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DENDRITIC AND SPINAL PATHOLOGY OF THE PURKINJE CELLS FROM THE HUMAN CEREBELLAR VERMIS IN ALZHEIMER'S DISEASE

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SUMMARY

Background*: Alzheimer's disease constitutes one of the main causes of dementia. It is clinically characterized by memory impairment, deterioration of intellectual faculties and loss of professional skills. Furthermore changes in equilibrium and limb coordination are clinically demonstrable in persons with Alzheimer's disease. In the present study we tried to figure out possible changes of the Purkinje cells in Alzheimer's disease brains.*

Subjects and methods*: We studied the Purkinje cells from the vermis of the cerebellum in 5 Alzheimer' disease brains Golgi technique.*

Results*: In the Purkinje cells from the inferior surface of the cerebellar hemispheres severe dendritic and spinal pathology consisting of loss of distal dendritic segments and alterations of dendritic spine morphology can be noticed in Alzheimer's disease brains.*

Conclusions*: The morphological and morphometric estimation of the dendrites and the dendritic spines of the Purkinje cells from the inferior surface of the cerebellar hemispheres in Alzheimer's disease brains revealed substantial alterations of the dendritic arborization and marked loss of the dendritic spines, which may be related to cognitive impairment and motor deficits in Alheimer's disease.*

Key words: *Alzheimer's disease – cerebellum - Purkinje cells - Golgi method*

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INTRODUCTION

Alzheimer's disease constitutes one of the main causes of dementia in senium and presenium. It is clinically characterized by memory impairment, deterioration of intellectual faculties and loss of professional skills (Baloyannis 1993, Perry & Hodges 2000).

AD brains are characterized by significant atrophy, being most obvious in the temporal and parietal lobes (Baloyannis 1993). Light microscopy reveals deposition of senile plaques and neurofibrillary degeneration initially in the enthorhinal cortex, the hippocampus, and in the acoustic and visual cortex, in the frontal lobe and the cerebellum in the advanced stages (Baloyannis 1993, Wegiel et al. 1999, Larner 1997, Padurariu et al. 2012). Dendritic and spinal pathology, as well as loss of synapses are also key neuropathological features (Mavroudis et al. 2011).

The cerebellum has not be studied extensively in AD while had being considered as a motor center. Conversely there is evidence that this structure participates in emotional reactions (Heath et al. 1979, Weinberger et al. 1979), constant states of activity (Weinberger et al. 1980, Pollack 1997), and experience of rage, anger of fear (van Dongen et al. 1994, Silveri et al. 1994).

As we have shown in a previous study of ours the cerebellar cortex is characterized by a unique pattern of Alzheimer-type pathology, while there are only diffuse neuritic plaques and no neurofibrillary changes (Mavroudis et al. 2010). Furthermore, a loss of Purkinje cells and synaptic alterations in the mossy fibers, granule cell dendrites, parallel fibers 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 (Perry & Hodges 2000, Wegiel et al. 1999, Larner 1997, Fukutani et al. 1996).

The current study was carried out to investigate the alterations of the dendritic arborization and the dendritic spines of the Purkinje cells from the inferior surface of the cerebellar hemispheres in AD.

SUBJECTS AND METHODS

The present study is based on the morphological analysis of the dendritic arborizations of the Purkinje cells from the inferior surface of the cerebellar cortex from 5 patients suffered from Alzheimer's disease and from 5 healthy individuals who died accidentally and were used as normal controls.

All the brains have been offered by the Laboratory of Forensic medicine and Toxicology of the Aristotle University of Thessaloniki.

All the patients have a clinical diagnosis of Alzheimer's disease by a neurologist and Mini-Mental State Examination scores below 12.

Gross examination of the brains was performed by a neuropathologist who was blinded to the participant's cognitive scores and medical history. Tissue blocks from multiple neocortical regions, hippocampus, entorhinal cortex, amygdala, visual cortex, auditory cortex and cerebellum were embedded in paraffin. To assess Braak stage, sections at the range of 10μm were stained with the Gallyas method for neurofibrillary pathology while for staining of neuritic plaques the Bielchowsky method was used. Neuritic plaques scores were determined by semiquantitative estimation of neocortical plaque density using the CERAD criteria. All the Alzheimer's disease brains were on Braak stages V/VI, fulfilled the Khachaturian criteria for definite Alzheimer's disease and have indicative CERAD scores for Alzheimer's disease (Table 1).

