Published in final edited form as:

Neurobiol Aging. 2018 February ; 62: 34–44. doi:10.1016/j.neurobiolaging.2017.10.002.

Early Alzheimer-type lesions in cognitively normal subjects

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Abstract

Amyloid deposits and tau-immunoreactive neurofibrillary tangles, together with neuronal and synaptic loss, are the neuropathological hallmarks of Alzheimer's disease (AD). Both proteins are present in the normal brain during aging. However, the temporal sequence of their involvement in the onset of AD pathology remains controversial.

To define whether amyloid β protein deposits or tau protein lesions appear first during normal brain aging, we performed an immunohistological study on serial sections from 105 autopsy brains (age range: 40 to 104 years) from patients free of clinical signs of cognitive decline, using anti-tau (AT8) and anti-amyloid (4G8) antibodies in the hippocampus, entorhinal cortex, inferior temporal cortex (Brodmann area 20), prefrontal cortex (Brodmann area 9), occipital cortex (Brodmann areas 17 and 18), and in the brainstem.

All cases older than 48 years displayed at least a few neurofibrillary tangles, which appeared more frequently in the entorhinal than in the transentorhinal cortex. Tau pathology in these areas preceded tau inclusions in the brainstem. Furthermore, the first site of the apparition of tau pathology is inconsistent, being the entorhinal cortex in most cases, and in fewer cases, the transentorhinal region.

There was no case presenting with amyloid deposition in absence of neurofibrillary tangles, lending evidence to the fact that neurofibrillary tangles appear earlier than amyloid plaques during normal brain aging. However, the role of amyloid in promoting tau deposition cannot be excluded in some cases, but may not represent the sole mechanism of disease induction and progression.

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The authors declare no conflict of interest and thank Mrs. M. Surini-Demiri for excellent technical assistance.

Keywords

Brain aging; Alzheimer's disease; amyloid; neurofibrillary tangle; primary age-related tauopathy

Introduction

Alzheimer's disease (AD) is one of the most invalidating brain illness in terms of handicap, clinical evolution, treatment, and health costs. An estimated 5.4 million people have been reported to suffer from AD in 2016 in the US (Alzheimer Association, 2016). Amyloid deposits (senile plaques, SP), and neurofibrillary tangles (NFT) associated to neuronal and synaptic loss are the neuropathological hallmarks of AD (Akram et al., 2008; Bussière et al., 2003a; Bussière et al., 2003b; Davies, et al., 1987; Masliah et al., 1989; Scheff et al., 1990).

Several hypotheses have been formulated to explain the pathophysiology of AD. Evidence from animal models has concluded that tau is required for amyloid β protein (A β) toxicity *in vivo* and both proteins have strong interactions. The mechanisms of this interaction is likely variable, based on available evidence: A β may cause tau-dependent excitotoxicity of NMDA receptors; (Ittner et al., 2010; Nussbaum et al., 2012;), or tau-dependent microtubule loss (King et al., 2006), as well as impairment of long-term potentiation (Shipton et al., 2011). However, other studies showed that tau formation is a first step and plays a role in synaptic loss or neuronal death (Bloom 2014; Crimins et al., 2013; Criscuolo et al., 2017; Nieweg et al., 2015; Spires-Jones and Hyman, 2014).

Most clinicopathological studies of AD find that A β alone is not sufficient for developing dementia (for review see Nelson et al., 2012). Despite the strong association between known mutations (APP, PSEN1, and PSEN2), genetic risk factors (e.g., APOE ϵ 4) and amyloid plaque formation, only NFT distribution and severity show correlation with the clinical signs of AD (Baner et al., 1996; Bennett et al., 2004; Crystal et al., 1993; Giannakopoulos et al., 2003; Guillozet et al., 2003; Haroutunian et al., 1999; Imhof et al., 2007; Jellinger and Baner, 1998; Nelson et al., 2012; Nelson et al., 2007; Tomlinson et al., 1970; Xuereb et al., 2000). Severe NFT burden in association neocortical areas is consistently correlated with dementia. Interestingly, however, oldest old cases can show high densities of NFT, mainly in hippocampus, without clinical expression (Nelson et al., 2012).

