

REVIEW ARTICLE**The preclinical phase of the pathological process underlying sporadic Alzheimer's disease****Heiko Braak and Kelly Del Tredici**

Abnormal tau lesions (non-argyrophilic pretangle material, argyrophilic neuropil threads, neurofibrillary tangles) in select types of neurons are crucial for the pathogenesis of sporadic Alzheimer's disease. Ongoing formation of these tau lesions persists into end-stage Alzheimer's disease and is not subject to remission. The early pretangle disease phase is a focus of increasing interest because only abnormal forms of the microtubule-associated protein tau are involved at that point and, in contrast to late-stage disease when amyloid- β deposition is present, this phase is temporally closer to the prevailing conditions that induce the pathological process underlying Alzheimer's disease. Extracellular and aggregated amyloid- β may only be produced under pathological conditions by nerve cells that contain abnormal tau. One potential trigger for tau protein hyperphosphorylation and conformational change in Alzheimer's disease may be the presence of a non-endogenous pathogen. Subsequently, a predictable regional distribution pattern of the tau lesions develops in phylogenetically late-appearing and ontogenetically late-maturing neurons that are connected via their axons. It is hoped that hypotheses drawn from these considerations, as well as from recent tau dissemination models, from studies of variant tau conformers, and from tau imaging will encourage the development of new preventative and disease-modifying strategies.

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Abbreviations: HSV-1 = herpes simplex virus 1; NFT = neurofibrillary tangles; NT = neuropil threads

Introduction

The present review aims to summarize and discuss key features of the early stages of a pathological process that ultimately leads to clinically overt sporadic Alzheimer's disease in a small proportion of individuals. As in many other conditions, the early stages of this process remain asymptomatic. The overall process, which may begin in

young adulthood and childhood (Braak and Del Tredici, 2011a; Braak *et al.*, 2011; Duyckaerts, 2011; Elobeid *et al.*, 2011) is protracted; however, it is relentlessly progressive and irreversible (Montine *et al.*, 2012; Braak and Del Tredici, 2015a). Much later, with advancing age, some individuals cross a barely detectable threshold to the symptomatic phase (Sabbagh *et al.*, 2010; Albert *et al.*, 2011; Yaffe *et al.*, 2012). The prevalence of symptomatic cases

rises with increasing age and, for this reason, societies with longer life expectancies are subject to a growing Alzheimer's disease burden (Brookmeyer *et al.*, 2007; Mayeux and Stern, 2012).

The Alzheimer's disease-associated pathological process is almost entirely confined to the human CNS (Arnold *et al.*, 2010; Dugger *et al.*, 2013). Reliable morphological features of the Alzheimer's disease-related tau lesions make it possible to detect a typical lesional distribution pattern within the CNS, and these lesions appear to proliferate in a predictably systematic manner (Table 1, Figs 1A–D and 4B–F). Moreover, the developmental pattern and progression of the lesions also repeat phylo- and ontogenetic maturational phases of the CNS, however, this time in reverse (Braak and Braak 1996; Reisberg *et al.*, 1999, 2002). Accordingly, late-maturing structures are frequently the ones that display the disease-associated tau lesions particularly early. Phylogenetically older and ontogenetically early-maturing components, on the other hand, prove to be resistant. Here, we focus chiefly on those structures that first emerged late phylogenetically, i.e. during the period when the human species was evolving out of higher primate precursors (Rapoport, 1988, 1990, 1999; Rapoport and Nelson, 2011).

A shared hallmark of the structures that are vulnerable to the Alzheimer's disease-related process is that they serve 'higher' CNS functions characteristic of the human brain: either structures of the cerebral cortex itself or components of subcortical nuclei that diffusely project to and optimize complex functions of the human cerebral cortex (O'Donnell *et al.*, 2012). On the other hand, none of them are absolutely essential for the preservation of the 'primitive' brain functions required for existence, so that their partial loss or partial impairment does not jeopardize life expectancy. The following section is intended to briefly summarize the aforementioned hallmark structures serving higher CNS functions together with their major interconnectivities.

Anatomical considerations

The cerebral cortex of the mammalian brain consists of neocortical and allocortical components: neocortical areas are chiefly responsible for interactions with the external world and include primary fields (core fields) that are encircled by both first order association areas (belt areas) and high order association areas (Braak, 1980; Amunts and Zilles, 2001; Zilles and Amunts, 2010). The allocortex receives olfactory information and includes the olfactory bulb and related areas as well as important limbic system structures, such as the hippocampal formation and entorhinal region (Braak and Braak, 1992; Braak *et al.*, 1996; Amunts *et al.*, 2005). Together with these cortical regions, a functionally unified group of subcortical nuclei evolved, the ascending projections of which terminate diffusely in neocortical layers I–III and V–VI and in the allocortex. These subcortical nuclei (magnocellular nuclei of the basal

Table 1 Absence of tau lesions in $n = 10$ and development of early tau pathology in $n = 1885$ of 2366 cases by decade

Age (years)	Zero (AT8)	a–c (AT8)	1a–1b (AT8)	NFT I–II (Gallyas)
0–9	6	1	0	0
10–19	2	16	2	2
20–29	2	21	23	20
30–39		11	51	32
40–49		5	76	86
50–59		3	68	242
60–69			34	424
70–79			10	430
80–89			1	282
90–100				45
Total	10	57	265	1563

forebrain, oral nuclei of the raphe system, locus coeruleus) enhance cortical performance (Curcio and Kemper, 1984; German *et al.*, 1987, 1992; Dringenberg, 2000; Aston-Jones and Cohen, 2005; O'Donnell *et al.*, 2012).

In lower macrosmatic (endowed with a highly developed sense of smell) mammals, the neocortex usually is small and mainly consists of primary fields in contrast to the belt and association areas that are much less developed (Fig. 2A). The allocortex is well developed: major input to the entorhinal region originates from olfactory structures, whereas input from the neocortex is sparse. Special transition zones between the temporal neocortex and the allocortical entorhinal region do not exist. The subcortical nuclei with axons that project to and boost the performance of the cerebral cortex are not as well developed (Fig. 2A) as they are in microsmatic (endowed with a weakly developed sense of smell) primates and in humans (Fig. 2B).

In humans and higher primates, the belt areas and, in particular, the high order association areas of the neocortex expanded considerably in size (Fig. 2B). In addition, the primary fields became subject to an ever greater degree of differentiation and functional sophistication (Braak, 1980). By contrast, most allocortical structures devoted to olfactory processing are rudimentary compared with the corresponding areas in macrosmatic mammals (compare Fig. 2A with 2B), whereas the hippocampal formation, entorhinal region, and related areas are highly developed (Amunts *et al.*, 2005). The entorhinal region receives sparse information from olfactory areas. The transentorhinal region appears for the first time in microsmatic primates, where it is largest in the human brain and decreases markedly in size further down the primate scale (Braak and Braak, 1992). The transentorhinal region guides data from the neocortex to the entorhinal region (Fig. 2B). In fact, the hallmark of the human entorhinal region is the massive input it receives from the neocortex and the output of this information to the hippocampal formation and

