American Journal of Alzheimer's Disease & Other Dementias®
25(7) 585-591

DOI: 10.1177/1533317510382892
http://aja.sagepub.com
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Morphological Changes of the Human Purkinje Cells and Deposition of Neuritic Plaques and Neurofibrillary Tangles on the Cerebellar Cortex of Alzheimer's Disease

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Abstract

Alzheimer's disease is a neurodegenerative disorder, characterized by progressive decline in memory and in social performance. The morphological hallmarks of the disease are neuronal loss, loss of dendritic spines, neurofibrillary degeneration and neuritic plaques mainly in the hippocampus and the cortex of the cerebral hemispheres. This study is based on the morphological analysis of the cerebellar cortices of eight brains, 4 patients suffered from Alzheimer's disease and 4 normal controls, by Golgi method, as well as Nissl, Gallyas', Bielschowsky's, Methenamine Silver staining and Congo red methods. Although typical neuritic plaques were not seen in the cerebellar cortex and the diffuse plaques found in the cerebellum in far smaller proportion than plaques in the prefrontal and parietal cortices of the same cases, Golgi impregnation technique revealed a loss of Purkinje cells and a marked decrease in the density of dendritic arborization.

Keywords

Alzheimer, Purkinje, cerebellum, golgi method, diffuse plaques

Introduction

Alzheimer's disease (AD) constitutes one of the main causes of cognitive impairment in the presenium and senium.[1] The neuropathological spectrum of AD comprises neuronal loss, as well as loss of dendritic spines, neurofibrillary degeneration, and neuritic plaques mainly in the hippocampus and the cortex of the cerebral hemispheres.[1,2] The cerebellar cortex is characterized by a unique pattern of Alzheimer-type pathology, while there are only diffuse neuritic plaques and no neurofibrillary changes.[3] However, a loss of Purkinje cells and synaptic alterations in the mossy fibers, granule cell dendrites, parallel fibres and Purkinje cell dendrites with substantial loss of dendritic spines, and considerable decrease in number of granule and Golgi cells in the granule cell layer have been reported by previous studies.[2,4-7]

The current study was carried out to investigate the alterations of the dendritic arborization and the dendritic spines of the Purkinje cells in AD and to correlate them with the presence of neuritic plaques and neurofibrillary degeneration.

Materials and Methods

Methods Selection

Although Golgi method is capricious and unpredictable, it has been used for more than 100 years for the study of the neuronal morphology and continues to provide a unique view of the neuronal cell. With Golgi method, the cell soma, as well as the dendrites, are clearly stained in brown and black and can be followed through to their entire length.

Nissl staining approach allows the visualization of all neuronal somata and was used to provide a more accurate account of neuronal densities and cortical thickness in each given brain area.

Gallyas staining has been used for the depiction of neurofibrillary tangles, while it shows almost the same sensitivity as the Thioflavin S staining and is more sensitive in neurofibrillary tangles (NFT) demonstration than PHF-1 or NFT immunostaining.[8]

Senile plaques are best seen with silver stains and immunocytochemistry for amyloid-β protein. Furthermore, for most practical and diagnostic reasons, they are divided into the "diffuse" or "preamyloid" plaques and the "classical" or "neuritic" plaques. Diffuse plaques stain with Bielschowsky and

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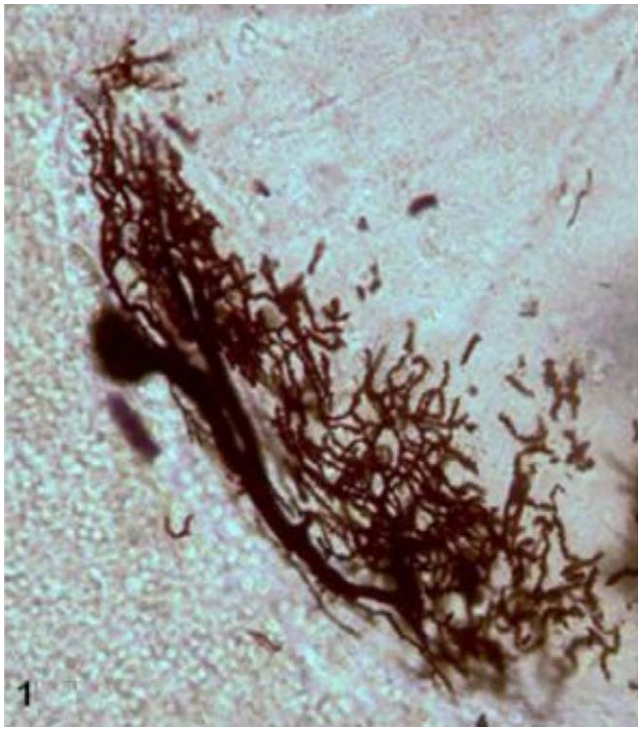

