

Morphological substrates of cognitive decline in nonagenarians and centenarians: A new paradigm? ☆

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Abstract

Brain aging is characterized by the formation of neurofibrillary tangles (NFT) and senile plaques (SP) in both cognitively intact individuals and patients with Alzheimer's disease (AD). The ubiquitous presence of these lesions and the steady increase of the prevalence of dementia up to 85 years have strongly supported a continuum between normal brain aging and AD. In this context, the study of nonagenarians and centenarians could provide key informations about the characteristics of extreme aging. We provide here a detailed review of currently available neuropathological data in very old individuals and critically discuss the patterns of NFT, SP and neuronal loss distribution as a function of age. In younger cohorts, NFTs are usually restricted to hippocampal formation, whereas clinical signs of dementia appear when temporal neocortex is involved. SPs would not be a specific marker of cognitive impairment as no correlation was found between their quantitative distribution and AD severity. The low rate of AD lesions even in severe AD as well as the weakness of clinicopathological correlations reported in the oldest-old indicate that AD pathology is not a mandatory phenomenon of increasing chronological age. Our recent stereological observations of hippocampal microvasculature in oldest-old cases challenge the traditional lesional model by revealing that mean capillary diameters is an important structural determinant of cognition in this age group.

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1. Introduction

The continued expansion of the elderly population and a growing awareness of age-related diseases such as dementia have prompted considerable interest in the study of the aging

human brain. The aging population is rapidly growing as a result of increased accessibility of advanced medical technology and declining birth rates. By 2020 it is estimated that at least 1 billion people will be older than 60 years representing more than 20% of the total population. The few individuals who reach very old ages are called “longevity outliers”. This particular group represents the fastest growing age group worldwide. For instance, people over age 85 accounts for 2.1% of the general population in France and this proportion is expected to increase to 3.3% in 2020 and 6.8% in 2050. Similarly, the number of centenarians increases steadily in the US population and their numbers are expected to grow to more than 400000 in the next 30 years (for review see [1]). This phenomenon has been also

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confirmed in New Zealand and Japan [2,3]. A well-known paradox is the high proportion of centenarians in the African American community (19.6 per 100000) in spite of a lower life expectancy at birth [4].

The growing evidence for a steady increase of the number of centenarians worldwide was paralleled by several community-based longitudinal and cross-sectional studies aiming to determine possible psychobiological particularities of these individuals [5–10]. These studies showed that the known predictors of mortality such as socio-demographic factors, smoking and obesity are less important in this age group. In contrast, levels of disability as well as depression are key determinants of both morbidity and mortality after 90 years. Centenarians are less prone to oxidative stress and are thought to have better antioxidant defences, nutritional status, immunologic profile, endocrinologic and metabolic characteristics than younger elderly cohorts [11,12]. Psychologically, they report greater satisfaction with life and social and family relations and display lower scores for anxiety and depression and better coping abilities compared to less elderly individuals [13]. In this age group, good health and not moving home are associated with greater intellectual activity, while extraversion and negative life events with greater social activity [14]. These data support the possibility that centenarians form a select cohort with relatively slow rates of aging and increased resistance to biological and psychological stress and age-related diseases such as cancer, stroke and heart disease.

2. Dementia in the oldest-old

Although prevalence and incidence data are still scarce in this age group, it has long been thought that very old age is associated with the highest prevalence of dementia [15]. Several earlier studies reported an increased prevalence of dementia with aging, to 100% in the 100-year age group [16,17]. Methodological biases were present in most of these studies and limit the validity of their conclusions. First, the samples included only a limited number of nonagenarians and centenarians. Second, the clinical diagnosis of dementia made by the physician was based on the global decline of cognitive performances rather than on a detailed analysis of each cognitive function leading to an overestimation of the prevalence of dementia in the oldest-old. In fact, recent epidemiological studies in larger cohorts of very old individuals showed prevalence rates which varied from 27 to 62% pointing to the fact that dementia is not inevitable in very old individuals (for review see [1,18]). In an epidemiological survey of 1694 patients who met criteria for probable or definite Alzheimer's disease (AD), Lautenschlager and colleagues [19] also reported that the risk of AD decreases significantly after age 90. Similar results were drawn by a community-based study of 402 individuals older than 85 years who have been assessed using structured interviews in Munich [20]. A relative resistance of centenarians to the degenerative process was also suggested by the observations