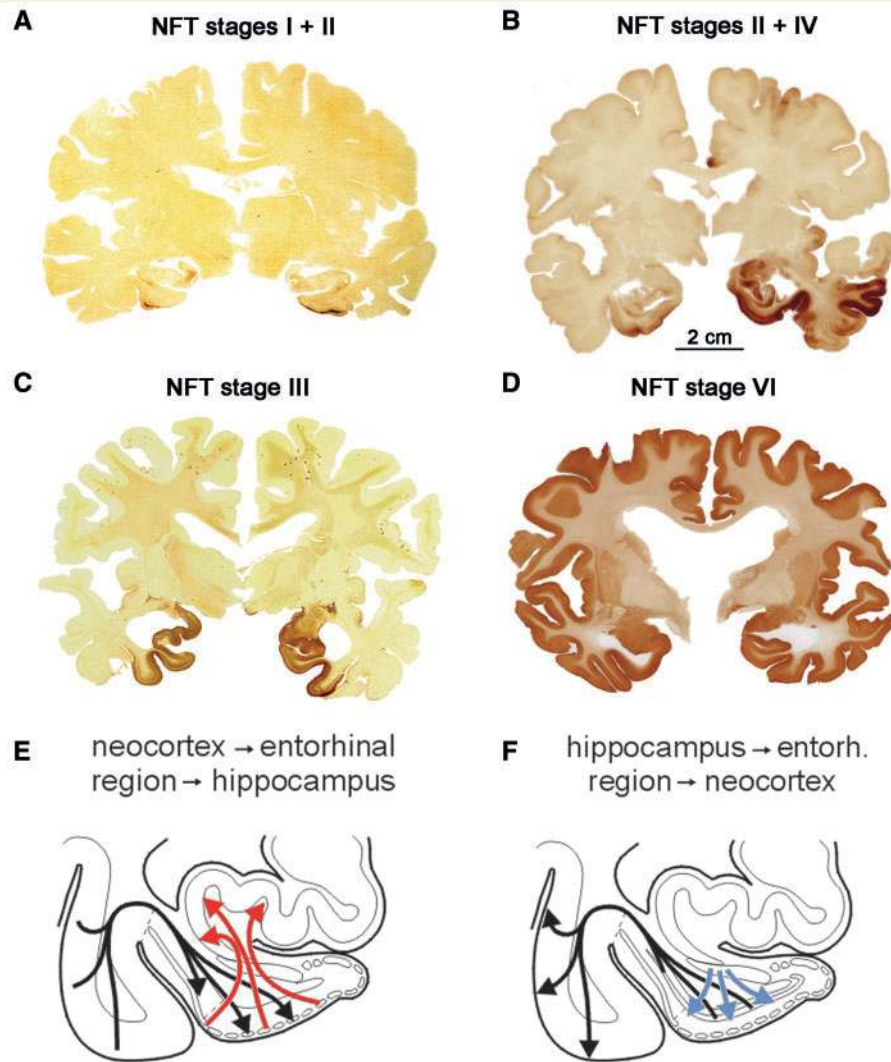


Figure 1 AT8-immunopositive NFT stages I–VI of the pathological process underlying sporadic Alzheimer's disease in coronal 100 μm sections cut perpendicular to the intercommissural axis. **(A)** NFT stages I (left) and II (right) in a cognitively unremarkable 80-year-old female, who died of myocardial infarction. The tau pathology develops first and foremost in the most superficial cellular layer (layer pre- α) of the transentorhinal (stage I) and entorhinal cortex (stage II). **(B)** NFT stages II (left) and IV (right) in a 75-year-old cognitively impaired male, who died of a metastatic pulmonary neoplasm. Differences between hemispheres usually do not exceed more than one NFT stage. The existence of such cases makes it very unlikely that a separate 'age-related non-Alzheimer's disease-related' tauopathy exists, in which, purportedly, the tau lesions intrinsically do not progress beyond the limits of the entorhinal cortex and hippocampus. **(C)** NFT stage III in a 90-year-old female (cause of death: malignant pancreas neoplasm). The section is cut through the hippocampal formation at the level of the uncus (left) and through the amygdala (right). Here, the Alzheimer's disease-related tau changes are seen not only in the transentorhinal and entorhinal cortices but also in the medially adjoining amygdala and hippocampal formation, as well as in laterally adjoining portions of the basal temporal neocortex. **(D)** NFT stage VI in a 72-year-old severely demented female patient with Alzheimer's disease (cause of death: aspiration pneumonia). Note that all portions of the cerebral cortex become involved in the late-phase of the Alzheimer's disease process. Scale bar in **B** applies to **A**, **C** and **D**. **(E** and **F)** Major entorhinal connectivities. **(E)** Neocortical afferences (black arrows) converge on the transentorhinal and entorhinal layer pre- α , whence the data are transferred via the perforant path (red arrows) to the hippocampal formation. The border between the entorhinal and transentorhinal regions is marked by a dashed grey line. **(F)** A dense back-projection from the subiculum in the hippocampal formation terminates in the entorhinal deep layer pre- α (blue arrows) and is transferred from there back to the neocortex (black arrows). Adapted with permission from Braak H and Del Tredici K, *Alzheimers Dement* 2012; 8: 227–33 and *Adv Anat Embryol Cell Biol* 2015a; 215: 1–162.

beyond (frontal neocortex) (Fig. 2B). In this evolutionary context, the functionally unified group of subcortical nuclei also developed new subdivisions, and the cortical projections from these more recent subdivisions reached the newer cortical structures, e.g. the transentorhinal

region, as well as the predominantly neocortically-oriented portions of the entorhinal region (Fig. 2B) (for such 'integrated phylogeny' see Rapoport, 1988, 1990, 1999).

To connect areas within the neocortex, pyramidal cells of layers II–III of primary sensory areas give rise to projections

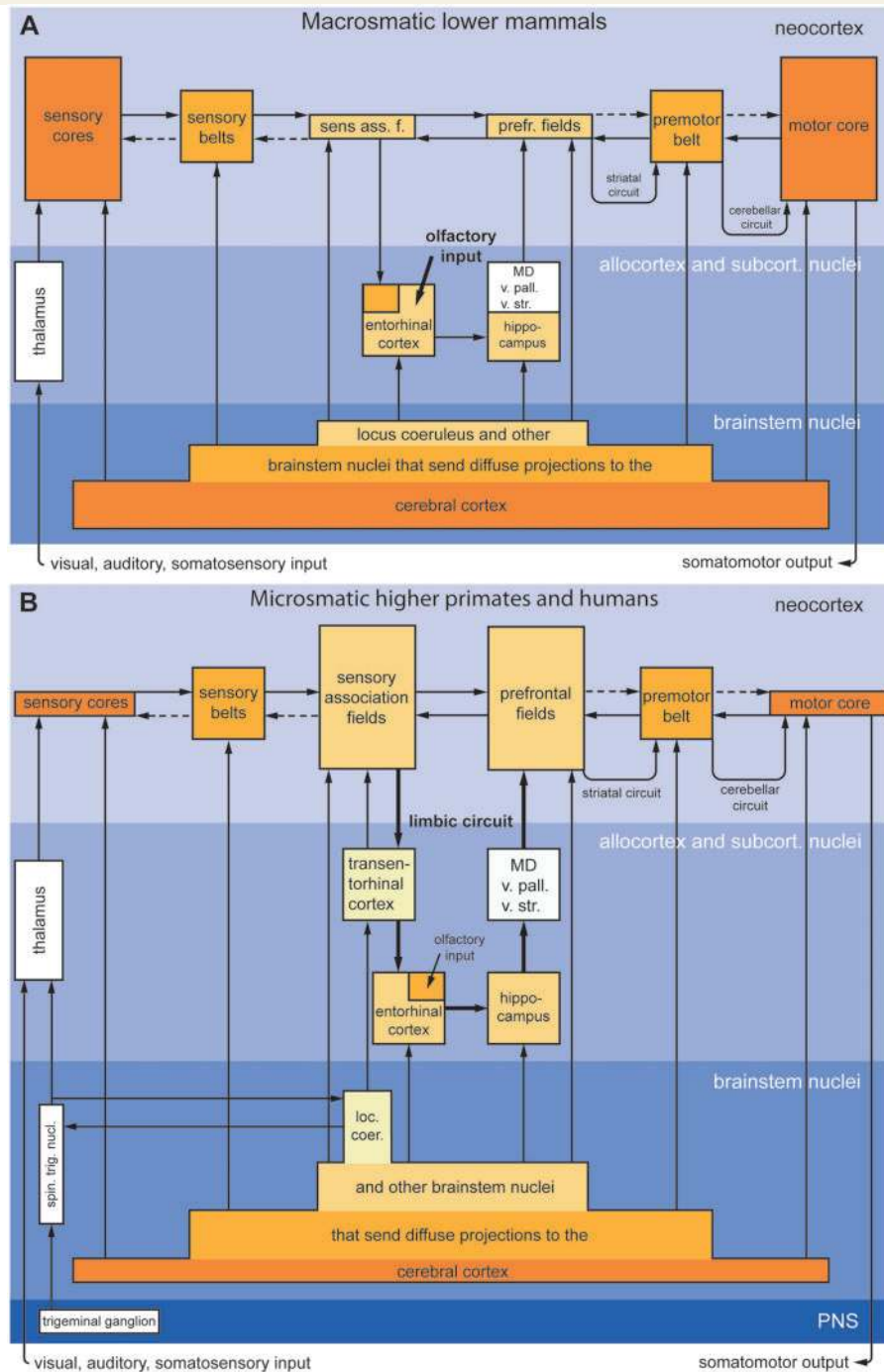


Figure 2 Two diagrams comparing the major interconnectivities between cortical regions that receive diffuse input from select subcortical nuclei in lower macrosmatic mammals (A) and in higher primates and humans (B). Visual, auditory, and somatosensory data flow via the thalamus to respective primary core fields of the neocortex and via projections to sensory belt areas and a variety of high order sensory association fields. From there, they are conducted via long and sparsely myelinated cortico-cortical tracts to the prefrontal association fields. Major routes for projections in the opposite direction are provided by the striatal and cerebellar circuits supplemented by cortico-cortical pathways (dashed arrows) that transmit the data through premotor areas to the primary motor cortex. Select data enroute from high order sensory association fields to the prefrontal cortex diverge from this data stream and eventually converge on the transentorhinal and/or entorhinal regions, thereby constituting the afferent arm of the limbic circuit. Projections from the entorhinal region and hippocampal formation (i.e. the efferent arm of the limbic circuit) reach the prefrontal cortex via the ventral striatum, ventral pallidum, and mediodorsal thalamus. (A) In lower macrosmatic mammals, the neocortex is small and mainly consists of primary fields. The belt areas and association fields are less well developed (shown here by the smaller size of these regions). The phylogenetic advance is indicated by degrees of shading, ranging from dark orange ('old') to light orange and dark yellow ('new'). The allocortex is highly developed in these animals. Olfactory data are processed predominantly in the entorhinal region, which receives only weak input from the barely developed high order sensory

(continued)

that reach layer IV of first order sensory association areas (belt areas). Similar types of neurons located in these areas project to the high order sensory association areas (Rockland and Pandya, 1979; Barbas, 2007). From there, the data are conveyed via long cortico-cortical projections to layer IV of the prefrontal neocortex (Fig. 2A and B). The neocortical layer IV is specialized at receiving data and distributing them radially to the remaining cell layers (Bannister, 2005). Thus, the projections that are directed from core or belt areas to high order association fields are comparable to precise thalamo-cortical projections that likewise preferentially terminate in layer IV.