Figure 1. Purkinje cells from the cerebellar cortex of Alzheimer's disease brains, demonstrating substantial loss of tertiary and secondary dendritic branches. Golgi Silver staining method. Magnification ×400.

Methenamine Silver staining. Wisniewski et al, in a comparative study of 4 staining methods on the detection of neuritic plaques, demonstrated that Bielschowsky's method revealed the same number of neuritic plaques in comparison to immunostaining with monoclonal antibody (4G8, IgG2b) to β-amyloid.[9] According to Yamaguchi et al Bielschowsky's method is more effective than β protein immunostaining in the visualization of the diffuse types of plaques.[10] Furthermore, Methenamine Silver staining is more specific to the amyloid of the primitive and typical types of senile plaques than Periodic Acid Modified Methenamine stain (PAM) stain, results similar to that of the β protein immunostain[11,12] and is the most sensitive method for detecting the presence of the diffuse type of senile plaques.[13]

Materials and Methods

This study is based on the morphological analysis of the cerebellar cortex of 10 brains (5 from patients who suffered from AD and 5 healthy senile individuals who died accidentally) kindly offered to us from the laboratory of Forensic medicine and Toxicology of Aristotelian University of Thessaloniki.

Histological criteria for the diagnosis of AD were those outlined by the National Institutes of Health/American Association of Retired Persons (NIH/AARP) Research Workshop on the Diagnosis of Alzheimer's Disease.[14] All cases fulfilled the histological criteria for AD.

The brains were immersed immediately after the excision from the skull in a fixing solution of 10% of formaldehyde in room temperature and remained there for 25 days. Then, we selected specimens from the vermis and from the superior surface of the cerebellar hemispheres including part of the dentate nucleus, for silver techniques and Nissl staining. Sections from the frontal and the parietal cortices were also examined in all 5 AD and 5 control cases.

For Golgi method, the specimens were immersed in a dilution of potassium dichromate (7 g of potassium dichromate in 300 mL of tap water) at room temperature. They remained in that solution for 1 week and then they were immersed in aqueous solution of 1% silver nitrate where they remained for 1 more week at a temperature of 15°C in photoprotected environment.

After the fixation, the specimens were embedded in low-melting point paraffin and cut with a slicing microtome at thick sections at the range of 100 μm. Some of the sections were poststained with 10% methylene blue according to Golgi-Nissl method, and after a rapid differentiation, they were covered with entelan and studied in a Carl Zeiss Axiostar Plus photomicroscope. Adjacent sections from each brain were dehydrated and embedded in low-melting point paraffin. Then they were cut with a vibrotome in sections at the range of 8 μm and were used for Gallyas method,[15] Methenamine Silver staining, Bielschowsky method, Congo red method, and Nissl staining.

The morphological criteria taken into account for the selection of the neurons were integrity and dark homogeneous impregnation throughout the extent of the dendrites, cell bodies located in the middle part of the section thickness to minimize the number of branch segments cut off the plane of the section, and relative isolation from other impregnated cells, blood vessels, and silver deposits (artifacts) placed nearby.[16]

Neuronal tracing was carried out using the Neuro J plug-in in Image J application and each of the selected cells was analyzed according to Sholl's method of concentric circles[17]; 6 concentric spheres were drawn at 40-μm intervals centered on the cell bodies, and dendritic intersections with each sphere were counted. This procedure provided a measure of dendritic density as a function of distance from the cell body.[17]

Spine counts were carried out on the dendrites of the Purkinje neurons at standard magnifications of ×1000. Visible spines were counted on 10 segments (10 μm in length) of the dendritic field. The first 5 segments were located on secondary dendrites, and the second group of segments was located on tertiary dendritic branches.

