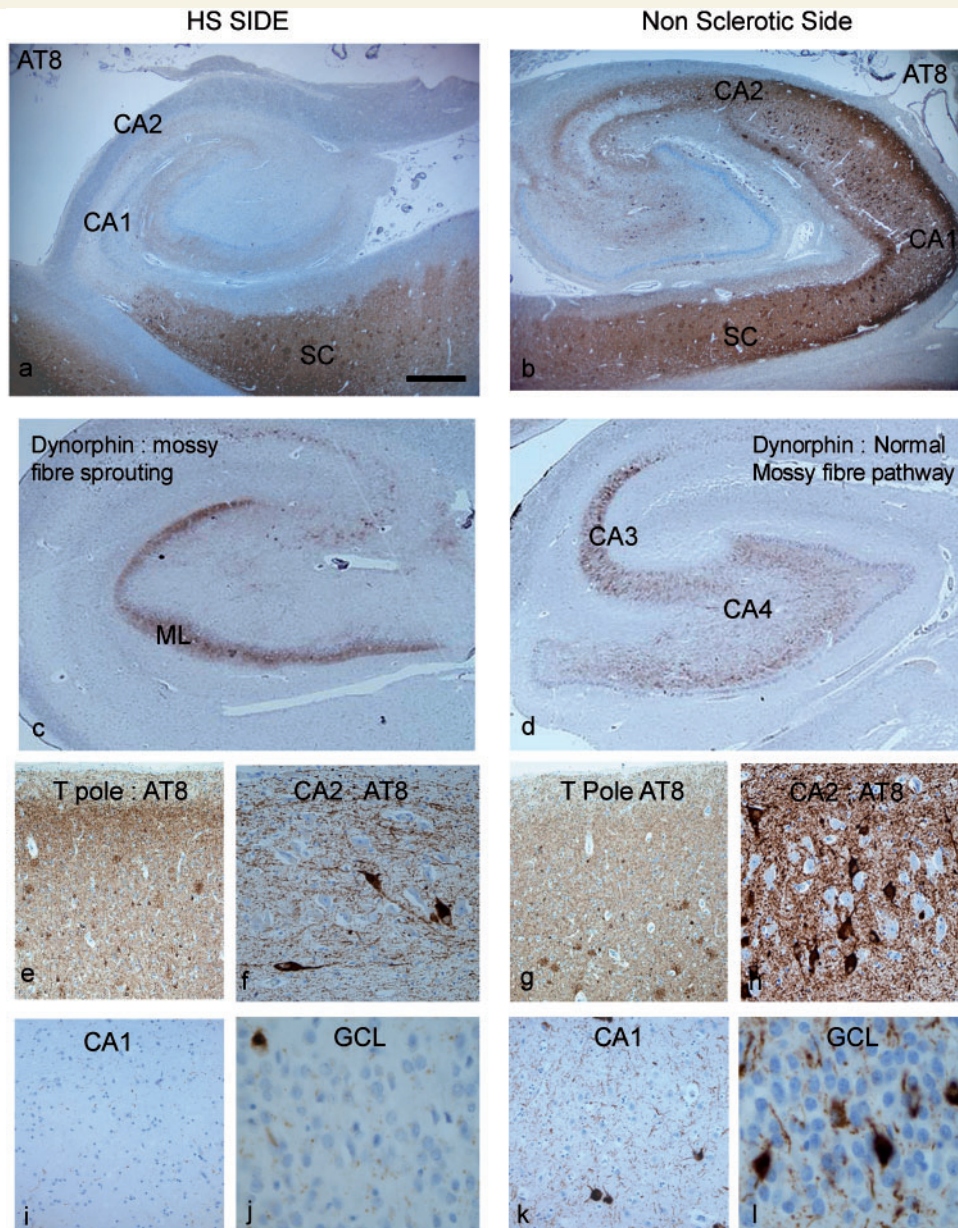


Figure 3 AT8 staining in cases with unilateral hippocampal sclerosis. The figures on the *left* show staining in the sclerotic hippocampus (HS) with the paired corresponding non-sclerotic hippocampus on the *right*. (a and b) Case of overall Braak stage V demonstrating abrupt transition of staining for AT8 between the subiculum and CA1 with a relative lack of staining in CA1, corresponding to the region of neuronal loss on the sclerotic side; AT8 positivity in CA2 and the molecular layer of the dentate gyrus is present, albeit reduced, compared with the non-sclerotic hippocampus. (c and d) Dynorphin stained sections of the corresponding hippocampi shown in a and b highlighting the normal mossy fibre projection pathway on the non-sclerotic side projecting to neurons in CA4 and CA3 (d). In contrast, mossy fibre sprouting into the molecular layer (ML), with reduced staining in CA3 and CA4, is observed with dynorphin on the side with sclerosis (c). Comparison between b and d also highlights that the region corresponding to the mossy fibre trajectory is relatively late for AT8 pathology. (e and g) Temporal pole sections (representing a hippocampal cortical projection region) showing similar AT8 staining between left and right hemispheres in a case with asymmetrical hippocampal sclerosis (case with Braak stage V). (f and h) CA2 sector (case with Braak stage IV) showing reduced staining in the side with sclerosis and similarly for CA1 sector (i and k; case with Braak stage III) and the granule cell layer (GCL) (j and l; case with Braak stage IV). Scale bar: a–d = 1000 μ m, e–l = 100 μ m. SC = subiculum.

sclerosis in epilepsy (Thom *et al.*, 2009). In addition, the paucity of AT8 labelling in the region of sclerosis including threads, ghost tangles or astrocytes supports the view that the sclerosis predated the tau accumulation. All these features are evidence that favours