In fact, recent studies have revealed that neuropathology of the normal aging brain is fairly complex with the description of primary neuronal (Crary et al., 2014; Jellinger and Baner, 1998) and astroglial tauopathies (Crary et al., 2014; Kovacs et al., 2016). These cases underline that during aging multiple factors are likely to result in diverging neuropathological features.

The goal of our study was to examine which of NFT or SP appears first during normal brain aging, to identify and describe the localization of the earliest neurodegenerative lesions. For this purpose, a cohort of 105 brain autopsies from cognitively intact cases encompassing a broad age range (40–104 years) was examined for the presence of amyloid deposition and NFT.

Materials and Methods

Brain specimens were obtained from authorized autopsies at the Division of Geriatrics, University Hospitals of Geneva, Switzerland. Only cognitively intact subjects from the Geneva brain collection (N = 105; 46 male/59 female, age range 40–104 years, postmortem delay 31.6 ± 22.5 years) were included in this study (Kövari et al., 2011). The presence and severity of cognitive decline was evaluated by the Clinical Dementia Rating Scale (CDR) (Hughes et al., 1982). All cases with clinically documented cognitive decline, dementia (CDR >0.5), and any pathological lesions such as vascular lesion, tumors, meningitis, encephalitis, or other neurodegenerative diseases were excluded. Clinical neuropsychiatric data were used to assess the cognitive status of the individuals and family history was recorded to exclude early-onset dementia.

The demographic characteristics of the cohort are presented in Tables 1 and 2. Age ranged from 40 to 104 years (64.4 ± 19.1). There were 46 males (mean age 59.4 ± 16.1 years; 40 to 98 years) and 59 females (mean age 68.4 ± 20.5 years; 44 to 104 years, $p < 0.02$ ANOVA). The cause of death was available in 84 cases; most patients (29 cases) died from metastatic carcinoma without brain involvement, followed by pulmonary or cardiovascular causes, such as pulmonary embolism, bronchopneumonia, respiratory insufficiency (22 cases), myocardial infarcts, and cardiac failure (21 cases). Other patients died following septicemia, gastrointestinal pathologies, or acute subdural hematoma (12 cases).

For the present study formalin-fixed tissue blocks were taken from the left hippocampus together with the inferior temporal cortex (Brodmann area 20), prefrontal cortex (Brodmann area 9), occipital cortex (Brodmann areas 17 and 18), and the brainstem at the levels of the substantia nigra and locus coeruleus including the raphe at this level.

The tissue blocks were embedded in paraffin and 12 μm -thick adjacent sections were stained with hematoxylin-eosin or with antibodies against tau (AT8; Pierce Biotechnology, Rockford, IL, USA, 1:1,000, that recognizes the Ser202 and Thr205 epitopes) and A β (4G8, Signet Laboratories, Dedham, MA, USA, 1:2,000, that recognizes amino acids 18–22 of the A β protein).

In absence of tau- or A β -immunoreactive (ir) lesions or in cases with only few lesions, serial sections on the first histological slide were taken from the block, the entire paraffin block was cut into serial sections and every 20th and 21th slide were used for tau and A β immunohistochemistry, respectively. Lesions observed on the microscope were then mapped onto schematics of each level from the series manually. NFT, tau-ir neurites and SP were plotted in different colors for each case and each level separately on the schematics that included the hippocampus, and the entorhinal and inferior temporal cortex (Fig. 1 and 2). Representative examples of differences among cases are summarized on a mid-hippocampal level shown in Figure 3.

In all cases, the neuropathological severity of tau-ir lesions and amyloid deposition was determined using the Braak staging for NFT and the Thal phases for SP (Braak and Braak, 1991; Thal et al., 2002).

Results

Among the younger cases (age range 40–48 years; mean age 45.8 ± 2.2 years; Table 1), 5 out of 35 were devoid of tau pathology in the anterior hippocampus, entorhinal, and transentorhinal cortex, and all of the other regions examined. All subjects older than 48 years displayed at least some NFT (Fig.4).

In most cases, NFT appeared earlier in the entorhinal than in the transentorhinal cortex (for example see Fig. 5, 98 years old patient). NFT appeared in different mediolateral position, even in different levels of the entorhinal cortex (Figs. 1–5). NFTs outnumbered pretangles in all of the examined regions. In the absence of hippocampal tau deposition no NFT were observed in any neocortical region or in the brainstem in the examined cases. On serial sections of the same hippocampus, depending on the antero-posterior level, the NFTs are localized throughout the entorhinal cortex, distributed without an evident pattern (Fig. 1). The increase in tau burden according to age was also reflected by the Braak stages for NFT. For example, cases with severe tau deposition (Braak stages V: three cases, and VI: one case) had a mean age of 95.7 and 98 years (Table 1).