of Howieson and collaborators [21] who performed a longitudinal study of 31 non-demented individuals older than 80 years and reported a preservation of cognitive abilities during a follow-up period of 5 years. Furthermore, a lack of association between AD and apolipoprotein E allele epsilon 4, a major risk factor for late-onset AD in younger cohorts, has been demonstrated in centenarians [22–25]. However, one may argue that these epidemiological findings reflect only the fact that younger cohorts are at higher risk to develop AD and do not necessarily support differential neuronal aging in this age group. During the period 1990–2000, case-control neuropathological studies provided the first lines of evidence supporting the presence of distinct patterns of neuronal vulnerability to the degenerative process in centenarians.

3. Patterns of AD-related lesion distribution in the oldest-old: early contributions

From a neurobiological point of view, the study of oldest-old individuals may permit to define the spectrum and extent of changes in brain morphology that occur with normal brain aging and assess correlations between the neuropathological definition of normal brain aging and clinical development of dementing process [26–28]. Brain aging is characterized by the formation of neurofibrillary tangles (NFT) and senile plaques (SP), as well as neuronal and synaptic loss in both cognitively intact individuals and patients with AD. In non-demented cases, NFT are usually restricted to the hippocampal formation, whereas the progressive involvement of the association areas in the temporal neocortex parallels the development of overt clinical signs of dementia. In contrast, severe SP formation may take place in several neocortical areas in the presence of very mild cognitive impairment and there is no correlation between the quantitative distribution of SP and severity of AD (for review see [29,30]). With regard to neuronal loss, stereological analyses have revealed age-related decreases in total neuron number of 30% and 50% in the dentate hilus of the hippocampus and subiculum, respectively, between ages 13 and 85. Conversely, no neuronal loss was found in CA1-3 fields and entorhinal cortex where AD lesions are also observed [31–33]. These studies also showed that in AD, there is an additional depletion of neuronal cell bodies in the dentate hilus and subiculum, as well as a massive reduction in the numbers of pyramidal neurons in the CA1 field and layers II and V of the entorhinal cortex [31–35]. Moreover, earlier and recent studies have shown a neuronal reduction in temporal, inferior and superior parietal and frontal cortices of AD cases [35,36].

Does the pattern of lesion distribution and neuronal loss change in extreme aging? A first observation concerns the presence of several cases with minimal AD pathology and preserved cognitive functions [26,28,37]. These individuals, also called “supernormal centenarians”, represent a rare phenotype relatively protected from AD pathology and may be thus an example of successful aging near the upper age