an epilepsy-associated, rather than Alzheimer's disease-associated, pathogenesis of hippocampal sclerosis in our series. We identified hippocampal sclerosis in 40% overall, bilateral in 48%, which is comparable to a previous series of 650 post-mortem epilepsy cases

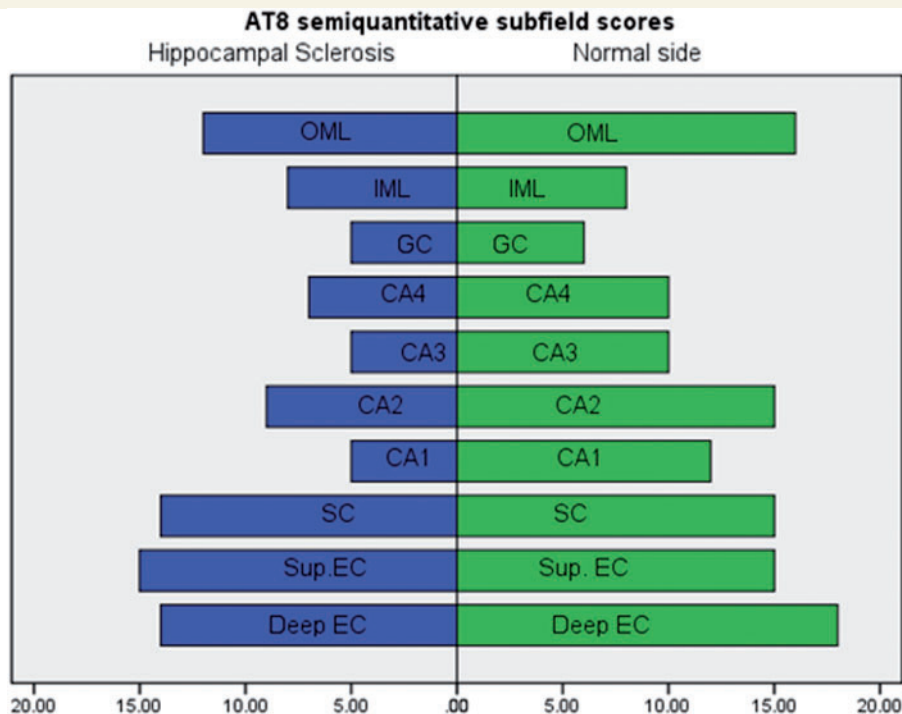


Figure 4 Distribution of AT8 pathology in hippocampal subfields (CA4, 3, 2 and 1), dentate gyrus granule cells (GC), internal molecular layer (IML) and outer molecular layer (OML)] subiculum (SC) and entorhinal cortex (superficial and deep cortex). The AT8 was scored semiquantitatively as: 0 = no staining, 1 = occasional neuropil threads and tangles, 2 = moderate numbers of neuropil threads and tangles, 3 = extensive deposition of neuropil threads and tangles. The accumulative count for six cases with unilateral hippocampal sclerosis and overall Braak stage of III–V are compared graphically for the side with sclerosis compared with the contralateral non-sclerotic hippocampi. There was overall significantly reduced AT8 labelling on the sclerotic side ($P = 0.007$), the greatest reduction of labelling on the sclerotic side involving CA1, CA4 and CA3 subfields where greatest neuronal loss is perceived.

that documented hippocampal sclerosis in 30.5%, with bilaterality in 56% (Meencke *et al.*, 1996). Hippocampal sclerosis patterns reflected those reported in surgical temporal lobe epilepsy series (Bruton, 1988; Blumcke *et al.*, 2007; Thom *et al.*, 2010a), albeit with greater representation of atypical patterns as previously noted (Thom *et al.*, 2009). There was no association between the presence or pattern of hippocampal sclerosis and the Braak stage.