The thickness of the molecular layer of the cerebellar cortex, as well as the diameter of the cell soma, has been measured in Nissl-stained specimens.

The density of the Purkinje cells was counted in Nissl-stained specimens on 50 pictures at standard magnifications of ×40, using the cell counter plug-in of Image J. For each of the brains, the density of the pyramidal cells of the fifth layer of the prefrontal cortex has also been counted on blocks of 150 × 150 μm, to compare the neuronal loss in the cerebellar and in the cerebral cortices, respectively.

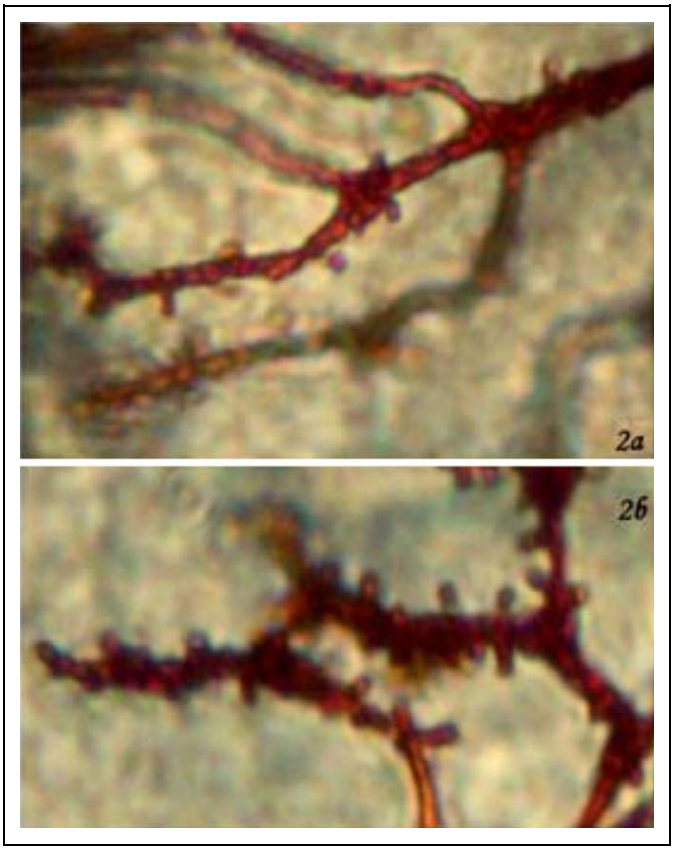

Figure 2. The density of the dendritic spines of the Purkinje cells of Alzheimer's brains (A) was significantly decreased, compared with the normal controls (B). Golgi Silver staining method. Magnification ×1000.

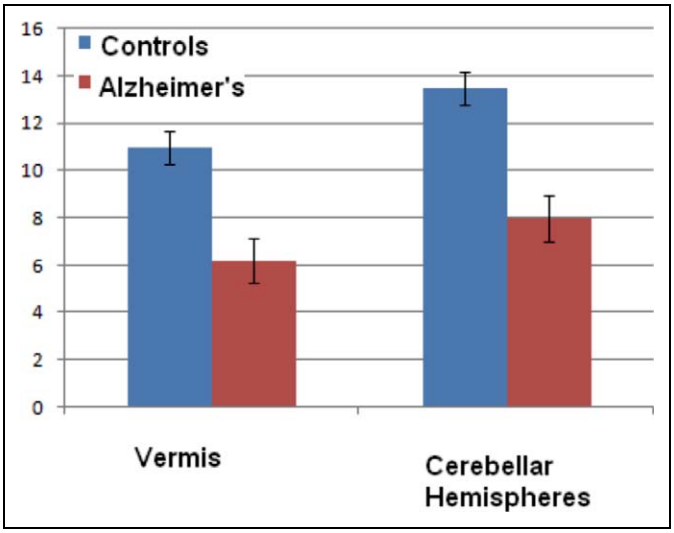

Figure 3. Spine density of the Purkinje cells from the cerebellar hemispheres and the vermis of the cerebellum of the Alzheimer's disease brains and normal controls ($P < .0018$). Error bars indicate standard deviation.