In younger subjects (40–48 years old) 32 cases out of 35 did not show amyloid deposition in any examined region. The severity of amyloid deposition was variable and independent of age, as some young cases showed more severe amyloid burden than older ones (Fig. 6). In this regard, Thal phase 1 cases had the highest mean age at 94.75 years, but younger cases, for example a 63 years old patient, had Thal phase 3 (Table 2). Table 3 shows the distribution of Thal phases at different Braak stages for NFT; interestingly, severe amyloid deposition was present in two cases with low tau pathology and two cases with Braak stage IV were free of amyloid deposition. However, A β deposition was the most frequent in the inferior temporal neocortex (Brodmann area 20; 25.7 % of all cases).

In the majority of cases, the earliest occurrence of tau pathology was observed in the entorhinal cortex, in complete absence of A β in the same area (Fig. 7, first row). However, in the 5 cases without NFT, no amyloid deposition was observed in the hippocampus and neocortical areas excepting only one case showing a slight amyloid deposition in the occipital cortex.

Even in the oldest-old persons, in contrast to NFT pathology, A β deposition was not always present. In these cases also, NFT were more frequent in the entorhinal cortex (100%), followed by the hippocampus, inferior temporal, superior frontal, and occipital cortex. Among the 100 other cases showing any degree of tau pathology in the entorhinal cortex only 25 presented with amyloid deposition in this region (Table 4). Both diffuse and neuritic plaques were present, latter mainly in cases with more severe tau pathology. In the brainstem 13.5 % of cases had tau inclusions. In all of these cases NFT were present in the locus coeruleus and all of these subjects showed NFT in the entorhinal cortex.

Discussion

Our study assessed the temporal and regional relationships of the appearance of AD-related neuropathological lesions in the normal aging brain. Brains from a wide age range and

without cognitive decline were studied, including relatively young cases permitted the assessment of neuropathology well before the onset of AD or mild cognitive impairment.

A key aspect of our study pertains to the temporal relationship of the appearance of NFT and SP in the cognitively intact aging brain. A considerable number of the cases were young at time of death (62 cases between 40 and 65 years), enabling us to evaluate AD pathology at early stages. This is a significant advantage over previous studies on the neuropathology of normal aging, including some of our own work (Bouras et al., 1994), which were based on cases of a higher average age, in which the burden of both NFT and SP is already high, giving essentially no possibility to determine which of these two lesions appears first (Bennett et al., 2004; Bouras et al., 1994; Caselli et al., 2010; Davis et al., 1999; Middleton et al., 2011). The present data suggest that NFT always precede SP in the normal aging brain. NFT are already present at Braak stage I at an age of 52.6 years on average (Table 1). Most importantly, in this sample of 105 autopsies, we found no case with A β pathology in absence of NFT. However, 76.2% of all cases showed no A β pathology while a high proportion, 95.2%, of them had NFT. In contrast to NFT – present in 100 out of 105 brains, even in young cases — we observed SP only in fewer than half of them, with a much higher average age. This constitutes, in our view, strong evidence that NFT appear during normal brain aging before amyloid deposition. In a study of a cohort with a young age of death, Braak and Del Tredici have reported comparable results regarding the precedence of NFT over SP (Braak and Del Tredici, 2011). Their series of 42 individuals shows however some differences compared to our study. They proposed subcortical regions such as the locus coeruleus and nucleus subcoeruleus as the site where NFT appear first. In contrast to their data, serial sections of these regions in our series revealed no cases with brainstem NFT in absence of entorhinal and transentorhinal tau deposition. These results confirm the recent data of Okamoto et al., (2017) who could not confirm that tau pathology begins in the brainstem, and propose a simultaneous appearance in hippocampus and LC. It is important to keep in mind that in the present study, serial sections of all brain regions under study were assessed to attain a higher probability of observing rare lesions. With this approach, lesions indeed begin in the entorhinal cortex and then propagate to other cortical areas. This is agreement with our previous findings in a quantitative neuropathological evaluation of a large series of elderly patients that described the distribution of lesions in aging and AD (Bouras et al., 1994). All of the cases were found to have NFT in the entorhinal cortex and most cases had some NFT in the CA1 field of the hippocampus and subiculum. In addition, the extent of amyloid deposition showed an important variability in the non-demented group: SP never occurred in the absence of NFT and when both present, they occurred in different cortical areas. Conversely, several cases contained NFT without amyloid deposition (Bouras et al., 1994). Similarly, Hyman et al. reported in 409 autopsies of patients older than 50 years that only 6 cases had SP without NFT, although 115 had NFT with no SP (Hyman and Gómez-Isla, 1997).