For the way back, the prefrontal neocortex and high association sensory areas generate projections that are directed towards the primary fields (Fig. 2A and B). These projections mostly originate in the deep neocortical layers V–VI and end diffusely in layers I–III and V–VI of their target areas, whereas layer IV receives only sparse contacts (Barbas and Rempel-Clower, 1997). The cortico-cortical projections from high order association fields to belt or core areas are much less focused and effective than projections in the opposite direction; in this respect, these projections resemble the diffuse projections from the subcortical magnocellular nuclei of the basal forebrain, oral nuclei of the raphe system, and locus coeruleus. In the frontal lobe, these projections ultimately transmit data from the prefrontal fields by way of the premotor areas to the primary motor field, which is the major intersection for relaying motor programmes to premotor and motor neurons in the lower brainstem and spinal cord (Fig. 2B). Because the data flow from prefrontal and premotor fields to the primary motor area lacks precision, parallel circuits direct most of the data into the striatal and cerebellar circuits, which integrate the basal ganglia, the thalamus, many nuclei in the lower brainstem, the cerebellum, and the spinal cord into the regulation of cortical output (Heimer *et al.*, 1991).

Limbic circuit components (the transentorhinal and entorhinal regions, the hippocampal formation) and regions beyond intervene in this data flow at a critical point,

namely, where exteroceptive data are conveyed from high order sensory association areas to the prefrontal cortex (Fig. 2B). Some of these data leave the mainstream, converge through multiple neocortical relay stations, and are siphoned via the transentorhinal region to the entorhinal region. Outer entorhinal layers—particularly the cellular islands of layer pre- α ('grid cells')—(see Hafting *et al.*, 2005; Moser and Moser, 2013; Moser *et al.*, 2014) (Fig. 3A–C) project along the perforant path to the hippocampal formation (Fig. 1E), whereas a back-projection from the hippocampus terminates in the deep entorhinal cellular layer pri- α that relays information back to the neocortex (Braak *et al.*, 1996) (Figs 1F and 3B). Efferent portions of the hippocampus, in turn, project to the ventral striatum, and from there the information is guided through the ventral pallidum and magnocellular mediodorsal thalamic nuclei to the medial and orbitofrontal portions of the prefrontal neocortex (Braak and Del Tredici, 2015a) (Fig. 2B).

Thus, the limbic circuit makes the neocortex the chief source of information for the human limbic system. During evolution from lower macrosmatic mammals to microsmatic higher primates and humans, the neocortex expanded remarkably—this step was accompanied by a thorough reorganization of its interconnectivities with the centres of the limbic circuit (compare Fig. 2A and B). The major vestige of this process is an enormous expansion and sophistication of those limbic circuit centres that predominantly receive neocortical input and generate output to the neocortex (Figs 1E, F and 2B). As a functional unit, the transentorhinal and entorhinal regions in higher primates and humans provide an interface between the sensory neocortex, the hippocampal formation, and the prefrontal neocortex. The increase of neocortex-dependent transentorhinal and entorhinal fields occurred at the expense of the previously predominant territories involved in processing olfactory data. A similar developmental process took place within the functionally unified group of subcortical nuclei with diffuse cortical projections: Newer portions arose within the already-existing nuclei and

Figure 2 Continued

association areas of the neocortex. Special transition zones (transentorhinal region) between the temporal neocortex and the allocortical entorhinal region do not exist. **(B)** By contrast, the neocortex of microsmatic higher primates and humans is well developed, as shown by the growing size of the belt areas (light orange) and, in particular, the high order association areas (deep yellow). Allocortical structures devoted to olfactory processing are rudimentary, whereas the limbic regions of the allocortex (hippocampal formation and entorhinal region) are highly developed. The entorhinal region receives limited olfactory input. The transentorhinal region is seen for the first time in primates and attains its largest extent in the human brain (light yellow). It guides data from the neocortex to the 'newly' organized entorhinal region. Thus, the human entorhinal region predominantly receives its input from the neocortex while exerting major influence via the hippocampal formation (and areas beyond) on the prefrontal neocortex. Thick black arrows depict these strong afferent and efferent arms of the limbic circuit. The subcortical nuclei with axons that project to and boost the performance of the cerebral cortex develop in parallel with these advances of allo- and neocortical regions and generate new internal subdivisions. These developments are indicated in the lower third of the diagrams by differences in the shading of these subdivisions ranging from dark orange ('old') to light orange and dark yellow to light yellow (most 'recent'). The diagram also displays the potential route by means of which an HSV-1 infection could reach the locus coeruleus. Allocortex and subcort. nuclei = allocortex and subcortical nuclei; loc. coer. = locus coeruleus; MD = mediodorsal thalamic nuclei; PNS = peripheral nervous system; prefr. fields = prefrontal fields; sens. ass. f. = sensory association fields; spin. trig. nucl. = spinal trigeminal nucleus; v. pall. = ventral pallidum; v. str. = ventral striatum.

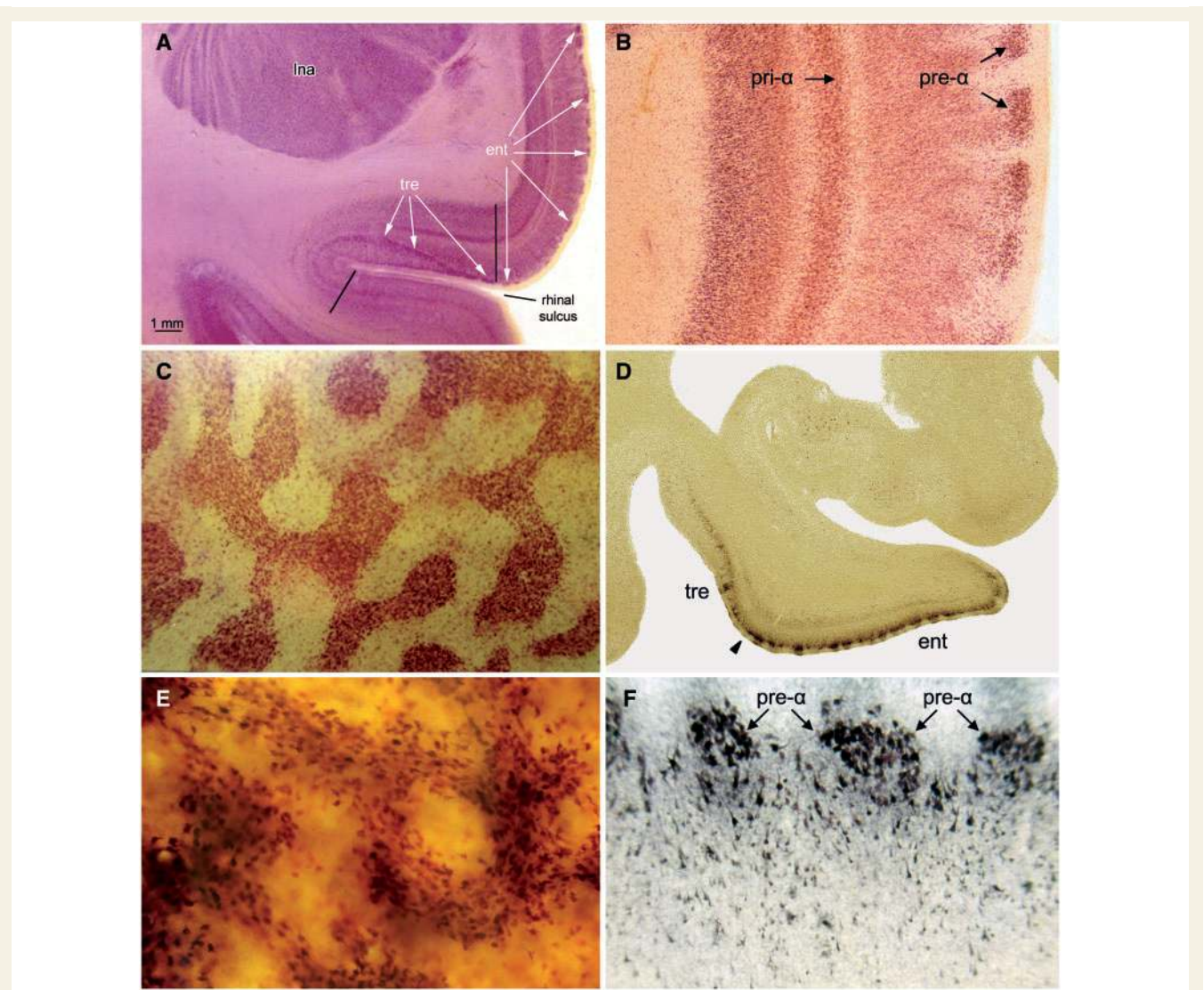


Figure 3 The transentorhinal and entorhinal cortex. (A) Found only in higher primates and humans, the transentorhinal region (tre, borders thereof indicated by black lines) is located deep in the rhinal sulcus and is here recognizable by means of the intensely stained medium-sized neurons characterizing the obliquely descending layer pre- α (white arrows). By contrast, the entorhinal region (ent, white arrows) extends over the surface of anterior portions of the parahippocampal gyrus. Coronal section, 200 μ m, aldehyde fuchsin and Darrow red. (B) Detail micrograph showing the distinctive and highly complex lamination pattern of the entorhinal region. Arrows point to the characteristic cellular islands of layer pre- α and one of the deep layers, pri- α . Coronal section, 200 μ m, aldehyde fuchsin and Darrow red. (C) This overview section cut tangentially to the surface of the entorhinal region provides a bird's-eye view of the cellular islands in layer pre- α of a 48-year-old female (control). The neuronal patterns in layer pre- α of the right and left entorhinal regions are so unique that it is possible to distinguish one individual from another. Section = 200 μ m, aldehyde fuchsin and Darrow red. (D) NFT stage II of the Alzheimer's disease-related pathological process in a 47-year-old non-demented female (control case). Note the oblique descent of the selectively involved layer in the transentorhinal region (modified pre- α neurons) accompanied by severe involvement of layer pre- α in the entorhinal region and beginning involvement of the deep layer pri- α . The hippocampal formation is still uninvolved. Coronal section, 100 μ m, Gallyas silver-iodide stain for neurofibrillary lesions. (E) NFT stage III in a tangential section through layer pre- α of the entorhinal region showing NFT-bearing neurons in layer pre- α . Section = 200 μ m, Gallyas silver iodide for neurofibrillary lesions. (F) In the course of the Alzheimer's disease process, the cellular islands of layer pre- α (arrows) sustain increasingly severe damage. By end-stage disease, the destruction of layers pre- α and pri- α effectively 'disconnects' the hippocampal formation and entorhinal region from the neocortex. Section = 100 μ m, Gallyas silver-iodide method for neurofibrillary lesions. Inna = lateral subnucleus of the amygdala; pre- α = layer α of the entorhinal external principal layer; pri- α = layer α of the entorhinal internal principal layer. Adapted, in part (A, B, D), with permission from Braak H and Del Tredici K, *Adv Anat Embryol Cell Biol* 2015a; 215: 1-162.