limit of life. Several neuropathologic analyses postulated that in contrast to younger cases where dementia is mainly related to severe NFT formation within adjacent components of the medial and inferior aspects of the temporal cortex, oldest-old individuals display a preferential involvement of the anterior part of the CA1 field of the hippocampus whereas the inferior temporal and frontal association areas are relatively spared [34,37]. The first contributions in this field have considered that the extent of NFT development in the hippocampus is the key determinant of dementia in the very old. For instance, Hauw et al. [38] examined the NFT distribution in the cerebral cortex of 12 centenarians (1 case with AD), and found higher NFT densities in the CA1 field in the demented patient than in cognitively intact centenarians. In their study of 27 non-demented centenarians and younger AD cases, Mizutani and Shimada reported that demented patients had dramatically higher NFT densities in the dentate hilus of the hippocampus, whereas no difference was observed in the entorhinal cortex and the subiculum [28]. More recent studies attempted to define the exact cognitive impact of NFT, SP, and neuronal loss in this age group (see Table 1). We found a significant difference in NFT densities in the anterior CA1 field, but not in the posterior CA1 field and entorhinal cortex, between demented and non-demented very old patients and suggested that nonagenarians and centenarians may show a specific subregional distribution of NFT within the CA field [26,34,39]. Two recent reports led to discrepant conclusions. In their study of 19 centenarians (including 4 AD cases), Garcia-Sierra et al. [40] found a substantial NFT involvement of hippocampus and entorhinal cortex supporting the notion of a limbic dementia in the oldest-old. Using a semiquantitative assessment of AD lesions, the longitudinal Oregon Brain Aging study reported that NFT and SP densities in neocortical areas were significantly related to cognitive scores [27]. Moreover, it has been reported that overt clinical signs of AD in oldest-old individuals requires a progressive damage of areas 7, 22, 23 and 24 suggesting a displacement of NFT, such that parietal and cingulate cortex are more affected than is usually the case in AD, whereas superior frontal and inferior temporal association areas are relatively preserved [34,41]. Unlike younger cohorts where SP formation does not correlate with neuronal depletion and cognitive status [42–44], earlier and more recent studies suggested that SP densities in the neocortex are related to the degree of neuronal loss and severe AD in the oldest-old [27,34]. Moreover, the extensive neuronal loss in the hippocampal formation reported in younger AD series [45] appears to be confined to the layer II of the entorhinal cortex in nonagenarians and centenarians [34].

Three main methodological issues explain the discordance between these contributions. First, most of them compared controls to severe AD cases without taking into account cases with questionable and mild dementia. Second, estimates of AD-related pathology were performed with non-stereological methods which are subject to sampling bias.

Most importantly, AD pathologic hallmarks are usually studied separately without taking into account the well established interaction between amyloid deposition, NFT formation and neuronal loss [for review see [29,30]]. Using rigorous stereological design, we recently analyzed the patterns of AD lesion distribution and clinicopathological correlations in a prospectively documented series of nonagenarians and centenarians. In the following chapters, we summarize these data and critically discuss their pertinence in the context of the debate regarding the relationship between AD pathological process and normal brain aging.

4. Hippocampal AD-related pathology after 90 years: sparing of the entorhinal cortex and CA1 field

Our first stereologic study included 12 patients older than 90 years who died and were autopsied in the Departments of Geriatrics and Psychiatry of the University of Geneva School of Medicine [46]. All cases underwent neuropsychological assessment within the last 6 months prior to their death and a Clinical Dementia Rating (CDR) scale score was available for all of them. This analysis revealed several differences in lesion distribution within the hippocampus and entorhinal cortex between oldest-old individuals and previously analyzed younger cohorts of elderly persons [47,48]. In particular, the progression of NFT formation across the different CDR groups was significantly slower in nonagenarians and centenarians (from 1 to 17% in the entorhinal cortex and 1.7 to 37% in the CA1) compared to younger cases [from 4 to 79% in the entorhinal cortex and 3 to 80% in the CA1 field [48]]. Interestingly, the only community-based neuropathologic study of oldest-old individuals also confirmed this pattern of NFT distribution in the CA1 field [27]. The relative resistance of the hippocampal formation to NFT development in this age group was even more striking in respect to the entorhinal cortex. In agreement with our previous observations in centenarian brains [34], even cases with moderate dementia display only mild NFT formation in this area with more than 80% of preserved neurons. This contrasts with the results of several previous studies in younger samples which demonstrated that the entorhinal cortex is more severely affected and involved earlier in the degenerative process than other hippocampal regions [30,41,49,50]. Our cases also displayed significantly lower total amyloid volume in the areas studied compared to that reported in younger series [47]. Similarly, the magnitude of neuronal loss in the entorhinal cortex and CA1 field in these cases was also significantly lower than that reported in younger AD cases [35,48,51–53]. In this latter group, the number of layer II entorhinal cortex neurons is thought to decrease even by 60% in patients with CDR 0.5 and by 90% in severe AD cases [35]. In the CA1 field, a depletion of 38% to 69% was also reported [35,48,51–53]. These data also imply that, like AD pathologic changes, neuronal loss is less prominent in the oldest-old even in the presence of AD [34]. In conjunction with the observations of Itoh and