Table 1. Demographic features and Braak & Braak staging of the brains used in the present study

Brain	Age	Gender	Braak & Braak stage
AD1	67	Male	V/VI
AD2	65	Female	V/VI
AD3	72	Female	V/VI
AD4	69	Male	V/VI
AD5	76	Male	V/VI
NC1	86	Male	I/II
NC2	72	Female	0
NC3	75	Female	0
NC4	80	Female	0
NC5	69	Male	0

Only one of the brains used as normal controls was on Braak stage I/II, the rest having no NFTs and SP s at all (Table 1).

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 (Khachaturian 1985). All cases fulfilled the histological criteria for AD.

The brains after the excision from the skull were immediately immersed in a formaldehyde 10% fixing solution where they remained for at least 25 days. Then we excised small parts from the cerebellar cortex at the level of the vermis, which have been used for Golgi method. The specimens were immersed in a dilution of potassium dichromate (7 g of potassium dichromate and 1ml of formaldehyde 37% in 300 mL of tap water) at a temperature of 18^{o}C. 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^{o}C in photoprotected environment. Afterwards the specimens were embedded in low-melting paraffin, cut with a slicing microtome at thick sections at the range of 120μm, covered with entellan and studied with a Carl Zeiss Axiostar plus light microscope.

The morphological criteria that were used for the selection of 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 (Jacobs et al. 1997).

For each one of the brains 10 cells were selected. The morphological study and morphometric analysis of the selected cells were carried out with the aid of Image J application on 100 digital images which were taken using an Amscope digital microscope camera.

Furthermore the selected cells were analyzed according to Sholl's method of concentric circles (Sholl 1953); 15 concentric circles were drawn at 15μm intervals centered on the cell bodies, and dendritic intersections with each circle were counted. This procedure provided a measure of dendritic density as a function of distance from the cell body. Furthermore the distance of peak of the development of the dendritic arborization (critical value) and the dendrite maximum which is the maximum of intersections in the peak of the development of the dendritic tree were calculated (Sholl 1953).

Spine counts were carried out on the dendrites of the Purkinje neurons at standard magnifications of X1000. Visible spines were counted on 10 segments (10 mm in length) of the dendritic field. The first 5 segments were located on tertiary dendrites, and the second group of segments was located on quaternary dendritic branches. Dendritic spines were classified according to their morphology in long neck ones and short stubby spines. The densities of dendritic spines, as well as their size were estimated manually with the help of Image J software.

The thickness of the molecular layer of the cerebellar cortex, as well as the diameter of the cell soma, and the number of Purkinje cells has been measured in Nissl-stained specimens with the help of Image J application (Figure 1). Adjacent sections were cut in a microtome at the range of 8μ and used for Holzer method, in order to figure out if there is increased gliosis in AD brains.

Statstical Analysis

A Student's t-test was used to determine whether significant differences existed across the independent parameters from Purkinje cells from normal controls and AD brains (significance was taken as $P<0.05$). Furthermore, a Pearson correlation test was carried out in order to figure out if there is any correlation between Purkinje cell number and Purkinje cell dendritic complexity variables.

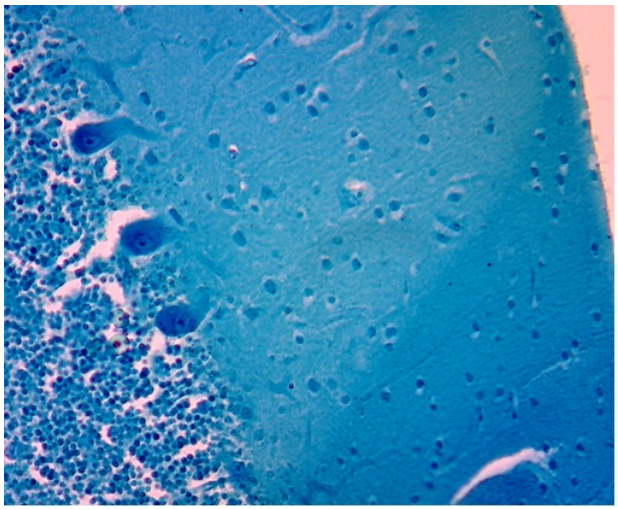

Figure 1. The number of Purkinje cells and the thickness of the molecular layer were estimated in Nissl method (Nissl method, Magnification 100X)

RESULTS

Purkinje cells did not exhibit autolytic changes described by Williams et al. (1987).