According to Iseki et al., the type of tau deposition (pre-tangle, NFT or ghost cell) could be different depending on amyloid burden and the regions of the hippocampus that are involved (Iseki et al., 2002). The observation that NFT appear well before SP in the normal aging brain has a major implication for the understanding of events that lead to developing AD. In our series only 9 cases out of 105 that had a Braak NFT stage of IV or higher (see Table 1).

Of these, only a couple showed significant amyloid deposition. However, it remains that all cases showing plaques had NFT, while a few cases had only tau pathology in absence of amyloid. Interestingly, 5 cases devoid of tau lesions had no amyloid deposition. Based on the data presented in table 3 a role of amyloid in promoting tau deposition cannot be excluded in certain cases, but this may not represent a universal mechanism. Our study did not investigate the presence of oligomeric forms of amyloid and should be extended to include more cases with Braak NFT stages IV and higher. Our data contrast with the observation of Davis et al. (1999) who concluded that A β should be the earlier step in AD pathology, whereas NFT appear second and correlate better with brain changes later on. The findings of the present study on normal aging, and also of earlier clinicopathological correlations in AD brain (Arriagada et al., 1992; Giannakopoulos et al., 2003; Guillozet et al., 2003; Hyman and Gómez-Isla, 1997), indicate that tau-related pathology not only appears first in the aging brain but also correlates with cognitive decline, while A β -related pathology does not. The absence of any distinguishable colocalization pattern in the initial stages of tau and A β pathology in the subjects of our study suggests that both lesions develop and progress independently in the normal brain. A β might however play a role in triggering clinical manifestations of AD at a later stage, yet this view is difficult to uphold given the existence of “tangle-only dementia” or “tangle-only AD” with no A β involvement, a well-known clinical and pathological entity (Bancher, et al., 1996; Yamada, 2003; Yamada, et al., 1996). Tangle-only dementia cases also reinforce the importance of tau deposition in developing dementia (Jellinger and Bancher, 1998).

Recently, cases of tau deposition in normal aging, NFT presence with little or no A β pathology were classified as primary age-related tauopathy (PART). PART is a neuropathological entity presenting clinically as normal cognition or only with mild cognitive decline but without clinical dementia (Crary et al., 2014; Jellinger et al., 2015; Josephs et al., 2017; Weller et al., 2015). The sample size of our study and the sensitivity for the detection at the single lesion level proposes that the cases qualifying as definite PART is as high as 72% and 15% as probable PART given the concomitant A β pathology (Table 3). Introducing PART as a common neuropathological entity, distinct from the neuropathological forerunners of AD may be compelling to explain the high prevalence of subjects with NFT without SP as well as clinical tau-only dementia and thus partially reconcile the amyloid cascade hypothesis with the well-established clinicopathological relevance of NFT in AD (Crary, 2016). Thus, PART should represent a wider aspect of normal or pathological aging a part of which, for some reason, also develops SP and subsequent AD. In fact, recent evidence suggests that key molecular and genetic characteristics of AD, namely the frequency of APOE4 allele, the presence of TDP-43-positive inclusions, Lewy bodies, and hippocampal sclerosis occur at a lower frequency in PART than in late-onset AD (Josephs et al., 2017). No subject had SP without NFT in our sample. Consequently, NFT appearing after SP as the main distinguishing feature of AD pathology from PART should be excluded. It remains plausible that in both PART and AD, initial pathology only involves NFT and patients develop SP later. As in all cases (all non-demented) of our series after age of 48 years NFTs were present, and 66% of them were free from amyloid deposition, these cases correspond to the description of PART. Based on this

observation, certain cases of PART could be considered as a form of clinically normal brain aging.