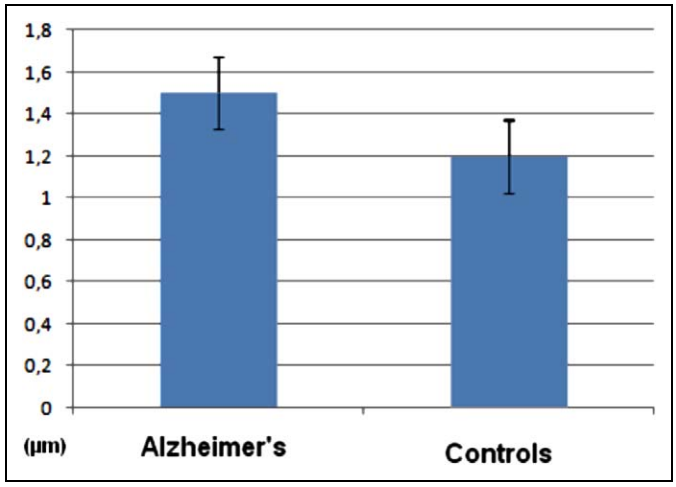

Figure 4. Length of dendritic spines of the Purkinje cells from Alzheimer's disease brains and normal controls. Error bars indicate standard deviation.

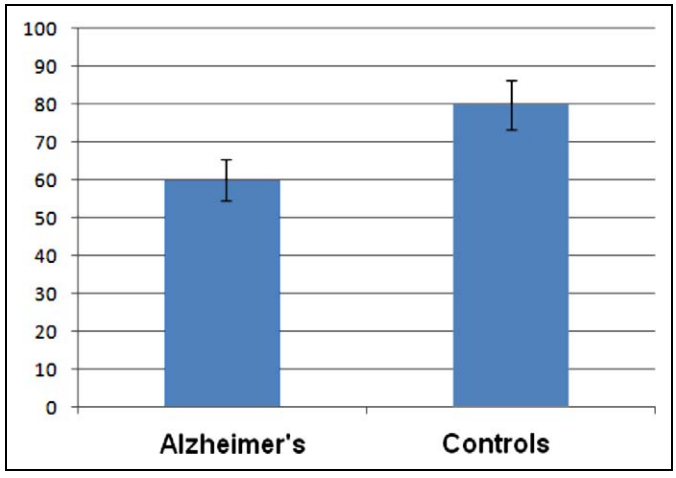

Figure 5. The percentage of the depth of the molecular layer that is covered by the dendritic arborization of the Purkinje cells in Alzheimer's disease and in normal controls. Error bars indicate standard deviation.

The density and the size of the senile plaques were measured, using the Image J program on cortical blocks of 150 × 150 μm.

For the statistical analysis, we used the Student's *t* test (significance was taken as $P < .05$).

Results

Golgi Silver staining

Golgi-stained tissue did not exhibit the autolytic changes described by Williams et al.[18] The Golgi impregnation technique revealed a substantial loss of tertiary and secondary dendritic branches of the Purkinje cells in the brains of the patients who suffered from AD in comparison with normal

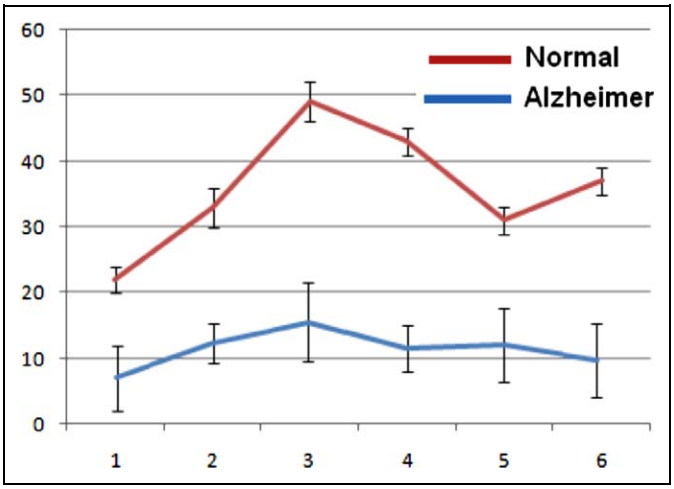

Figure 6. Concentric cycles' analysis of the dendritic arborization of the Purkinje cells demonstrated marked decrease in the density of dendritic arborization in Alzheimer's disease brains ($P < .0098$). Error bars indicate standard deviation.