There were no differences in the thickness of the molecular layer between AD brains and normal controls. Holzer method showed severe gliosis in AD brains.

Purkinje cells from AD brains showed a noteworthy restriction of the dendritic arborization (Figures 2A, 2B) due to loss of terminal dendritic branches, while the number of primary and secondary dendrites per neurons did not exhibit significant difference between the two groups of the study. Sholl's analysis demonstrated marked decrease of the number of intersections in the distal circles (Figure 3). The peak of the development of the dendritic arborization occurred at 85μm from cell soma for AD brains and at 130μm for the normal controls. Dendrite maximum was 21 and 56 respectively.

The area that is covered by the dendritic field for each of the Purkinje cells was considerably decreased in AD brains as indicated in Figure 4.

The total number of dendritic spines per Purkinje cell of AD brains was by 50% decreased, compared to normal controls (Figure 5, Figure 6). The majority of the remaining spines on AD brains were of the short-stubby type, while the Purkinje cells of normal controls bear spines of the long-neck type and only a few from the short-stubby type.

Although there was loss of Purkinje cells in AD brains, it was without statistical significance. On account of the loss of Purkinje cells in AD brains, empty baskets are usually seen in Golgi method.

Pearson correlation test did not exhibit any correlation between Purkinje cell numbers and Purkinje cell dendritic complexity and spine variables.