established contact with the more recently emerging or reorganized cortical fields (Fig. 2B). In view of these evolutionary considerations, the question arises whether it is meaningful to study all pathological features and

mechanisms of the Alzheimer's disease-related pathological process in rodents, inasmuch as these mammals do not possess the specialized and late-maturing vulnerable types of nerve cells and late-maturing cortical regions that

become involved in humans during Alzheimer's disease (Fig. 2A).

The aforementioned late-maturing centres of the human limbic circuit not only control subordinate centres for endocrine and autonomic regulation (Benarroch, 1993); they also are integral to the maintenance of learning, memory, and emotional equilibrium. In addition, they influence somatomotor activity. As custodians of memory and learning, the centres of the limbic circuit function as a neuronal bridge linking the external and internal worlds (Braak and Del Tredici, 2015a). In the healthy human brain, these limbic circuit centres, the neocortex, and the subcortical nuclei with diffuse cortical projections function harmoniously. The neocortex specializes in precise and initially unfiltered analyses of exteroceptive data. However, if data are to be retained in memory or categorized as significant, they need to be 'sorted out' of the larger stream of incoming non-essential data. Cooperation between the neocortex and the centres of the limbic circuit makes such discrimination possible. This fact is significant for comprehending the irreversible consequences of the cumulative brain destruction that develops, especially in the transentorhinal and entorhinal regions, during the Alzheimer's disease-related process (Figs 1A–D and 3D–F).

Aggregation of key susceptible proteins

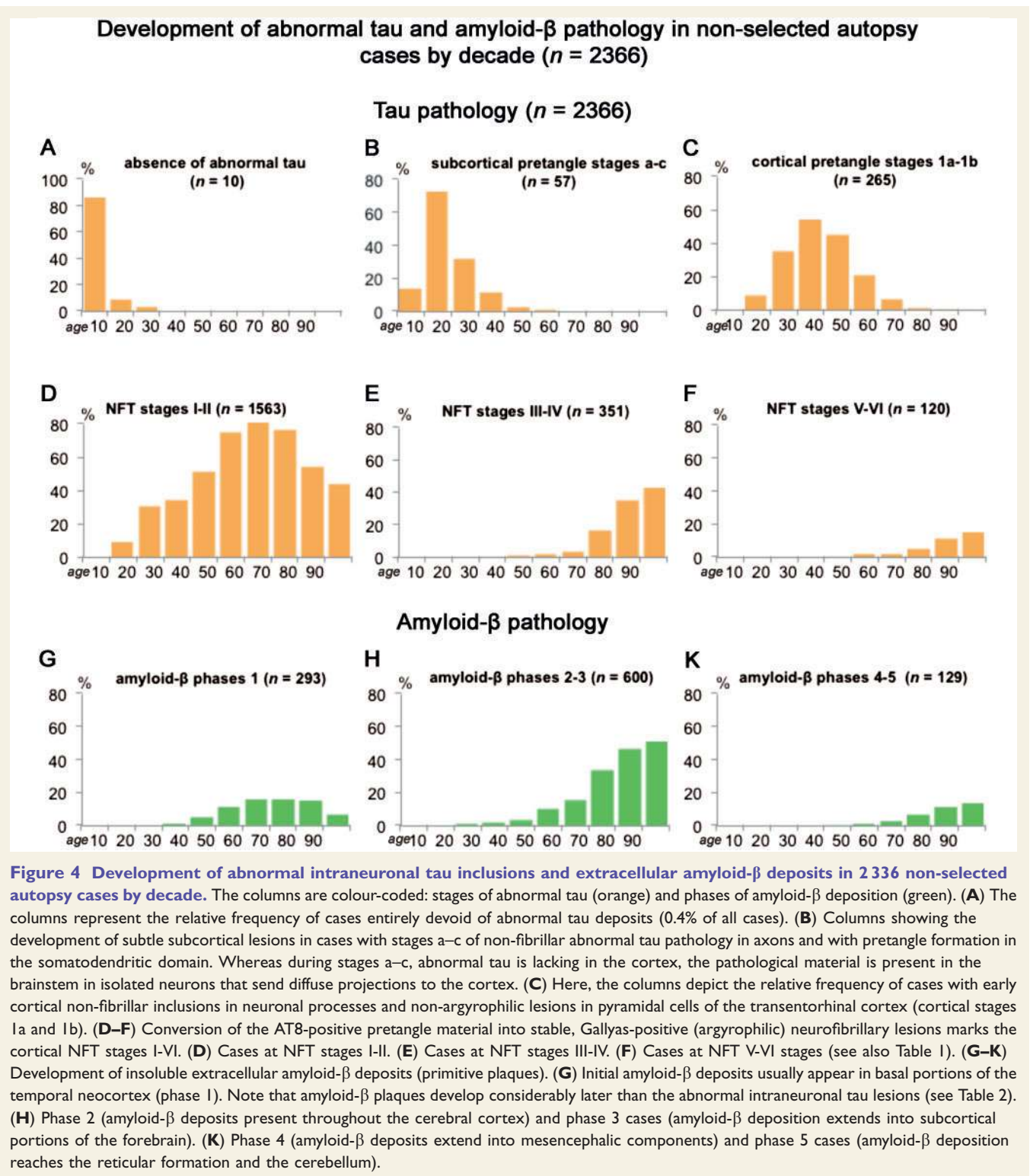
Aggregates consisting of two different pathological proteins, abnormal tau and amyloid- β , are central to the process underlying Alzheimer's disease (Duyckaerts *et al.*, 2009; Montine *et al.*, 2012; Nelson *et al.*, 2012; Spillantini and Goedert, 2013). Among its other functions, the normal tau protein binds transiently to axonal microtubules and stabilizes them (Ballatore *et al.*, 2007). Abnormal tau ultimately leads to the formation of intraneuronal inclusions that do not develop in all neuronal types with the same degree of probability but originate only in a very few and well-characterized neuronal types, which, as previously pointed out, as a functional group boost the performance of the cerebral cortex. Subsequent to the appearance of the tau aggregates (Fig. 4B–F), deposits of the pathological protein amyloid- β (Fig. 4H–K) appear in extracellular spaces of predictable sites within the CNS (Haass *et al.*, 2012; Cohen *et al.*, 2015). Once both abnormal protein types are present in aggregated form, they accumulate gradually in the brain tissue because they are highly resistant to degradation or removal.

The majority of the few select neuronal types that are vulnerable to the Alzheimer's disease process are components either of the cerebral cortex itself or of subcortical nuclei that diffusely project there. Virtually all of these vulnerable neurons generate a long, late-myelinating, and weakly myelinated axon, whereas projection neurons with a sturdily myelinated axon as well as those with a short

axon and practically all types of short-axoned interneurons (Benarroch, 2013) do not develop tau pathology. Among the exceptions to this rule are the large cholinergic interneurons in the striatum and, occasionally, chandelier cells in high-order association areas of the temporal neocortex (Braak and Del Tredici, 2015a). In Alzheimer's disease, the pathological process essentially involves neurons that are phylogenetically recent developments and reach their full maturity late in life. These nerve cells often show lifelong signs of immaturity (Arendt *et al.*, 1998; Arendt, 2000; Bartzokis, 2004; Braak and Del Tredici 2004, 2012b, c; Rapoport and Nelson, 2011; Arendt *et al.*, 2015a).

Tau protein pathology commences in humans much sooner than was previously thought to be the case (Fig. 4B) and it then systematically progresses without remission in extent and severity into increasing numbers of yet uninvolved regions of the CNS (Arnold *et al.*, 1991; Braak and Braak 1991, 1997; Fewster *et al.*, 1991). The pathological process involves highly differentiated nerve cells that suspend their cell cycle for purposes of survival (Herrup and Yang, 2007). Such postmitotic cells possess a large number of 'strategies' and mechanisms for repairing transient damage. As a result, these neurons survive and their functions persist as long as the individual lives, although owing to the development and presence of the pathological tau inclusions during Alzheimer's disease they do so at the cost of more and more functional restrictions (Morsch *et al.*, 1999; Stokin and Goldstein, 2006). The outcome of the Alzheimer's disease process, therefore, is not determined by a massive loss of nerve cells but, instead, by the existence of enormous numbers of nerve cells that survive with limited functionality. Only when the number of impaired and dysfunctional neurons has passed beyond a given threshold do the higher functions of the human CNS become noticeably curtailed.