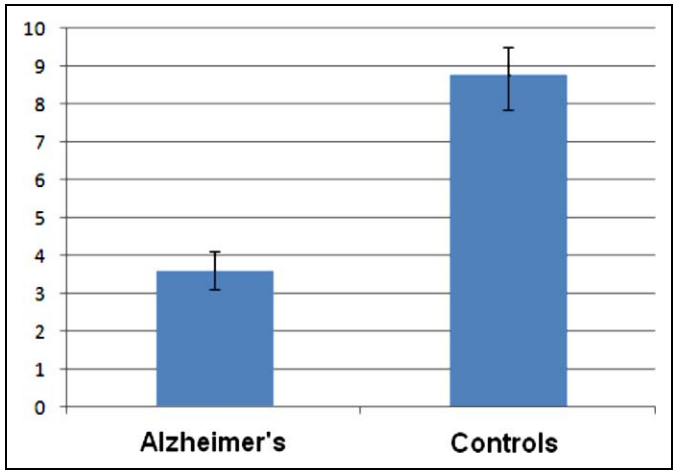

Figure 8. Purkinje cells' density on the vermis of the cerebellum of the Alzheimer's disease brains and normal controls ($P < .001$). Error bars indicate standard deviation.

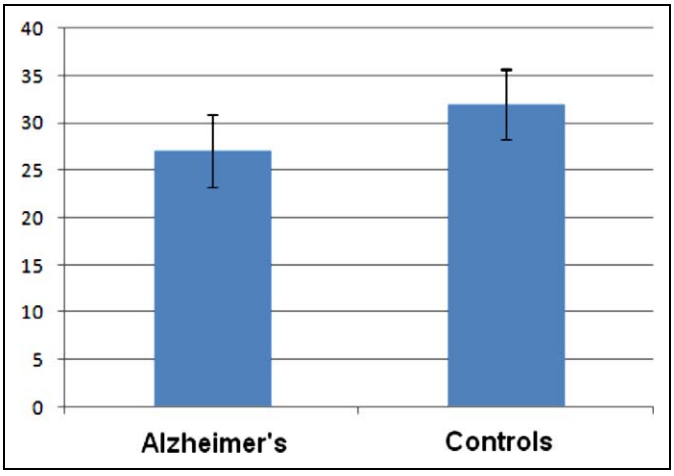

Figure 7. The diameter of the cell body of the Purkinje cells (in μm) from Alzheimer's brains and nondemented senile individuals. Error bars indicate standard deviation.

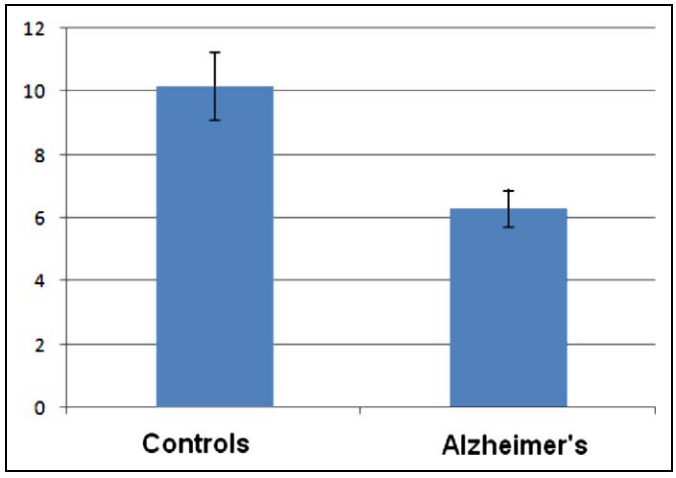

Figure 9. Purkinje cells density on the hemispheres of the cerebellum of the Alzheimer's disease brains and normal controls ($P < .0006$). Error bars indicate standard deviation.

controls and a marked decrease in the density of dendritic arborization (Figure 1).

The density of the dendritic spines of the Purkinje cells of AD was by 43% decreased (Figures 2A, B, and 3; $P < .0018$), compared with the normal controls, however, the length of the spines was increased by 16% (Figure 4, $P > .001$).

In the AD brains, the dendritic arborization of the Purkinje cells covered a maximum of 61% of the depth of the molecular layer, while in the nondemented senile brains the percentage came up to 80% (Figure 5, $P > .016$).