Table 1
Neuropathological studies in the oldest-old from 1995 to 2006

Author	Hospital based	Community based	Aged over 90	Cognitive status	AD pathology	Vessels
Giannakopoulos et al. [37]	x		29	10 non demented, 19 demented	Dementia severity was related to SP formation; differential distribution of NFT compared to younger cohorts	No information
Troncoso et al. [90]		x	6	3 possible AD, 1 probable AD, 2 definite AD	Correlation between cognitive decline and amyloid deposits in neocortex; absence of SP in non demented cases	No information
Giannakopoulos et al. [32]	x		50	13 non demented, 15 MCI, 22 demented	Dementia in very old age requires both high NFT densities in the anterior CA1 field and progressive damage of the neocortex; dissociation between NFT and neuronal loss	No information
Itoh et al. [52]	x		13	5 non demented, 8 MCI	No relationship between hippocampal NFT and cognitive status	Cerebral amyloid angiopathy in 53.8% of cases
Green et al. [25]		x	15	6 non demented, 2 MCI, 7 demented	Strong positive correlation between rate of cognitive decline and AD lesions	No information
Garcia-Sierra et al. [38]	x		19	15 non demented, 4 demented	The density of intracellular NFTs in the entorhinal cortex correlates with mental status	No information
Leuba et al. [91]	x		21	10 non demented, 9 MCI, 2 AD	Very old age does not influence NFT formation in the primary visual system	No information
Neuropathol. Group of medical research council cognitive function and ageing study [92]		x	65	29 non demented, 36 demented	Extensive overlap of intermediate Alzheimer-type pathology among demented and non demented very old cases	No correlation between vascular disease and dementia
Iseki et al. [93]	n.s.		6	6 demented	No difference in AD-related hippocampal pathology between oldest-old and younger cases	No information
Silver et al. [7]		x	14	6 non demented, 4 AD demented	Absence of relationship between AD lesions and cognition in 4 cases	No information
Thal et al. [94]	n.s.		3	1 non demented, 1 MCI, 1 unknown	AD-related pathology is related to small vessel changes due to cerebral amyloid angiopathy	Strong correlation between the extension of small vessel lesions and cognitive status
Snowdon [95]		x	3	2 non demented, 1 demented	No clinicopathological correlations	No information
von Gunten et al. [44]	x		12	4 non demented, 2 MCI, 6 demented	Decreased NFT numbers and amyloid volume in hippocampus compared to younger series; only mild neuronal loss in CA1 field and entorhinal cortex in demented centenarians	No information
Prohovnik et al. [96]	x		81	18 non demented, 11 MCI, 52 demented	Dementia severity is unrelated to neuropathological markers; decreased SP densities in very old AD cases	No information
Bouras et al. [69]	x		19	4 non demented, 4 MCI, 11 demented	Total amyloid volume was not related to cognitive status; significant relationship between NFT number and CDR scores in the CA1 field, but not in the entorhinal cortex	Mean capillary diameters in entorhinal cortex predict more than 30% of CDR variability

n.s. not specified, MCI: mild cognitive impairment.

collaborators [54] who first reported only mild synaptic loss and cerebral amyloid angiopathy in centenarians, these findings give additional experimental support to the notion that the occurrence and progression of AD-related pathologic changes are not a sine qua non concomitant of increasing aging [27,34,39,55].