It is important to note in this context that the 'pace' of the Alzheimer's disease-related process is subject to considerable interindividual variation. The rate of progression in some is so aggressive that the clinical picture of Alzheimer's disease emerges quickly; however, in most individuals, the process develops so gradually that clinical signs of Alzheimer's disease do not develop (Fig. 4D). The process is, as it were, Janus-faced: the majority are asymptomatic; only a few become demented (Ferrer, 2012). It has been proposed that the presymptomatic phase of Alzheimer's disease, which we believe spans decades (Braak *et al.*, 2011; Braak and Del Tredici, 2014, 2015a), may be a ubiquitous condition associated with ageing, i.e. an age-related non-Alzheimer's disease-related tauopathy (Crary *et al.*, 2014). Should this be true, however, and should there be a tauopathy that does not progress beyond the early phase, i.e. beyond neurofibrillary tangle (NFT) stage III (when amyloid- β deposits are frequently absent), then evidence would also have to exist showing that, in the short or long term, such 'non-Alzheimer's disease-related' cases would display no other lesions except remnants of NFTs in brain tissue—so-called 'tombstone tangles' or 'ghost tangles'—in the



absence of fresh tau lesions. To date, however, no one has presented evidence of such a phenomenon (Braak and Del Tredici, 2014; Duyckaerts *et al.*, 2015). The idea that the here-described presymptomatic phase is intrinsically ‘non-Alzheimer’s disease-related’ requires the problematic definition of the point at which such tau inclusions convert from being non-Alzheimer’s disease-related to Alzheimer’s

disease-related lesions. Moreover, in addition to the morphological evidence for the existence of a single Alzheimer’s disease process, recent molecular findings indicate that the underlying pathological process is not necessarily a heterogeneous one: experimentally, the conformational states of the misfolded tau protein derived from patients with Alzheimer’s disease that propagated to cause the formation

Table 2 Comparison of tau stages with phases of amyloid- β deposits

Amyloid- β phases	Tau zero	Tau stage		NFT stage	
		a–c	1a–1b	I	II
0	10	57	248	687	266
1	0	0	11	141	103
2	0	0	6	128	134
3	0	0	0	22	74
4	0	0	0	2	6
5	0	0	0	0	0
Total	10	57	265	980	583

of new tau inclusions were not only remarkably stable (e.g. ‘prion-like,’ and see below) but also produced chiefly a single cell strain as opposed to other tauopathies (Sanders *et al.*, 2014).

Thus, the presymptomatic phase is arguably integral to the Alzheimer’s disease process and without it both types of proteinaceous lesions that characterize the disorder cannot progress to or beyond the threshold eventually leading to the symptomatic phase. The situation is similar to that of atherosclerosis in coronary artery disease, where endothelial abnormalities occur early and increase in severity on an interindividual basis for decades in the absence of symptoms: angina, myocardial ischaemia, and acute myocardial infarction first manifest themselves only in late-stage disease (Graham *et al.*, 2007; Matsuzawa and Lerman, 2014; Smith *et al.*, 2015).

The following sections deal with the clinically mute phase of the Alzheimer’s disease-associated pathological process. More so than the neuropathologically more complex late Alzheimer’s disease stages, this early phase shows quite clearly the circumstances that prevail when the disease process is set in motion for the first time and subsequently promotes its systematic progression, possibly also owing to the intervention of additional mechanisms or conditions. The initial phase is also crucial for all efforts directed at developing new causal and therapeutic strategies because without a more profound knowledge of the events that transpire during the clinically silent phase, such strategies will not become accessible or prove effective.

Early subcortical lesions (stages a–c)

The earliest phase of the Alzheimer’s disease process is characterized by the abrupt appearance of pathologically altered tau aggregates in vulnerable neurons of a few select CNS predilection sites (Köpke *et al.*, 1993; Hyman and Gómez-Isla, 1994; Goedert and Spillantini, 2006; Goedert *et al.*, 2006; Mandelkow *et al.*, 2006, 2007;

Alonso *et al.*, 2008; Iqbal and Grundke-Iqbal, 2008; Iqbal *et al.*, 2009; Mandelkow and Mandelkow, 2012). The lesions can be visualized using immunoreactions directed against abnormally phosphorylated tau protein (AT8) (Mercken *et al.*, 1992; Montine *et al.*, 2012). Continual formation of such a material takes place from the beginning until the end-phase of Alzheimer’s disease (Braak and Del Tredici, 2015a).

Abnormal tau material does not commence development haphazardly or with the same degree of probability in the various types of vulnerable nerve cells. Rather, the Alzheimer’s disease process appears to begin in specific subcortical nuclei (the magnocellular nuclei of the basal forebrain, oral nuclei of the brainstem raphe system, and the locus coeruleus) that generate diffuse projections via weakly myelinated, long, and richly branching axons to numerous forebrain sites, particularly high order association areas of the neocortex (Curcio and Kemper, 1984; Marcyniuk *et al.*, 1986a; Zweig *et al.*, 1989; German *et al.*, 1987, 1992; Busch *et al.*, 1997; Sassin *et al.*, 2000; Parvizi *et al.*, 2001; Lyness *et al.*, 2003; Marien *et al.*, 2004; Mesulam, 2004; Mesulam *et al.*, 2004; Haglund *et al.*, 2006; Grudzien *et al.*, 2007; Weinschenker, 2008; Benarroch, 2009; Grinberg *et al.*, 2009; Sara, 2009; Simic *et al.*, 2009; Braak and Del Tredici, 2011a, 2012a; Braak *et al.*, 2011; Elobeid *et al.*, 2011; Schliebs and Arendt, 2011; Chalermpananupap *et al.*, 2013; Trillo *et al.*, 2013; Arendt *et al.*, 2015a).

In most cases, the first visible pathological changes in histological sections occur in neuromelanin-containing (noradrenergic) projection cells of the locus coeruleus, where they are initially confined to proximal segments of long axons (stage a) (Table 1 and Fig. 4B) (Braak and Del Tredici, 2011a, 2015a). Without light microscopically detectable precursors, a hyperphosphorylated and slightly aggregated tau protein abruptly appears in the proximal axon of isolated neurons in brains of remarkably young individuals. The pathological material is amenable to fixation in formaldehyde, fibrillar, and its aspect is homogeneous. The abnormal material appears to be more viscous than soluble hyperphosphorylated monomeric tau and it shifts very slowly into more distal portions of the axon (Braak and Del Tredici, 2011a, 2015a). The idea that the pathological process may begin preferentially in the brainstem and, more specifically, in projection neurons of the locus coeruleus, is supported by evidence from studies showing that, apart from this site, none of the other CNS regions that are known to become involved during the course of Alzheimer’s disease displayed the presence of AT8-immunoreactive aggregates (Braak and Del Tredici, 2011a; Elobeid *et al.*, 2011). Because such cases often are exceptionally young, study cohorts should include young individuals (Attems *et al.*, 2012; Arendt *et al.*, 2015a).

In mature neurons, a large proportion of the natively soluble and unfolded tau protein is localized in the axon. Although the protein is produced in the cell soma, it then is redistributed—provided it is fully functional—into the

axonal compartment (von Bergen *et al.*, 2005; Iqbal *et al.*, 2009). There, tau is directed by kinases and phosphatases, and it alternately binds and disengages from axonal microtubules (Yu *et al.*, 2009). A dynamic equilibrium exists between the less highly phosphorylated forms of the protein that are bound to microtubules and the highly soluble and hyperphosphorylated forms that swim freely in the axoplasm (Ballatore *et al.*, 2007). The transiently unbound monomeric hyperphosphorylated tau molecules generally remain unprecipitated in fixatives, such as formaldehyde, and are not visualized by conventional immunoreactions. In short, hyperphosphorylation of monomeric tau *per se* is not deleterious. It originates by means of endogenous axonal kinases and is upregulated in large amounts without toxic effects on the CNS in various species of mammals during hibernation as well as under anaesthesia (Run *et al.*, 2009; Arendt *et al.*, 2015b).

By contrast, the aggregated tau material found in projection neurons of the locus coeruleus of young persons, including those who were neurologically unremarkable, is radically different: the material changes its conformation (Falcon *et al.*, 2015), forfeits its solubility, and persists in a hyperphosphorylated state. More importantly, it is no longer subject to regulation within the axonal metabolism and it becomes completely resistant to proteosomal recycling, autophagy, or other endogenous cellular removal mechanisms (Kovacech *et al.*, 2010). Based on currently available evidence, it only can be surmised that a conformational change (Falcon *et al.*, 2015), in conjunction with a sudden increase in size of such abnormal tau molecules and with their reduced soluble state, fundamentally distinguishes this pathologically aggregated species of tau from the transiently hyperphosphorylated tau that occurs under physiological conditions (Weaver *et al.*, 2000; Maeda *et al.*, 2007).