Sholl's Analysis

We used Scholl analysis to measure the number of intersections as a function of distance from the soma.

Concentric cycle's analysis of the dendritic arborization of the Purkinje cells demonstrated marked decrease in the density of dendritic arborization in AD brains (Figure 6, $P < .0098$).

We found that the peak dendritic development of the Purkinje cells occurred at 90 μm from the cell soma for AD brains and 120 μm for the normal controls and the dendrite maximum was 16 and 49, respectively.

Nissl Staining

The diameter of the cell body of the Purkinje cells of the AD brains was decreased by 15% at most compared with that of the nondemented senile individuals (Figure 7, $P > .001$). The cell soma diameter that emerged from the morphometrical study in Golgi impregnation was estimated as being quite

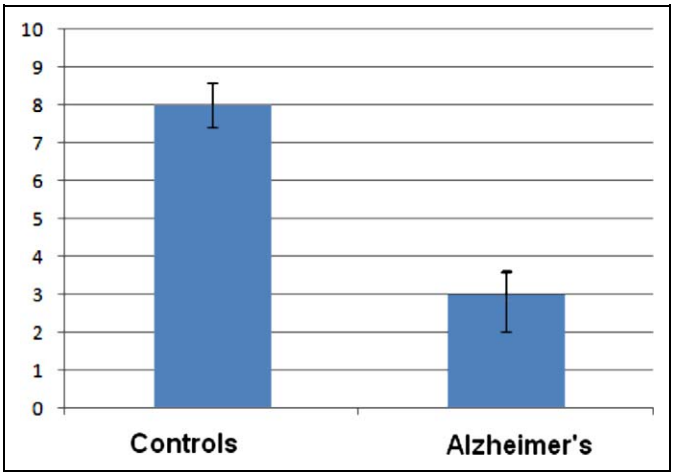

Figure 10. Pyramidal cells' density (per 10000 μm^2) on the frontal cortex of normal controls and Alzheimer's disease brains ($P < .0009$). Error bars indicate standard deviation.

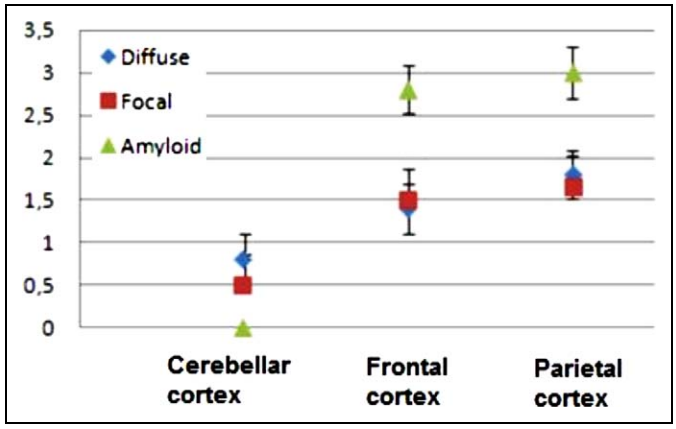

Figure 11. Plaques densities (per 10000 μm^2) of the cerebellar, frontal and parietal cortex, in Alzheimer's disease brains and normal controls ($P < .001$). Error bars indicate standard deviation.

lower than the diameter, which has been revealed by older studies.[19]

There were no differences in the thickness of the molecular layer between the 2 groups of the study.

The population of the Purkinje cells counted in each block was impressively lower in the AD group, even to an extent of 62% down, in comparison to the nondemented individuals, as this has been noticed in the Nissl counterstaining (Figures 8 and 9, $P < .001$). Respectively, in the prefrontal cortex, the population of pyramidal cells was diminished by 70% in AD brains (Figure 10, $P > .001$).

NFT and Plaque Density

Gallyas technique revealed neurofibrillary degeneration in 30% of the Purkinje cells and some large glutaminergic neurons of the dentate nucleus.

Two types of plaque-like deposits were demonstrated in AD cerebellar cortex. Diffuse plaques in the molecular layer and compact amyloid cored plaques in the Purkinje and granular cell layers. Diffuse plaques in the molecular layer of cerebellar cortex were not detected in normal aged controls. Typical neuritic plaques were not seen in the cerebellar cortex of AD brains.