Another intriguing result of this study was the clear dissociation between total neuron numbers and AD-related lesions in the hippocampal formation. Although this is expected for amyloid deposits based on previous work in elderly individuals younger than 85 [for review see [44]], it may be more surprising for NFT numbers, given that

several stereologic and non-stereologic quantitative studies have reported strong relationships between NFT counts and neuron loss in the hippocampal formation and neocortex suggesting that NFT-dependent neuronal loss may be the rule in the cerebral cortex [35]. In contrast, non-NFT-related mechanisms of neurodegeneration may determine neuronal depletion in this age group [34]. Although these mechanisms remain largely speculative, recent contributions postulate that apoptosis, oxidative stress and excitotoxic mechanisms play a key role in inducing neuronal death that would predate NFT formation in some regions [for review see [52]].

The most striking finding of this study was the relative paucity of correlations between AD pathological hallmarks in the hippocampal formation and clinical status after 90 years. Only a modest percentage of the CDR variability was explained by NFT counts in CA2-3 (18%) and dentate gyrus (17%). In contrast, neither Nissl-stained neuron numbers nor total amyloid volume was significantly related to CDR score in both univariate and multivariate models. In this respect, it is worth noting that in spite of the clear neuronal loss observed in cases with moderate to severe dementia, total neuron numbers in the entire sample did not significantly predict cognitive status. Moreover, NFT numbers in the CA2-3 fields and dentate gyrus predict at the best less than 25% of CDR variability indicating that independent morphometric variables may decisively contribute to the cognitive decline in this age group [56].

Besides AD-related pathology, structural parameters of the cerebral vasculature have been thought to determine cognitive performances in the elderly. In the 90's, several age-related alterations of the microvascular ultrastructure (such as perivascular collagen deposits, atrophy of endothelium, basement membrane thickening and pericyte degeneration) as well as qualitative changes in microvascular structure (such as glomerular loops and twisted capillaries) have been described both in the aging brain and in AD [for review see [57–60]]. In contrast, quantitative analyses of structural parameters in brain capillaries led to controversial data. Increased age-related capillary density attributed to tissue shrinkage in the human neocortex and hippocampus has been reported earlier [61], but more recent animal and human studies challenged these findings [62–66]. Similarly, an age-related increase of capillary diameters and decrease of capillary length has been found in the aging human hippocampus [67] but not in neocortical areas [65,68]. As for AD-related lesions, the discordance of the previous observations mainly reflects methodological biases related to the use of density-based estimates of microvascular parameters. Recently, the development of modern design-based stereological techniques allowed for an accurate assessment of age-related changes in the capillary network [69,70]. Using this design, we explored the morphometric characteristics of the capillary network in nonagenarians and centenarians as well as their impact on cognition.

5. Microvascular morphology in the oldest-old: a new correlate of cognitive status?

This second stereologic study included 19 very old individuals with various degrees of cognitive impairment. Besides estimates of total NFT and neuron numbers as well as total amyloid volume, we also assessed total capillary length, number, and length-weighted mean diameters in the CA1 and entorhinal cortex [71]. Both mean diameters and total capillary numbers were strongly related to total neuron numbers in the CA1 field and entorhinal cortex. In a multivariate model including total NFT and capillary numbers (or diameters) in the CA1, both variables significantly explained neuron number variability. In contrast, there was no significant association between total capillary length and neuron number in the areas studied. No relationship was found between AD-related lesions and capillary morphological parameters in the CA1 and entorhinal cortex. These results revealed that total capillary numbers may explain more than 40% of the neuron number variability in the CA1 and entorhinal cortex, supporting a strong relationship between microvascular changes and AD-related neuronal depletion. Importantly, previous studies postulated that decreases in capillary number and diameter could disrupt the balance between energy requirements and cerebral blood supply, rendering the brain more vulnerable to oxidative stress damage and ultimately neuronal death [58,72,73]. In particular, an early animal study showed that chronic brain hypoperfusion in rats induces ultrastructural capillary changes in CA1 field which were accompanied by a substantial compromise of spatial memory [74]. Supporting a primary role of cerebral hypoperfusion in triggering AD-related pathology, a single photon emission computerized tomography study revealed the presence of regional cerebral perfusion abnormalities which preceded clinical symptoms in presenilin-1 mutation carriers [75].