In the same context, it again should be emphasized that aggregated tau only develops in nerve cells that are not absolutely essential for basic human survival. As such, the limited life expectancy of human beings during the era when they were evolving out of higher primate species does not speak for deleterious effects either in conjunction with the tendency to produce abnormal tau inclusions or in connection with unfavourable consequences owing to biologically programmed laws of evolution associated with abnormal tau production (Braak and Del Tredici, 2015a). The unanticipated and precipitous beginning of the pathological process is hardly conceivable in young brains, where the endogenous axonal kinases and phosphatases function dependably. Why, then, should they spontaneously trigger the production of pathological tau aggregates and thereby contribute to severe axonal dysfunction in otherwise healthy neurons? Could 'non-endogenous' kinases entering the nerve cell from outside (e.g. viral kinases) pose a potential danger and possibly not only hyperphosphorylate tau but also induce a conformational change in the protein (Ball, 1982; Jamieson *et al.*, 1991; Wozniak *et al.*, 2009a; Itzhaki and Wozniak, 2010; Wozniak and Itzhaki, 2010; Deruelle and Favoreel, 2011; Ball *et al.*, 2013; Itzhaki, 2014)? The

viral pathogen concept is at least consonant with the observation that the earliest aggregated tau molecules consistently appear first in the proximal axon, inasmuch as the bulk of the tau protein available to a cell is localized in the axon and also because viral kinases leave the cell soma to become redistributed to the axon.

One mechanism for a possible viral induction of the pathological process is the penetration of susceptible neurons by a herpes simplex type 1 (HSV-1) viral infection. Might reactivation of a quiescent HSV-1 infection in the trigeminal ganglia be followed by spreading of the virus along pathways from the ganglia to the spinal trigeminal nucleus in the lower brainstem (Fig. 2B)? Nociceptive neurons in layer 1 of this nucleus receive input from the locus coeruleus but also project to specific nuclei of the thalamus and send collaterals to the locus coeruleus (Fig. 2B). Because of these bidirectional connectivities, painful impulses prompt a general activation of the sympathetic nervous system. Thus, a reactivated HSV-1 infection with a widespread prevalence of seropositivity (Itzhaki *et al.*, 1997, 1998) could also spread to the locus coeruleus (Fig. 2B). Infection of the olfactory bulb, which receives direct axonal projections from the locus coeruleus, could provide an alternative route (Barnett *et al.*, 1993; McLean *et al.*, 1993; Becker, 1995). Once inside host cells of the locus coeruleus, the viral pathogen might be capable of inducing the conformational change and aggregation of the tau proteins in axons (Deruelle and Favoreel, 2011; Ball *et al.*, 2013). Thereby, it would have to be assumed that these changes would remain confined to nerve cell types susceptible to the development of the Alzheimer disease-related tau aggregates (among them, the projection neurons in the locus coeruleus) and would not occur in non-susceptible neuronal types, e.g. nerve cells of the trigeminal ganglion.

The abrupt formation of hyperphosphorylated and slightly aggregated tau results in secondary changes within the axonal microtubuli sometimes thought to result in their disintegration (Mandelkow *et al.*, 2006, 2007; Li *et al.*, 2007; Alonso *et al.*, 2008; Iqbal *et al.*, 2009; Cowan *et al.*, 2010). Remarkably, however, the host neurons fail to display immediate drastic or adaptive responses to the serious disturbances of their axonal cytoskeletons. Even severely altered axons remain intact for a very long period of time. Gradually, the abnormal tau material converts from a viscous mass into insoluble polymeric spindle-shaped inclusions interspersed by short inclusion-free axon segments, and this situation persists into the final stages of the disease process (Velasco *et al.*, 1998). The spindles are inert, i.e. they do not appear to change in size or shift in either direction.

In the somatodendritic compartment, by contrast, such aggregated tau material is converted into fibrillar and then argyrophilic inclusions, i.e. selectively positive in the silver-iodide method developed by Gallyas (Gallyas, 1971; Uchihara *et al.*, 2001a, b; Mandelkow *et al.*, 2007; Uchihara, 2007, 2011; Alonso *et al.*, 2008; Iqbal *et al.*, 2009; Kovacech *et al.*, 2010). Cells form proteinaceous

inclusions possibly as a ‘protective’ measure to produce stable fibrillar species and to sequester possibly noxious and partially soluble material (Ittner *et al.*, 2011). The axonal tau material, however, does not display the slightest propensity to such a conversion at this point. The absence of argyrophilia is a key feature that fundamentally distinguishes the axonal AT8-immunoreactive material from the subsequent ‘pretangle material’ that fills the somatodendritic compartment (Braak and Del Tredici, 2015a). The only exceptions are dystrophic neurites of neuritic plaques—such neurites frequently represent sparsely myelinated axon terminals, which, nevertheless, do contain argyrophilic fibrillar material (Struble *et al.*, 1982, 1985).

Presently little understood mechanisms must exist, whereby even a severely involved axonal cytoskeleton continues to fulfil at least some residual transport functions. After the death of the host neuronal soma and all of its dendrites and axons, the axonal inclusions disappear from the tissue. Extraneuronal thread-like structures—‘tombstone axons’ akin to ‘tombstone tangles’—do not exist, so that both the soluble and the insoluble intra-axonal material can be absorbed quickly. Notably, there are no signs of activated astroglial or microglial cells at sites where widespread axonal loss has occurred (e.g. in the perforant path).

Secondary to the formation of non-argyrophilic material in axons, pretangle material accumulates in the somatodendritic compartment of noradrenergic coeruleus neurons (stage b) (Baner *et al.*, 1989; Matsuo *et al.*, 1994). The pathological material in the somatodendritic compartment develops so rapidly that it is not possible to identify a specific site whence it might originate. Given the preceding development of the axonal tau inclusions, the axon might be deprived of an adequate supply of ‘normal’ tau, and for this reason it is unlikely that the tau molecules henceforth undergoing aggregation in the cell soma come from the axon. Granted, deprivation of normal tau in the axon may stimulate renewal of tau synthesis in the cell soma; but, to what end? The somatodendritic compartment contains microtubules that normally are stabilized by microtubule-associated proteins other than tau. Accordingly, the newly synthesized tau might not find a sufficient number of binding sites in the cytoplasm of the cell soma where abnormal hyperphosphorylation and conformational changes could occur and then prompt pretangle formation.

Initially, small amounts of the newly formed and to a certain extent still soluble pretangle material are seen as droplets (Braak and Del Tredici, 2015a). These minimally aggregated forms of irreversibly hyperphosphorylated tau may adversely influence their surroundings (Kopelkina *et al.*, 2012). As an evasion tactic, involved neurons may rapidly convert the material into larger aggregates that push into the open spaces between the organelles of the cell soma. From then on, the material becomes insoluble, fibrillar, and strongly argyrophilic (Matsuo *et al.*, 1994). These argyrophilic neurofibrillary inclusions produce neuropil threads (NTs) in dendritic processes of involved cells and NFTs in the cell somata.

In the locus coeruleus, a dismantling of the abnormal tau or signs of cellular distress (e.g. swelling of the neuronal soma, displacement of the Nissl material, and/or the cell nucleus to the periphery) are not observable. In other words, neuronal death there is not imminent during this phase. Nerve cells obviously tolerate the presence of both the pretangle material and the argyrophilic NTs/NFTs remarkably well. Thus, the viscous abnormal material is acutely toxic only at first and briefly, whereas the insoluble material is not incompatible with long-term neuronal survival, although survival is not synonymous with optimal cell functioning. The avoidance of cell death in the short term is no guarantee for intact cell function in the long run. For example, NFT-bearing noradrenergic neurons manage to survive for decades during the pathological process, and a remarkable number do so even in its final stages, although their functions become prematurely impaired (Mann *et al.*, 1980; Tomlinson *et al.*, 1981; Ichimiya *et al.*, 1986; Marcyniuk *et al.*, 1986b; Busch *et al.*, 1997; Morsch *et al.*, 1999; but see Lyness *et al.*, 2003; McMillan *et al.*, 2011).

One of the hallmarks of the Alzheimer’s disease process is that the lesions consistently develop in the same temporal sequence and according to the same regional distribution pattern with very little interindividual variability. The progression of the tau pathology during the course of the disease has made possible a neuropathological staging of the process (Table 1 and Fig. 4) (Arnold *et al.*, 1991; Braak and Braak, 1991; Fewster *et al.*, 1991; Duyckaerts *et al.*, 2015). The earliest involved neurons are those in the locus coeruleus, and the tau lesions then reach the noradrenergic coeruleus neurons of the contralateral brainstem, so that the pathological process becomes symmetrical soon after its onset (stage b). Thereafter, additional nuclei with diffuse cortical projections become involved (stage c) (Fig. 4B) (Braak *et al.*, 2011; Braak and Del Tredici, 2015a; Iba *et al.*, 2015).

Initial cortical lesions (stages Ia, Ib, I and II)

It cannot be overlooked that neurons in which AT8-immunopositive material accrues are not necessarily located directly in the vicinity of initially involved coeruleus nerve cells but either in the contralateral coeruleus or in the cerebral cortex. This indicates that the disease process does not progress (or spread) via direct contacts on the part of involved cell somata or their dendrites to immediately adjacent cells. Instead, it is remarkable that all of the regions that become involved in the Alzheimer’s disease process are interconnected by means of axons. This interconnectivity indicates that physical axonal contacts between involved nerve cells play a key role in the pathogenesis of Alzheimer’s disease (Saper *et al.*, 1987; Pearson, 1996; Braak and Del Tredici, 2011b, 2015b; Liu *et al.*, 2012; Wu *et al.*, 2013; Ahmed *et al.*, 2014; Dujardin *et al.*, 2014).