These observations suggest that the Braak and Braak (1991) scheme may not be universal and that PART or other entities within normal brain aging populations occur and may have a different clinical and neuropathological presentation. For example there are cases without significant pathology in brainstem, and with first appearance in the EC, or without amyloid pathology in the presence of tau AD-type pathology (Mungas et al., 2014; Nelson et al., 2009). As such brain aging is likely determined by diverse factors, genetic or environmental, which may result in these differences in neuropathological presentation.

Neuritic tau pathology, which was present in a large number of our cases, is known to be a good correlate of cognitive decline (Vana et al., 2011). Because all of our cases were non-demented it is difficult to assess a direct correlation between the severity of neuritic changes and cognitive deficits. Our observations reveal that there exists a substantial degree of variability in the apparent amount of neuritic lesions across cases, ages, and genders, suggesting that it may represent a precursory pathological sign of worsening of the disease in some of them. A larger-scale quantitative analysis including subjects with definite AD will be required to clarify this issue.

A recent study on a sample from the Geneva brain collection, spanning over a century, has demonstrated that amyloid burden in that entire population and in the patients with AD has significantly decreased over the last decades, while NFT burden increased in parallel to the prevalence of clinical AD in recent years (Kövari et al., 2014). This fact further weakens the argument of a pivotal causal role of A β in AD. A possible role of A β in AD pathology could be through the involvement of its soluble and oligomeric forms that were not detected in the present study. Benilova et al. (2012) reviewed evidence in favor of this possibility (Cerf et al., 2011; Cleary et al., 2005; McLean et al., 1999; Pike et al., 1991). In any case, the appearance of NFT well before SP could have a major implication for AD treatment and, notably, prevention strategies that should target NFT early on.

Our results also demonstrate that pathology corresponding to all of Braak stages may be present in a normal brain. In the older subjects of our study, even high numbers of NFT may be present, despite intact cognition, and these cases could be considered as PART. Our data confirm the observation that some oldest-old cases can present with severe NFT pathology without clinical symptoms (Bennett et al., 2009; Corrada et al., 2012; Giannakopoulos et al., 1996; Giannakopoulos et al., 1993; Hyman and Gómez-Isla, 1997; Imhof et al., 2007; Maarouf et al., 2011; Middleton et al., 2011; Mufson et al., 2016; Price and Morris, 1999). This observation may be related to a relative resistance or “neuronal reserve” indicating by the number of functionally impaired neurons (Bussière et al., 2003a; Bussière et al., 2003b; Davis et al., 1999; Giannakopoulos et al., 1996; Giannakopoulos et al., 1993; Imhof et al., 2007). Taken together, these findings propose that the spectrum of AD neuropathology may be present in normal brain aging.

The distribution pattern of amyloid deposits turned out to be of limited importance from a clinical viewpoint. Amyloid burden, in form of senile plaques has been consistently reported

to be non-correlated with cognitive status (Arriagada et al., 1992; Giannakopoulos et al., 2003). Several studies have described intact cognition in elderly people despite a high amyloid plaque load (Bussière et al., 2002; Corrada et al., 2012; Hyman and Gómez-Isla, 1997; Maarouf et al., 2011). In the present study, the stages of A β pathology according to Thal et al. (2002) show no relationship with cognitive decline, as amyloid deposition was often seen in non-demented subjects. This heterogeneity of amyloid load and the variation in different Braak NFT stages was earlier quantified with stereological methods (Bussière et al., 2002). In contrast to NFT, SP appear first in neocortical areas. It is also important to note that most subjects (77/105) had no A β pathology. In view of the variability of severity of the degenerative pathology across the age range, the A β and tau pathology may in fact not be related (Guillozet et al., 2003). In conclusion, our results show that NFT are likely to be the first lesion to appear during brain aging, as early as 40 years of age and point to a prominent role of tau-related mechanisms in the development of AD.

Acknowledgments

This study was supported in part by the Maria Zausi Memorial Foundation through the Greek National Scholarship Foundation (ST) and by NIH grant P50 AG005138 (PRH).