Tau accumulation in both Alzheimer's disease and normal ageing progresses through the hippocampus in a stereotypical sequential and hierarchical fashion (Braak *et al.*, 2006; Duyckaerts *et al.*, 2009; Frost *et al.*, 2009), which may reflect anterograde propagation along neuroanatomical pathways (Duyckaerts *et al.*, 2009; Lace *et al.*, 2009). Six distinct stages of hippocampal involvement have been proposed that correspond with known connections between the hippocampus proper and the entorhinal cortex (Lace *et al.*, 2009). Tau accumulation commences in CA1/subicular border, then to the outer followed by inner molecular layer of the dentate gyrus, next CA1 and CA2 and lastly CA4/3. In the present series, six patients with unilateral hippocampal sclerosis, reorganization of the mossy fibre pathway as confirmed with dynorphin immunohistochemistry (Houser *et al.*, 1990) and higher Braak scores (III–V) showed asymmetry of AT8 staining in hippocampal subfields. Reduced AT8 staining on the sclerotic side was apparent in CA1 with the accumulation in

other subfields paralleling that on the preserved side, albeit reduced. Known hippocampal projection regions, including the frontal and temporal polar cortex, appeared symmetrically affected between hemispheres in these cases. In addition, in the non-sclerotic hippocampus, direct comparison between AT8 and dynorphin sections highlighted that neurons in the trajectory of a normal mossy fibre pathway [a component of the indirect hippocampal pathway with major input from the entorhinal cortex (Duvernoy and Cattin, 2005)], were relatively delayed in tau accumulation compared with adjacent subfields. These findings could argue against the 'pathway propagation' theory of tau accumulation but favour intrinsic cellular 'time-switches' or other mechanisms for this selective neuronal vulnerability of neurodegeneration.

There is an intriguing convergence between cellular regulatory pathways and factors that determine normal cortical development which, when deregulated, can lead to neuronal degeneration (Bothwell and Giniger, 2000; Wang and Liu, 2008; Mattsson *et al.*, 2010). Cdk5 is one such developmental regulatory protein (Lim and Qi, 2003), also pivotal in tau hyper-phosphorylation (Iqbal and Grundke-Iqbal, 2008). Cdk5 has been previously shown to be abnormally expressed in epilepsy-associated developmental pathologies such as focal cortical dysplasia (Sen *et al.*, 2008). Whether brains harbouring malformations are more vulnerable to superimposed neurodegenerative processes has been little explored. We have previously noted premature neurofibrillary