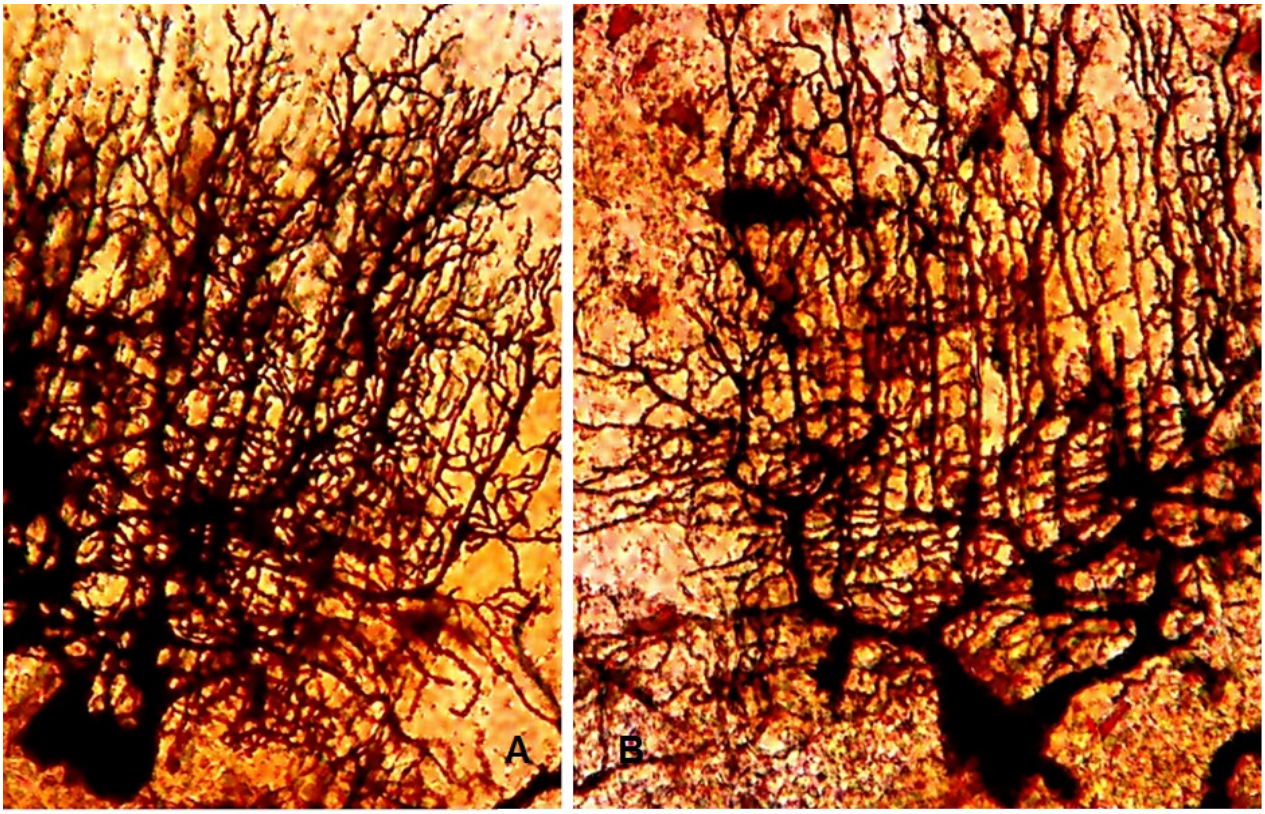

Figure 2A. Purkinje cells from the vermis of the cerebellum from a normal control brain.
Figure 2B. Purkinje cells from the vermis of the cerebellum from an AD brain, exhibiting severe loss of distal dendritic branches (Golgi method, Magnification 100X)

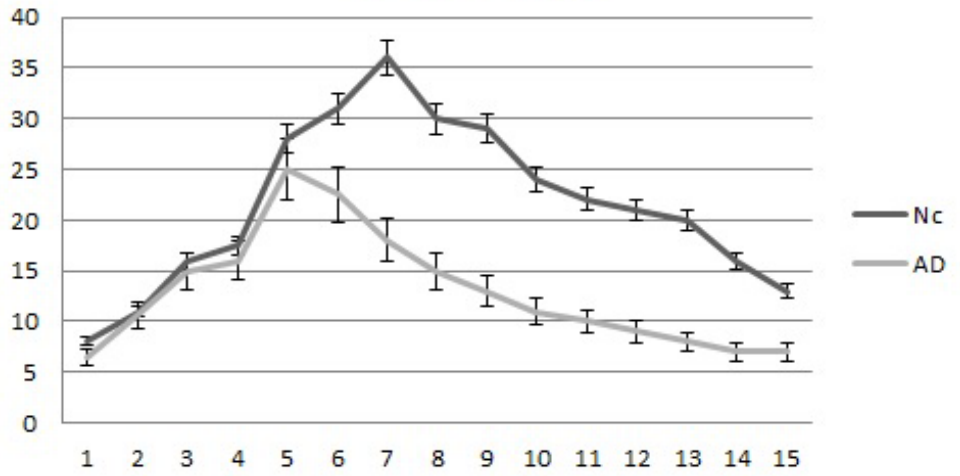

Figure 3. Sholl's analysis of the dendritic trees from AD brains (white line) and Normal controls (black line) ($p<0.01$, error bars indicate standard deviation)

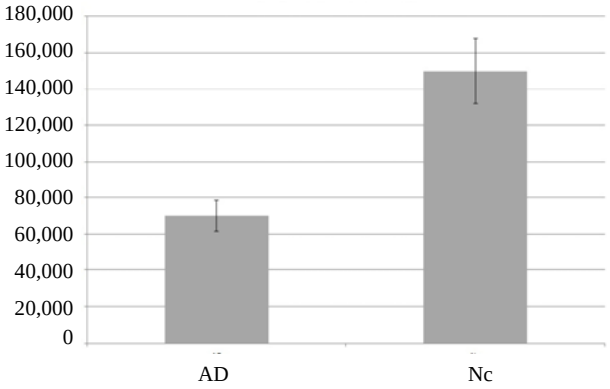

Figure 4. Dendritic field area covered by the arborization of the Purkinje cells from AD brains and Normal controls ($p<0.01$, error bars indicate standard deviation)

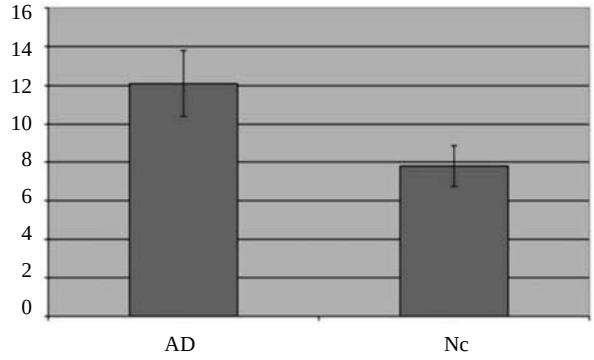

Figure 5. Spinal density (number of spines per 10μm) from the Purkinje cells from AD brains and Normal controls ($p<0.01$, error bars indicate standard deviation)