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Highlights

- We examined Alzheimer's-related lesions in cognitively normal aging
- A high variability in the localization of the first tau lesion is observed
- Neurofibrillary tangles appear earlier than amyloid plaques in normal aging

Figure 1.

Schematic representation of a series of antero-posterior levels of the hippocampus (A to F) as an example of the aleatory distribution of tau-ir lesions in a non-demented subject. (NFT blue triangles; tau-immunoreactive neurites orange lines). Schema of the hippocampal levels adapted from (Duvernoy, 2005)

Figure 2. Random distribution of tau-ir lesion on the serial section of the anterior hippocampus (NFT blue triangles; tau-immunoreactive neurites orange lines; scale bar 5 mm).

Figure 3.

Representative examples of localization of NFT (blue triangles), tau-ir neuritis (orange lines) and SP (yellow dots) mapped onto a photo of the anterior hippocampus in clinically control cases of different ages (scale bar: 5 mm). (CA1 = CA1 sector of the hippocampus; EC = entorhinal cortex; TrEC = transentorhinal cortex; TC = temporal cortex)

Figure 4. Interindividual heterogeneity of NFT distribution in the hippocampus and the temporal neocortex in non-demented people of different ages (AT8 tau immunohistochemistry; scale bar: 5 mm).

Figure 5.

Interindividual heterogeneity of NFT distribution in the different level of the entorhinal cortex in different ages of non-demented people (A–F, AT8 tau immunohistochemistry; scale bar on F: 5 mm). Representative examples of neuritic tau pathology in the different regions of the hippocampus. G: CA1 sector of the hippocampus, H: subiculum, I: entorhinal cortex, J: temporal neocortex; AT8 tau immunohistochemistry; scale bar on J: 200 μ m)

Figure 6. Interindividual differences in A β depositions in the hippocampal formation and the temporal neocortex in non-demented people of different ages (4G8 amyloid immunohistochemistry; scale bar: 5 mm).

Figure 7.

NFT and A β deposition in the hippocampal formation and the temporal neocortex in oldest old non-demented people. First line: 95 years old female, second line: 95 years old female, third line: 96 years-old female subjects. Left column: anti-tau (AT8); right column: anti-amyloid (4G8) immunostaining (A – F; scale bar: 5 mm). High magnification of neurofibrillary tangle (G) and cored senile plaque (H) (G: AT8 tau immunohistochemistry; H: 4G8 amyloid immunohistochemistry; scale bars 50 μ m on G, 100 μ m on H)

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Table 1

Mean ages according to Braak stages for NFT

	N	Mean age	SD	Min	Max
Stage 0	5	45.80	2.17	43	48
Stage I	43	52.60	12.47	40	97
Stage II	35	67.94	15.40	44	98
Stage III	13	82.31	16.54	49	104
Stage IV	5	88.60	14.31	63	95
Stage V	3	95.67	0.58	95	96
Stage VI	1	98	–	98	98

Stage 0: Cases without NFT

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Table 2

Mean ages according to Thal phases for SP

	N	Mean age	SD	Min	Max
Without SP	77	57.01	14.33	40	104
Thal phases					
Phase 1	4	94.75	6.13	87	102
Phase 2	11	77.45	18.86	45	98
Phase 3	10	89.00	9.84	71	98
Phase 4	3	85.33	19.34	63	97

Table 3

Distribution of subjects according to NFT and amyloid pathology combined

		Thal phases				
		Without SP	Phase 1	Phase 2	Phase 3	Phase 4
Stage I	38	1	2	1	1	1
Stage II	29	0	3	2	1	1
Stage III	3	2	4	5	0	0
Stage IV	2	1	0	1	0	0
Stage V	0	0	2	1	0	0
Stage VI	0	0	0	1	0	0
Total	72	4	11	11	2	2

Numbers represent the percent of the total number of subjects presenting any type of pathology

Table 4

Percentages of cases with tau and amyloid pathology*

	EC	HP	TC	FC	OC	LC
NFT	100	62	47	5	1	14
SP	25	17	27	23	23	0

* Percentages of NFT and A β deposition in the hippocampus and neocortical areas in the 100 cases presenting with tau pathology in the entorhinal cortex
EC = entorhinal cortex; HP = hippocampus, TC = temporal cortex, FC = frontal cortex, OC = occipital cortex, NFT = neurofibrillary tangle, SP = senile plaque