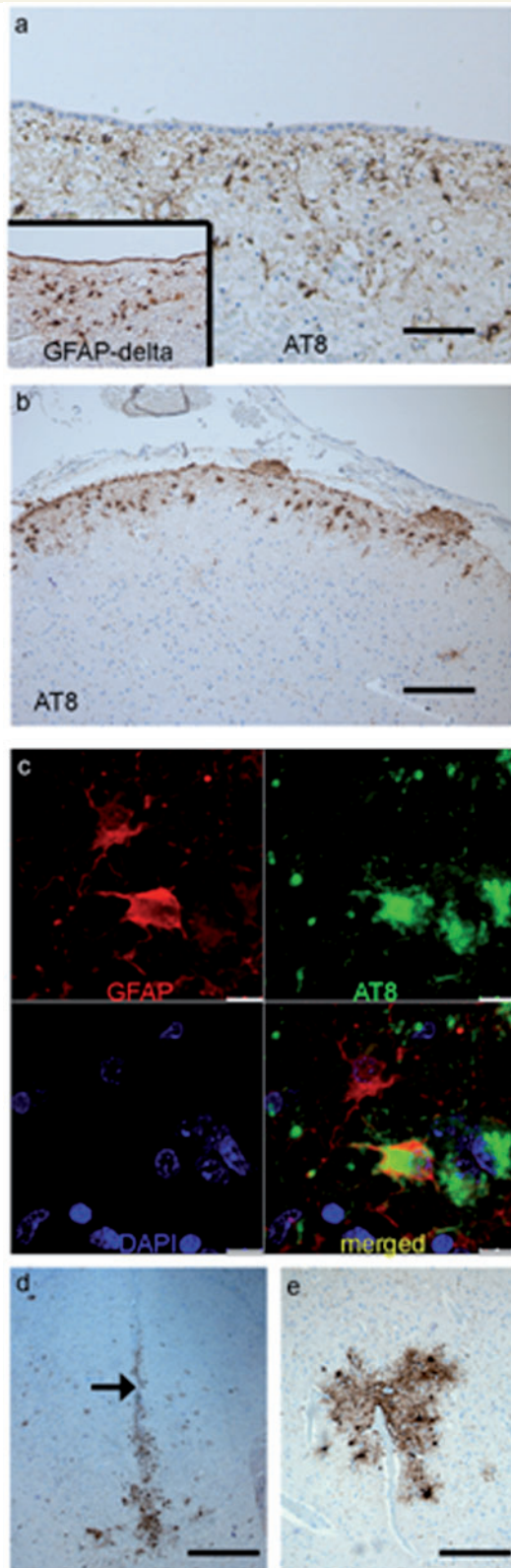


Figure 5 Astrocytic AT8. (a) Periventricular region with AT8 positive astrocytes in the subependymal region with corresponding region on GFAP-delta stained section from the same case shown in inset with similar distribution of morphologically similar cells. (b) AT8 staining showing astrocytic cells in cortical layer I and subpial region. (c) Confocal showing co-localization

tangle accumulation in the dysmorphic neurons of focal cortical dysplasia in patients with epilepsy (Sen *et al.*, 2007a). Overall, in the current series the presence of malformation of cortical development was not associated with higher Braak staging. A further study of susceptibility to neurodegenerative processes within the regions of cortical malformation is required.

Our study also highlights that in around half of the cases in the series, cognitive decline was not associated with tau accumulation, suggesting that other factors may play a role in cognitive decline associated with epilepsy. Developmental delay is a feature of some childhood-onset epilepsies although the influence of this on subsequent dementia is uncertain (Helmstaedter and Elger, 2009), and likely to be both heterogeneous and complex. In our series, a history of learning disability was not associated with a higher Braak stage. Furthermore, widespread cortical volume changes may be detected on MRI (Keller and Roberts, 2008), which may also relate to cognitive decline (Hermann *et al.*, 2008b). In a recent small series of patients with epilepsy and hippocampal sclerosis without neurodegenerative disease, gliosis preferentially involved frontal, temporal and orbitofrontal cortices, which we argued also reflected subtle traumatic brain injury (Blanc *et al.*, 2011). Classifications of epilepsies also recognize the existence of the concept of 'epileptic encephalopathy' where cognitive impairment, which may be progressive, is not explained by the underlying pathology at the light microscopic level but may be a manifestation of the seizures (Berg *et al.*, 2010). This is highlighted in the present series in three patients with Dravet syndrome with cognitive decline in which we have reported an absence of neuropathological or degenerative changes (Catarino *et al.*, 2011).

This study is limited in that we have, by necessity, studied cases with more severe epilepsy, which will not represent the broader epilepsy population. In addition, there are no genetic data available, in particular ApoE $\epsilon 4$ genotype; there is evidence that effects of head trauma are more severe in patients with this genotype (Friedman *et al.*, 1999; McKee *et al.*, 2009). Complete clinical data were not available in a proportion of cases, in particular for seizure frequency and total number of seizures. Clinical investigation patterns have varied over the 20-year-period of this brain collection. In a small number of cases, limited brain sampling at post-mortem did not allow a categorical exclusion of old trauma or vascular disease and such cases were not included in the analysis. For control data, we have used large published post-mortem series from a normal ageing, non-epilepsy population (Braak and Braak, 1997). This utilized silver methods rather than more sensitive AT8 immunostaining for Alzheimer's disease staging as currently recommended (Braak *et al.*, 2006). However, in a selection of cases, Gallyas silver staining showed a good correlation with AT8. In addition, as our Braak staging in young adults was very similar to the Braak (2006) study and we had few Braak V and no

between GFAP-positive astrocyte and AT8 in the periventricular region. (d) In a few cases, sulcal accumulation of tau was apparent in the neocortex and (e) perivascular tau distribution in the temporal neocortex. Scale bars: a, d, e = 50 μ m, b = 100 μ m, c = 10 μ m.

Braak VI stages overall, we consider that Braak staging has not been overestimated in the current study.

In conclusion, this study supports the occurrence of accelerated brain ageing in chronic epilepsy although progression to high Braak stages was infrequent, possibly because of higher rates of premature mortality. Traumatic brain injury, rather than seizures themselves, is identified as an associated factor for AT8 accumulation in this series, suggesting that it is important to protect the head in drug-resistant epilepsy causing falls. It is likely that Alzheimer's disease pathology is not the sole explanation for cognitive decline associated with epilepsy, the cause of which requires further investigation.

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Supplementary material

Supplementary material is available at *Brain* online.

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