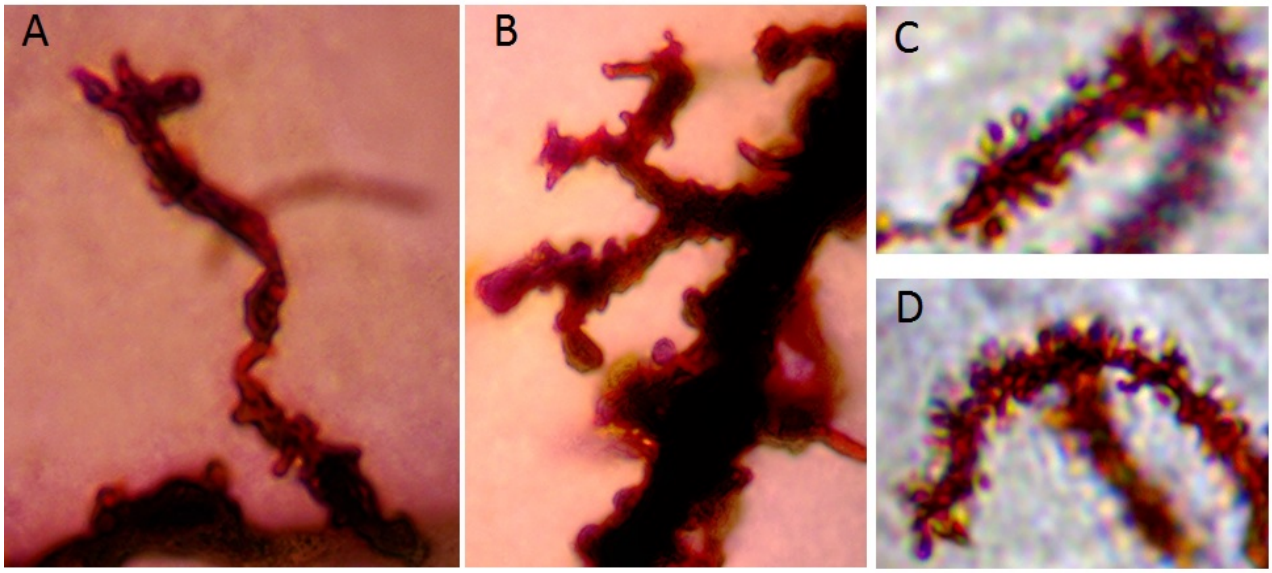

Figure 6. (A, B) Higher magnification from a part of the dendritic tree of a Purkinje cell from an AD brain. Marks the loss of tertiary and quaternary branches and the substantial loss of dendritic spines. (C, D) Higher magnification from parts of the dendritic tree of a Purkinje cells from a normal control brain (Golgi method, Magnification 1000X)

DISCUSSION

Cerebellum was thought to be spared by Alzheimer's disease, however recent studies showed a number of pathological changes, including loss of distal dendritic segments, decrease of the total number of dendritic spines, ubiquitin-immunoreactive dystrophic neurites and microglial proliferation of the Purkinje cells (Larner 1997, Mavroudis et al. 2010, Baloyannis et al. 2000, Dickson et al. 1990, Yamamoto & Hirano 1985, Fukutani et al. 1996). The present study confirms the involvement of the Purkinje cells in Alzheimer's disease neuropathology.

Distal dendritic segments and dendritic spines were prominently affected in Purkinje cells from Alzheimer's disease brains. These structures are the most plastic components of the dendritic arborisation (Michmizos et al. 2011); therefore these changes might be related to an impairment of mechanisms underlying neuroplasticity.