The plaques found in the cerebellum were detected in far smaller proportion than senile plaques in the prefrontal and parietal cortices of the same cases (Figure 11, $P > .001$).

Discussion

The cerebellum is a relatively less affected area in the AD brain, in comparison to the hippocampus and the cortex of the cerebral hemispheres.[9] However, a number of pathological changes has been reported in the cerebellum in AD in studies based mostly on immunocytochemical techniques, consisted of widespread deposits of diffuse amyloid, ubiquitin-immunoreactive dystrophic neurites, and microglial proliferation.[10-13]

In the senile human cerebellum, a decline of the total volume of the cortex has been assessed, due to loss of Purkinje neurons, degeneration of dendrites, belittlement in the size of the cell bodies, decrease in the density of the dendritic fields, focal storage of lipids within the dendritic arborization, and loss of dendritic spines.[14-17]

Morphometric analysis of the cerebellar cortex in Golgi staining broadens the neuropathological spectrum of AD in the cerebellum to include loss of secondary and tertiary dendritic branches of the Purkinje cells, substantial decrease in the number of Purkinje cells' dendritic spines, and decrease in the density of the Purkinje cells.[2,15,16]

Although Purkinje cells demonstrated significant dendritic and spinal pathology, the molecular layer of the cerebellar cortex is characterized by the absence of typical neuritic plaques and the presence of a few diffuse plaques.

The major difference between the 2 types of plaques is the nature of the amyloid β protein that is present. The Aβ peptide in diffuse plaques is not in the β-pleated sheet conformation that is representative of amyloids of all compositions.

Diffuse plaques in the cerebellum are positive for end specific monoclonal antibodies Aβ1-42 and Aβ1-40 negative.[20,21] The amyloid in the core of the neuritic plaques is composed of the β protein and is positive for both Aβ1-42(43) and Aβ1-40 end-specific monoclonal antobodies.

This view would suggest that there are distinct mechanisms involved in the deposition of amyloid β protein in diffuse and neuritic plaques and that the cerebellar environment plays a crucial role in the regulation of these mechanisms. This comes to agreement with Du et al who showed that metabolites of cerebellar neurons promote the expression of Aβ degrading enzymes and advance Aβ clearance.[22]

Only 30% of the Purkinje cells exhibit neurofibrillary degeneration, in comparison to 80% of the pyramidal cells of the frontal and 90% of them on the acoustic cortex. Nevertheless, Purkinje cells overall show important dendritic loss and spinal pathology.

Moreover, the degenerating axons in the cerebellum differ from the axonal degeneration commonly seen in the cortex of brain hemispheres probably due to the absence of marked tau pathology.[4,20]

These assumptions may suggest that there are more factors than Tau pathology and Aβ accumulation which lead to neuronal degeneration in AD.

Dendritic and spinal pathology in the cerebellum and other subcortical brain areas have been correlated by previous studies with mitochondrial alterations and fragmentation of the Golgi apparatus connecting the oxidative damage to AD pathogenetic mechanism.[19,23,24]

Furthermore vascular pathology and blood brain barrier abnormalities have been interrelated to loss of dendrites and dendritic spines in patients with AD.[25,26]

Conclusion

It is important to recognize that besides Aβ deposition and Tau pathology, there are more mechanisms, such as oxidative damage, vascular pathology, blood brain barrier abnormalities, and other still unidentified factors that participate in the neuronal degeneration in AD.

In other studies, some cylindrical forms of Hirano bodies in the perikaryon of Purkinje cells and senile plaques have been described,[27,28] but the conclusion of the current study is that the density of the plaques in the cerebellum is not as high as in the neocortex.

The loss of dendrites and dendritic spines leads to a substantial decrease of the synaptic contacts of the Purkinje cells with the parallel fibers and other cells of the cerebellar cortex, and this could contribute to the cognitive decline of AD.

The problem of the morphological and immunocytochemical differences between the cerebral and the cerebellar cortex in AD may lead to further investigation including the effect of the tau pathology and the accumulation of the α-β peptide in the environment of the cerebellar cortex and the biological properties of Purkinje cells in correlation with the biological parameters of the brain cortex.

Declaration of Conflicting Interests

The author(s) declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

The author(s) received no financial support for the research and/or authorship of this article.

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