The earliest cortical lesions consist of aggregated and non-argyrophilic, AT8-immunoreactive material. The lesions first appear in the transentorhinal cortex as radially aligned thread-like neuronal processes, most probably representing terminal portions of affected axons (cortical stage 1a). Thereafter, these lesions become accompanied by pretangle material-containing transentorhinal pyramidal cells (i.e. modified pyramidal cells that belong to the group of multipolar neurons in the superficial layer pre- α of the entorhinal cortex) (Fig. 3B and C)—occasionally denoted as entorhinal layer II (cortical stage 1b) (Table 1 and Fig. 4C). In contrast to the involvement of locus coeruleus projection neurons described in the previous section, the abnormal pretangle material in these cortical nerve cells develops at first in distal dendritic segments and, from there, fills the cell soma and, only then, the axon (Braak *et al.*, 1994; Iba *et al.*, 2015). The fact that during the onset of the subcortical pathological changes abnormal tau material originates solely in the proximal axon may be unique to the first neurons that become involved in the Alzheimer's disease process. By contrast, in all subsequently involved nerve cells (including cortical pyramidal cells), the initial tau lesions begin in the somatodendritic compartment.

It is difficult to comprehend how the somatodendritic compartment of these secondarily involved cortical pyramidal cells could be capable of producing, rather abruptly, normal tau in large amounts because the axons of these cells are already equipped with sufficient pools of the protein. An immediate relocation or displacement of tau backwards into the cell soma, i.e. 'missorting' (Thies and Mandelkow, 2007), at the expense of destabilizing the axonal cytoskeleton is highly improbable. Inasmuch as tau is generated *de novo* rapidly and in considerable quantities within the cell soma, its production at the 'wrong place at the wrong time' may be prompted from an external source, perhaps by abnormal signalling from pathologically altered axons of previously involved subcortical nuclei. Are there other, presently unknown, factors that contribute to this rapid *de novo* generation of the protein—for instance, pathogens that can be transmitted transsynaptically (e.g. viral particles; see Wisner *et al.*, 2011) or minute aggregates of hyperphosphorylated tau (but see Ball, 2003; Ball *et al.*, 2013)? As only a small fraction of the *de novo* synthesized tau finds binding sites in the somatodendritic compartment, the protein presumably persists there in the cytosol in a hyperphosphorylated state. Then, after exceeding critical concentrations, it aggregates and converts into pretangle material. Indeed, involved neurons appear within a relatively brief time span to be nearly filled with pretangle material (Braak and Del Tredici, 2015a).

The initially involved distal dendritic segments of cortical pyramidal cells, including those in the transentorhinal and entorhinal cortex, are phylo- and ontogenetically late-appearing structures that chiefly establish connections to other late-emerging and late-maturing nerve cells. These segments display a sudden and pronounced growth spurt (Coleman and Flood, 1987; Anderton *et al.*, 1998).

Prolonged longitudinal growth of dendrites can be a sign of immaturity, and this untimely phenomenon in the transentorhinal and entorhinal regions may be triggered by the Alzheimer's disease process. The distal-most dendritic segments of involved pyramidal cells become twisted, develop side-branches, and thereafter lose all connections to their proximal dendritic portions (Braak *et al.*, 1994). Such detached dendritic segments do not leave behind any remnants in the neuropil, and their loss, together with that of their synaptic connections, do not result in the deaths of the involved cortical pyramidal cells.

The transentorhinal region is usually the first cortical site where argyrophilic NTs/NFTs develop (NFT stage I) (Fig. 1A). At the same time, freshly involved non-argyrophilic projection cells (i.e. containing pretangle material) are always detectable there. Initially, the entorhinal region remains uninvolved or only minimally involved. During NFT stage II, the pathological process progresses into the entorhinal region, involving first the superficial entorhinal layer pre- α (Figs 1A, B and 3D) and, subsequently, the deep layer pri- α (Table 1, Figs 3D and 4D) (Braak and Braak, 1992). In addition, many cases reveal mild involvement of the first and/or second sectors of Ammon's horn in the hippocampal formation (NFT stage II).

The involvement of the pyramidal cells in layers pre- α and pri- α progresses slowly, causing extensive disruption within connectivities to and from the hippocampal formation (Braak *et al.*, 1996) (Fig. 1E and F). What begins as a circumscribed and eminently unspectacular cortical lesion in one or both hemispheres gradually isolates and, in late-stage Alzheimer's disease, 'disconnects' the hippocampal formation from the neocortex (Kemper, 1984; Delbeuck *et al.*, 2003; Reid and Evans, 2013). At the same time, the pathological process reduces the indirect influence of the hippocampal formation on the prefrontal neocortex by way of the ventral striatum/pallidum and mediodorsal thalamus (Fig. 2B). Thus, the first cortical tau lesions pave the way for the deterioration, curtailment, and loss of intellectual faculties and executive functions that emerge during the clinical phase of Alzheimer's disease.

With the exception of the abnormalities in distal portions of dendrites, the process described above for stages 1b–VI repeats itself in all subsequently involved cortical pyramidal cells located beyond of the transentorhinal and entorhinal regions: the somatodendritic compartments of these neurons pass through the phase of pretangle production prior to the development of argyrophilic NTs and NFTs, which characterize the ensuing stages of the pathological process (NFT stages I to VI) (Table 1, Figs 1A–D and 4D–F). As with the pretangle material, the development of NTs usually precedes that of NFTs (Braak and Del Tredici, 2015a). Gradually, NFTs occupy large portions of the cell soma. The fibrillar and argyrophilic tau lesions are much more robust than the pretangles and can displace the cell nucleus toward the periphery. NFTs also frequently protrude somewhat into the proximal segments of dendrites; however,

they do not extend into the axon hillock or into the axon (Braak and Del Tredici, 2015a).

Progression and possible propagation of the pathological process

The remarkable uniformity with which the Alzheimer's disease-related process progresses continues to be the subject of ongoing discussion. It is conceivable that defective axons of involved subcortical nuclei send aberrant signals to the cortical nerve cells on which they synapse, and that these signals induce or facilitate induction of the pathological changes within these pyramidal cells. Furthermore, involved axons might also transport viral particles and/or soluble but irreversibly hyperphosphorylated and slightly aggregated tau molecules—the axons might also be capable of releasing the material into the synaptic cleft (Henkins *et al.*, 2012; see, however, Ball, 2003; Ball *et al.*, 2013). Once taken up at the postsynaptic site, the material may cause template-induced misfolding and seeded aggregation of the abnormal tau protein in the recipient neuron (Brundin *et al.*, 2010; Frost and Diamond, 2010; Goedert *et al.*, 2010, 2014; Braak and Del Tredici, 2011b, 2015b; Jucker and Walker, 2011; Lee *et al.*, 2011; Prusiner, 2012; Clavaguera *et al.*, 2013b, 2014; Duyckaerts, 2013; Pooler *et al.*, 2013; but see also Beekes *et al.*, 2014). The conditions required to promote or enhance abnormal signalling and/or 'seeding' of tau are incompletely understood, but experimental evidence is available for the hypothesis that potential pathogens (including tau) can be transsynaptically transferred from one nerve cell to another and can induce the production of abnormal tau proteins in receptor cells (Clavaguera *et al.*, 2009, 2013a; Frost *et al.*, 2009; Sydow and Mandelkow, 2010; Guo and Lee, 2011, 2013; de Calignon *et al.*, 2012; Liu *et al.*, 2012; Walker *et al.*, 2013; Ahmed *et al.*, 2014; Dujardin *et al.*, 2014; Holmes *et al.*, 2014; Sanders *et al.*, 2014; Calafate *et al.*, 2015; Mirbaha *et al.*, 2015).

If the 'prion-like' hypothesis of neuron-to-neuron tau dissemination along axonal connectivities is tenable, the pathology beginning in select subcortical nuclei could propagate along their diffuse ascending projections to arrive at cortical predilection sites (e.g. transentorhinal/entorhinal regions, stages 1a, 1b, I and II). There, the routes of dissemination would split up and be directed medially, on the one hand, into the perforant path, which originates in layer pre- α and in other components of the outer principal layer (pre- β , pre- γ) (Figs 1E and 3), towards the hippocampal formation and, on the other, into the efferences originating in layer pri- α and other components of the layers pri- β and pri- γ (Figs 1F and 3) towards laterally adjacent high order sensory association areas of the basal temporal neocortex (stage III) (Fig. 1C). From this vantage point, axonal projections could reach additional high order

sensory association areas of the occipital and parietal lobes as well as the prefrontal neocortex (stage IV) (Fig. 1B). Finally, the pathological process could reach the first order sensory association areas, premotor fields (stage V), and even the primary fields of the neocortex (stage VI) (Fig. 1D). Any potential dissemination or transmission via cortico-cortical projections should be accompanied by the further expansion of the pathological process within the subcortical nuclei under consideration, whose axons project successively to previously uninvolved regions of the cerebral cortex—in other words, this would account for why the tau pathology in the locus coeruleus gradually becomes more severe at higher NFT stages (Attems *et al.*, 2012). Thus, vulnerable cortical neurons would be accessible via two pathways: cortico-cortical and coeruleo-cortical (or subcortico-cortical) projections. Whether this dual contact is one of the presumably multiple factors required for a successful propagation of the Alzheimer's disease-associated pathological process and/or for the formation of the stereotypical regional distribution pattern of the tau lesions, is still an open question.