Soluble Ab peptide and Ab oligomers unsettle neuroplasticity imbalance, resulting in an impairment of synaptic stabilization (Cullen et al. 1997, Wang et al. 2002) and loss of dendritic spines and synapses in Alzheimer's disease brains. Hyperphosphorylation of tau protein may also cause deleterious effects of neuroplasticity and may underlie its role in the aetiology of Alzheimer's disease (Maccioni et al. 1995, Mandelkow et al. 1995). Nevertheless the molecular layer of the cerebellar cortex is characterized by a unique pattern of pathology in Alzheimer's disease, which includes deposition of diffuse plaques and the absence of typical senile plaques, and only minor neurofibrillary changes (Wegiel et al. 1999, Mavroudis et al. 2010). Consequently there might be more mechanisms, such as oxidative damage, vascular pathology, blood brain barrier abnormalities that participate in the neuronal degeneration in AD (Baloyannis et al. 2000, Mavroudis et al. 2010).

Besides substantial spinal loss, the Purkinje cells from Alzheimer's disease brains bear short-stubby dendritic spines in 90%; in contrast to normal controls the Purkinje cells of which possess mainly the long-neck type of spines. Spinal shape is modulated by a number of proteins via regulation of the actin cytoskeleton (Jaworski et al. 2009).

It is important to emphasize that the cerebellar cortex is affected in Alzheimer's disease, despite the fact that neurofibrillary degeneration and typical senile plaques deposition are not prominent. Cerebellar cortex offers a valuable background for the further study of Alzheimer's disease pathogenesis is and future treatment strategies.

The loss of distal dendrites and dendritic spines seen in thick sections of Golgi stained material, leads to a substantial decrease of the synaptic area and synaptic contacts of the Purkinje cells and this could contribute to the cognitive decline of AD. Furthermore the inferior surface of the cerebellar vermis is related to muscle tone of the antigravity muscles of lower body, which are affected in Alzheimer's disease (Franssen et al. 1999). Therefore, changes in equilibrium and limb coordination that are clinically demonstrable in persons with Alzheimer's disease might be correlated to the present findings.

CONCLUSION

The morphological and morphometric estimation of the dendrites and the dendritic spines of the Purkinje cells from the inferior surface of the cerebellar hemispheres in Alzheimer's disease brains revealed substantial alterations of the dendritic arborization and marked loss of the dendritic spines, which may be related to cognitive impairment, equilibrium and limb coordination deficits in Alheimer's disease.

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Conflict of interest: None to declare.

References

1. *Baloyannis SJ, Manolidis SL, Manolidis LS: Synaptic alterations in the vestibulocerebellar system in Alzheimer's disease-a golgi and electron microscope study. Acta Otolaryngol. 2000; 120:247-250*
2. *Baloyannis SJ: Neuropathology of Dementia. Aristotelian University of Thessaloniki, 1993.*
3. *Cullen WK, Suh YH, Anwyl R, Rowan MJ: Block of LTP in rat hippocampus in vivo by beta-amyloid precursor protein fragments. Neuroreport 1997; 8:3213–3217.*
4. *Dickson DW, Wertkin A, Mattiace LA, et al.: Ubiquitin immunoelectron microscopy of dystrophic neurites in cerebellar senile plaques of Alzheimer's disease. Acta Neuropathol (Berl) 1990; 79:486-493.*
5. *Franssen EH, Souren LE, Torossian CL, Reisberg B: Equilibrium and limb coordination in mild cognitive impairment and mild Alzheimer's disease. J Am Geriatr Soc 1999; 47:463-9.*
6. *Fukutani Y, Cairns NJ, Rossor MN, Lantos PL: Purkinje cell loss and astrocytosis in the cerebellum in familial and sporadic Alzheimer's disease. Neurosci Lett 1996; 214:33-36.*
7. *Fukutani Y, Cairns NJ, Rossor MN, Lantos PL: Purkinje cell loss and astrocytosis in the cerebellum in familial and sporadic Alzheimer's disease. Neurosci Lett 1996; 214:33-36.*
8. *Heath RG, Franklin DE, Shraberg D: Gross pathology of the cerebellum in patients diagnosed and treated as functional psychiatric disorders. J Nerv Ment Dis 1979; 167:585-92.*
9. *Jacobs B, Driscoll L, Schall M: Life-span dendritic and spine changes in areas 10 and 18 of human cortex: a quantitative Golgi Study. J Comp Neurol 1997; 386:661-680.*
10. *Jaworski J, Kapitein LC, Gouveia SM, et al.: Dynamic Microtubules Regulate Dendritic Spine Morphology and Synaptic Plasticity. Neuron 2009; 61:85-100.*