In terms of clinicopathological correlations, mean capillary diameters in the CA1 field and entorhinal cortex were significantly related to CDR scores. In a univariate model, they explained respectively 19% and 31.1% of the cognitive variability. Importantly, these associations persisted in multivariate models where total neuron numbers, NFT numbers or amyloid volume were considered [71]. The biological significance of these findings remains unclear. A critical assumption that has to be made in this context is that the capillaries observed in postmortem material correspond to the structure in its *in vivo* state. Such a correlation has been reported [76,77] and it seems likely that instead of the recruitment of additional capillaries, increased cognitive load induces differential distribution of flow [78], heterogeneity in blood flow velocity [79] and changes in diameters [80]. Changes in the flow distribution are usually regulated in the capillary network itself through the local diffusion of nitric oxide [81], serotonin and other neurotransmitters [82,83]. Decreased capillary diameters may lead to impaired microcirculation within the hippocampal formation and thus

prevent adaptive responses to local changes in metabolic demands.

6. Conclusions

The debate whether there is continuity or discontinuity between normal brain aging and dementia has a long history and is not merely of academic interest. The hypothesis that AD is an aging-related condition is supported by the nearly ubiquitous presence of AD pathologic changes in the course of brain aging and the exponential increase of AD prevalence after 65 years of age. Contrasting with this conception of aging, the study of oldest-old individuals indicates that the occurrence of AD pathology is not a mandatory phenomenon of increasing chronologic age. In particular, the neuropathology of advanced aging strongly suggests that very old individuals with AD display a striking resistance to the neurodegenerative process and only mild neuronal loss in the hippocampal formation. Our hospital-based findings supporting a dissociation between the clinical expression and traditionally assessed AD pathology are consistent with the recent neuropathological observations of the New England Centenarian Study [7,84] and show the limits of the “lesional” model for explaining the expression of dementia symptoms in this particular age group. Furthermore, they offer a new perspective in the field of clinicopathological correlations by revealing the key cognitive impact of decreased capillary diameter, a structural but not lesional parameter of the aging brain.

The biological background of the increased resistance to AD lesion development after 90 years is still poorly understood. Thirty years ago, it was suggested for the first time that oldest-old people show genetic variations which influence basic mechanisms of brain aging resulting in a decreased susceptibility to age-associated diseases [85]. In particular, it has been proposed that nonagenarians and centenarians may lack the “disease genes” that predispose to fatal age-related diseases [86]. Alternatively, they may have genetic variations that confer protection against age-related illnesses (the “longevity-enabling” genes [87]). In this respect, the sparing of CA1 field and entorhinal cortex may be related to a genetically determined resistance of these neuronal subpopulations in very old people. Importantly, evidence from genetic studies of aging and AD implies that a number of susceptibility genes may modify or delay the onset of late-life brain failure. These gene families form a natural target for devising strategies to delay the onset of late-onset dementia [88]. More recently, the importance of genetic factors has been further stressed by studies of centenarian pedigrees showing increased relative survival probabilities in centenarian siblings compared to the general population [87]. The first community-based linkage and association studies identified two candidate genes predisposing to longevity. Both of them were related to lipoprotein synthesis suggesting that protection against cardiovascular diseases may be primordial to achieve extreme old age [89–91]. Although the relationship between these vascular “longevity-enabling” genes and AD-related patholo-

gy is not clear, the link between preserved cognition and brain capillary diameter points to the need for exploring the genetic determinants of the microvascular system in very old individuals. Moving away from the classical link between lesion and function, the research on extreme brain aging should focus on the mechanisms surrounding the activation of functional brain reserve. With the population of centenarians growing at such a rapid rate, complex longitudinal studies including functional neuroimaging, quantitative assessment of microvascular parameters in neocortical areas and differential gene expression may provide cues for a better understanding of both genetic and neurobiological dimensions of longevity.

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