The hypothesis of a neuron-to-neuron seeding and propagation via synapses with pre- and postsynaptic sites offers a parsimonious explanation for the predictable topographical distribution pattern of the tau pathology and the extraordinarily slow rate of disease progression in Alzheimer's disease (Braak *et al.*, 2011; Braak and Del Tredici, 2011b, 2015b; Duyckaerts *et al.*, 2015). Knowledge of the underlying mechanisms would mean that they could be influenced by interrupting or at least delaying the spread of the pathological process. The challenge would be to develop a causal therapy for Alzheimer's disease during the early phase when the process is confined to the brainstem and prior to the involvement of the cerebral cortex.

The amyloid- β protein

The end of the unusually protracted and tau-dominated early phase of the Alzheimer's disease process is marked by the gradual development of insoluble aggregations of amyloid- β (Braak and Braak, 1997; Braak and Del Tredici, 2014, 2015a) that chiefly precipitate in the extracellular spaces of the grey matter neuropil (Masters and Selkoe, 2012). The pathological protein is generated by an abnormal proteolytic processing of the amyloid precursor protein (APP) (Selkoe *et al.*, 2012). Numerous cells within and outside the CNS produce monomeric and soluble amyloid- β , which is rapidly degraded rather than precipitated by conventional formaldehyde fixation. By contrast, aggregated amyloid- β that is rich in cross- β sheet structures can be visualized using 4G8 immunohistochemistry or Campbell-Switzer pyridine silver staining (Masters and Selkoe, 2012; Montine *et al.*, 2012). Similar to argyrophilic tau aggregates, insoluble amyloid- β deposits are virtually confined to the grey matter of the CNS and occur there only at specific sites according to a distinctive

regional distribution pattern (Fig. 4G–K) (Thal *et al.*, 2000, 2002; Alafuzoff *et al.*, 2009).

APP undergoes vesicular anterograde transport within axons (Buxbaum *et al.*, 1998). Presynaptic boutons of the terminal axon contain enzymes necessary for APP degradation and for the production of aggregation-prone amyloid- β . As such, these presynaptic boutons most likely represent the major amyloid- β secretion sites (Koo *et al.*, 1990; Muresan and Muresan, 2008; Braak and Del Tredici, 2013). Notably, however, not all nerve cell types produce the pathological protein: for instance, plaques do not develop in the enteric nervous system or in the peripheral nervous system. Nor do healthy brains generally generate aggregation-prone amyloid- β . Indeed, abnormal amyloid- β species probably are produced only by select types of nerve cells and come into existence only under pathological conditions. They are found not only as plaque-like precipitations in the cortical neuropil and elsewhere (Fiala, 2007) but also as amyloid- β deposits lining basement membranes and/or smooth muscles of leptomeningeal as well as cortical arteries, arterioles, and, less frequently, capillaries (i.e. cerebral amyloid angiopathy) (Vinters, 1992; Attems *et al.*, 2011).

Predilection sites in the grey matter (e.g. cortical areas of the forebrain) can readily be distinguished from sites where amyloid- β deposition is absent or seldom (e.g. the pallidum). Amyloid- β deposition of 'primitive' (i.e. 'diffuse') plaques does not occur in the immediate vicinity of short-axoned neurons and, thus, it is unlikely that such nerve cells contribute to amyloid- β production. Development of amyloid- β plaques does not precede that of the tau lesions in Alzheimer's disease, nor is amyloid- β essential for tau progression (Schönheit *et al.*, 2004; Pimplikar, 2009; Dong *et al.*, 2012; Chételat, 2013; Moreno-Trevino *et al.*, 2015). Indeed, the tau-dominated phase of the pathological process reaches at least stage 1a before the first amyloid- β plaques appear in the basal temporal neocortex—at this early stage, tau aggregations are not detectable in pyramidal cells of the temporal neocortex (Table 2). Accordingly, the production of aggregation-prone amyloid- β in these cortical areas most probably originates solely from already dysfunctional diffusely projecting axons of brainstem nuclei that contain tau pathology and have a perturbed homeostasis (Braak and Del Tredici, 2013, 2015a).

The axon terminals of these nuclei display numerous local thickenings with only presynaptic sites that do not have postsynaptic counterparts. By means of these 'non-junctional varicosities,' they release their neurotransmitter substances diffusely into the interstitial fluid via volume transmission (Agnati *et al.*, 1995; Nieuwenhuys, 2000; O'Donnell *et al.*, 2012). In sporadic Alzheimer's disease, involved axons may be able to secrete aggregation-prone amyloid- β into this fluid through a similar mechanism (Braak and Del Tredici, 2013). It is also conceivable that HSV-1 viral DNA, which has been found in amyloid- β plaques (Wozniak *et al.*, 2009b), could penetrate into plaques in such a manner. This hypothesis is corroborated by

the correspondence between the topographical distribution pattern of the axonal networks of the diffusely projecting subcortical nuclei and the lesional pattern of the amyloid- β deposits. The pallidum, for instance, belongs to the few regions of the forebrain that remain virtually devoid of amyloid- β plaques, and this fact dovetails remarkably well with the finding that the pallidum does not receive axonal projections from the locus coeruleus. Moreover, because axons of the locus coeruleus project only within the CNS, a volume transmission mechanism would also explain why amyloid- β plaque formation remains confined to the CNS during Alzheimer's disease and does not develop in the enteric or peripheral nervous systems (Braak and Del Tredici, 2015a).

Concluding remarks

As a rule, attempts to develop therapeutic strategies for Alzheimer's disease currently are aimed at controlling the causal mechanisms and biochemical pathways leading to the aggregation and/or spreading of the tau inclusions and amyloid- β or at removing the abnormal proteins from the brain (Molnar *et al.*, 2009; Bulic *et al.*, 2010; Maarouf *et al.*, 2010; Wollen, 2010; Iqbal and Grundke-Iqbal, 2011; Pooler *et al.*, 2013). In practice, however, the results and implications for human disease have been highly unsatisfactory. While continuing to keep in sight the truly ambitious goals of prevention and developing therapeutic strategies, it also is needful to consider whether the timeline of the underlying pathological process can be influenced.

Should it prove to be the case that abnormal hyperphosphorylation and/or the initial conformational change and aggregation of tau in projection neurons of susceptible brainstem nuclei were to be caused by a viral pathogen, a timely antiviral therapy would be appropriate. The diagrams in Fig. 4B (stages a–c) suggest that such an intervention might make sense only in very young individuals. Immunization of children against HSV-1 infections (Becker, 1995; Petro *et al.*, 2015) and/or antiviral therapy could also be undertaken (Itzhaki, 2014) in large epidemiologically-based studies.

Although it is likely that nearly everyone bears within themselves traces of the Alzheimer's disease-related pathology (Fig. 4B–D), only a relative minority go on to develop mild cognitive impairment (Fig. 4E) or the clinical symptoms of end-stage disease (NFT stages V and VI) (Fig. 4F). Figure 4D, for instance, indicates that very considerable differences exist among individuals with regard to the timeline or chronological course of the disease stages: Whereas some reached the earliest NFT stages I–II already as teenagers, others were >90 years of age. This very broad spectrum of interindividual variability cannot be accounted for at present. It is conceivable, however, that differences in conformational variants (i.e. 'strains') of the misfolded tau and amyloid- β proteins contribute not only

to the degree of pathogenicity but also to the rate with which the pathological process progresses (Sanders *et al.*, 2014; Cohen *et al.*, 2015).

An additional key factor may also be the sequence, mentioned at the outset (see ‘Introduction’ section), whereby the lesional pattern and regional progression of the Alzheimer’s disease-related tau pathology repeat, in reverse, developmental phases of the human CNS (Braak and Braak, 1996; Reisberg *et al.*, 1999, 2002). Relatively speaking, neuronal types that are vulnerable to the Alzheimer’s disease process not only emerge late phylogenetically but also mature ontogenetically late in life. In such nerve cells, the development of the axonal myelin sheath is a post natal event, and maturation of the myelin sheath continues into late adulthood (Paus *et al.*, 2001; Bartzokis, 2004; Braak and Del Tredici, 2004; Ullén, 2009; Fields, 2011). Throughout this process, neuronal activity on the part of the maturing projection neurons provides the oligodendroglia with the decisive physiological stimulus to produce and sustain the myelin sheath (van der Knaap *et al.*, 1991; Ullén, 2009). This means that the greater the degree of neuronal activity, the thicker the myelin sheath becomes during the time of postnatal neuronal differentiation and, ultimately, the better protected against the Alzheimer’s disease process such nerve cells may become.

The question arises whether the maturation process of brain myelin can be improved by placing greater functional demands on susceptible nerve cells and their axons (Stern, 2009; Ullén, 2009; Meng and D’Arcy, 2012). For example, it is conceivable that concerted early efforts to acquire and hone fine motor skills (Bengtsson *et al.*, 2005; Schlaug *et al.*, 2005; Draganski and May, 2008; Erickson *et al.*, 2012; Gärtner *et al.*, 2013) and/or to expose youngsters to a bilingual or multilingual setting during the period of primary language acquisition (Freedman *et al.*, 2014), could augment the degree of post natal myelination and thereby, indirectly, postpone the pace with which the Alzheimer’s disease-related pathological process advances. Prospective longitudinal studies are needed to determine whether vulnerable projection neurons can be ‘reprogrammed’ through life-long learning to resist the pathological process longer and, in so doing, delay its progression (Barulli and Stern, 2013; Xu *et al.*, 2015). The demographics of ageing societies make it imperative that research efforts not be confined to preventing abnormal tau aggregation, cell-to-cell tau seeding, or amyloid- β production but, rather, expanded to include disease-modifying strategies which postpone the Alzheimer’s disease process in the long run (Braak and Del Tredici, 2012c; Trillo *et al.*, 2013).

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