11. *Khachaturian ZS: Diagnosis of Alzheimer's disease. Arch Neurol 1985; 42:1097-1104.*
12. *Larner AJ: The cerebellum in Alzheimer's disease. Dement Geriatr Cogn Disord 1997; 8:204-209.*
13. *Maccioni RB, Cambiazo V: Role of microtubule associated proteins in the control of microtubule assembly. Physiol Rev 1995; 23:1–27.*
14. *Mandelkow EM, Biernat J, Drewes G, Gustke N, Trinczek B, Mandelkow E: Tau domains, phosphorylation, and interactions with microtubules. Neurobiol Aging 1995; 16:355–362.*
15. *Mavroudis IA, Fotiou DF, Adipepe LF, Manani MG, Njau SD, Psaroulis D, Costa VG, Baloyannis SJ: Morphological changes of the human purkinje cells and deposition of neuritic plaques and neurofibrillary tangles on the cerebellar cortex of Alzheimer's disease. Am J Alzheimers Dis Other Demen 2010; 25:585-91.*
16. *Mavroudis IA, Fotiou DF, Manani MG, Njaou SN, Frangou D, Costa VG, Baloyannis SJ: Dendritic pathology and spinal loss in the visual cortex in Alzheimer's disease: a Golgi study in pathology. Int J Neurosci 2011; 121:347-54.*
17. *Michmizos D, Koutsouraki E, Asprodini E, Baloyannis S: Synaptic plasticity: a unifying model to address some persisting questions. Int J Neurosci 2011; 121:289-304.*
18. *Padurariu M, Ciobica A, Mavroudis I, Fotiou D, Baloyannis S: Hippocampal neuronal loss in the CA1 and CA3 areas of Alzheimer's disease patients. Psychiatr Danub 2012; 24:152-8.*
19. *Perry, RJ, Hodges JR: The relationship between functional and neuropsychological performance in early Alzheimer's disease. Alzheimer Dis Assoc Disord 2000: 14:1-10.*
20. *Pollack IF: Posterior fossa syndrome. Int Rev Neurobiol 1997; 41:411-32.*
21. *Sholl DA: Dendritic organization of the neurons of the visual and motor cortices of the cat. J Anat 1953; 87:387-406.*
22. *Silveri MC, Leggio MG, Molinari M: The cerebellum contributes to linguistic production: a case of agrammatic speech following a right cerebellar lesion. Neurology 1994; 44:2047-50.*
23. *Travis K, Jacobs B: Regional Dendritic Variation in Neonatal Human Cortex: A Quantitative Golgi Analysis. J Behav Neurosci Res 2003; 1:8-16.*
24. *van Dongen HR, Catsman-Berrevoets CE, van Mourik M: The syndrome of 'cerebellar' mutism and subsequent dysarthria. Neurology 1994; 44:2040-6.*
25. *Wang HW, Pasternak JF, Kuo H, Ristic H, Lambert MP, Chromy B, Viola KL, Klein WL, Stine WB, Krafft GA, Trommer BL: Soluble oligomers of ab-amyloid (1–42) inhibit long-term potentiation but not long-term depression in rat dentate gyrus. Brain Res 2002; 924:133–140.*
26. *Wegiel J, Wisniewski HM, Dziewiatkowski J, et al.: Cerebellar atrophy in Alzheimer's disease—clinicopathological correlations. Brain Res 1999; 818:41-50.*
27. *Weinberger DR, Kleinman JE, Luchins DJ, Bigelow LB, Wyatt RJ: Cerebellar pathology in schizophrenia: a controlled postmortem study. Am J Psychiatry 1980; 137:359-61.*
28. *Weinberger DR, Torrey EF, Wyatt RJ: Cerebellar atrophy in chronic schizophrenia. Lancet 1979; 1:718-9.*
29. *Williams RS, Ferrante RJ, Caviness VS Jr: The Golgi rapid method in clinical neuropathology: morphological consequences of suboptimal fixation. J Neuropath Exp Neurol 1978; 37:13-33.*
30. *Yamamoto T, Hirano A: Hirano bodies in the perikaryon of the Purkinje cell in a case of Alzheimer's disease. Acta Neuropathol (Berl) 1985; 67:167-169.*

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