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# Alterations of Mitochondria and Golgi Apparatus Are Related to Synaptic Pathology in Alzheimer's Disease

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Additional information is available at the end of the chapter

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## 1. Introduction

Alzheimer's disease (AD) is an insidiously progressive severe presenile and senile dementia, involving a number of cellular and biochemical mechanisms. AD affects millions of humans as the most common cause of cognitive decline worldwide, in addition to being a main medical challenge for aging population. From the clinical point of view, AD is mostly characterized by age-dependent inexorably progressing cognitive decline, affecting memory primarily associated with behavioral and mood disorders, which increasingly appear as the disease advances [1]. From the neuropathological point of view, AD is mostly characterized by selective neuronal loss [2, 3], marked synaptic alterations [4–6], morphological mitochondrial abnormalities [7, 8], tau pathology [9] resulting in neurofibrillary tangles (NFT) composed of hyperphosphorylated tau [10], inflammatory responses and by extracellular extensive deposits of polymers of A $\beta$  peptide, in the form of neuritic plaques, which are a main hallmark of AD [11, 12]. These are dispersed in the neocortex, the hippocampus, and many subcortical structures, which play an important role in cognition. In addition, AD is characterized ultrastructurally by organelle pathology involving mostly the microtubules, the mitochondria, and Golgi apparatus [13].

Most studies have revealed that the main pathological criteria for AD, namely the neuritic plaques and neurofibrillary tangles, can account for 40%–70% of the cognitive impairment seen in advanced age, though additional cerebrovascular changes [14, 15] contribute greatly in plotting the dramatic profile of AD.

The two main hypotheses of AD - the amyloid cascade hypothesis, introduced in 1991 [16, 17] and the Tau protein hypothesis, are still subjects of extensive research and debate. The production and accumulation of A $\beta$  peptide, a pathogenic factor leading to AD development,

are the result of the post-translational proteolysis of the APP [18], by concerted actions of  $\beta$ - and  $\gamma$ -secretases [19].

The amyloidogenic pathway for APP is initiated by  $\beta$ - site amyloid precursor protein-cleavage enzyme 1 (BACE- 1), resulting in the generation of the intermediate product sAPP $\beta$  [20].  $\gamma$  -Secretase activity is substantial for the cleavage of the transmembrane domain, releasing the A $\beta$  peptide and the APP intracellular domain. Generation of A $\beta$  peptide may occur in the endoplasmic reticulum (ER), trans-Golgi network [21], in lysosomes and on the surface of the cell, whereas its intracellular accumulation has been mostly detected in the majority of neurons in the endoplasmic reticulum, the mitochondria [22], the lysosomes, the multivesicular bodies, associated with synaptic pathology [23]. Many risk factors may affect APP protein metabolism and A $\beta$  peptide production, mainly in late-onset AD. In addition changes in cellular protein homeostasis and aspects of protein folding or misfolding [24] are also linked to AD pathogenesis [25].

All the biochemical phenomena, which may occur within the spectrum of pathogenetic mechanisms in Alzheimer's disease affect the brain metabolic activity reasonably, since increasing evidence from functional neuroimaging plead in favor of the global and regional disruptions in brain metabolism in advanced cases of AD [26].

From the etiological point of view, it would be hypothesized that the multiple genetic loci [27, 28], associated with familial Alzheimer's disease would plead in favor of the heterogeneity of the disease and support the idea that the phenomenological profile of Alzheimer's disease may be the final consequence of various metabolic, neurochemical, and morphological alterations, based on a broad genetic background [29]. Although the majority of familial or inherited AD, which manifests at an early age, are often associated with mutations in A $\beta$ PP [30], the numerous sporadic ones, which manifests usually at later stages of the life, are proved to be multifactorial, including induced expression of A $\beta$ PP [31] by pathological stimuli, environmental factors, as well as deprivation of trophic factors. The eventual accumulation of A $\beta$  peptide at synaptic terminals may be associated with synaptic damage, resulting in cognitive decline in patients with AD [32]. Moreover the increased risk of AD in sporadic cases, whenever a maternal relative is afflicted with the disease, pleads reasonably in favor of a maternally derived predisposition, which might be related to mutations of mitochondrial DNA (mtDNA) [33, 34].

### 1.1. Mitochondria in Alzheimer's disease

Mitochondria are highly dynamic ATP-generating organelles, which play an essential role in many cellular functions, such as alteration of reduction-oxidation potential of cells, free radical scavenging, intracellular calcium regulation and activation of apoptotic process. Mitochondria are unique amongst cellular organelles, since they dispose their own, spiral, double-stranded DNA (mtDNA), which is mostly inherited from the maternal line. The number of mitochondria is very high in neurons and especially in synaptic terminals, since they are the major energy generators for the cell biological processes and the synaptic activity, through tricarboxylic cycle and oxidative phosphorylation. The shape and size of mitochondria are not stable, since they undergo continual fission and fusion leading to their fragmentation or elongation accordingly.

Mitochondrial dysfunction might contribute to A $\beta$  neurotoxicity and is also associated with oxidative stress, which may play an important role in the early stages of pathogenetic mechanisms in AD [35-37], presumably prior to the onset of the cognitive dysfunction, since a substantial body of evidence suggests that mitochondria play a crucial role in ageing-related neurodegenerative diseases [38].

Mitochondria may be the target for amyloid precursor protein (APP) and A $\beta$  peptide, which might play an important role in impairing mitochondrial dynamics [39]. During AD processes accumulation of APP occurs mostly in the mitochondrial import channels, inducing mitochondrial functional impairment [40]. APP could not be processed to generate A $\beta$  peptide locally [41] although a fraction of active  $\gamma$ -secretase is associated with mitochondria [42].

In the mitochondria, A $\beta$  peptide uptake is mediated by the translocase, which is located in the outer mitochondrial membrane (TOM) [43]. Then A $\beta$  peptide is accumulated mostly in the outer mitochondrial membrane, the inter membrane and the matrix and interacts with large number of proteins inside mitochondria, leading eventually to mitochondrial dysfunction, whenever substantial amount of molecules of A $\beta$  peptide would be produced near mitochondria [44].

It is important to emphasize that mitochondrial alterations are associated with synaptic loss in AD patients, even before amyloid plaques are detected [32, 45]. Morphological and morphometric studies revealed that at early stages of AD the number of mitochondria in synaptic terminals is dramatically decrease and their structural pattern changes [45]. That modification might be attributed to enhanced nitrosative stress, generated by A $\beta$  peptide, leading to mitochondrial fission, which is followed by mitochondrial depletion, resulting in synaptic degeneration eventually [46].

## 1.2. Golgi apparatus in Alzheimer's disease

Golgi apparatus plays an important role in the pathogenesis of AD [13, 47], since it is associated with protein trafficking. All newly synthesized proteins, which are used for fast axoplasmic transport are processed practically through the vesicles and the cisternae of Golgi complex [48]. From the first its visualization by Camillo Golgi, in 1898, Golgi apparatus has been subject of intense morphological and neurochemical research.

The electron microscopy study of the mammalian Golgi apparatus has revealed that it consists of stapled cisternae, which serve for the modification of newly synthesized proteins and lipids. At the entrance site of the Golgi apparatus, namely the cis-Golgi, numerous clusters of vesicles and tubular structures form an intermediate chain between the smooth endoplasmic reticulum and the Golgi stack. The exit site, the so called trans-Golgi network (TGN) is the main site of sorting proteins to distinct cellular destinations. Bi-directional traffic between the Golgi apparatus and the endosomal system sustains the functions of the trans-Golgi network (TGN) in secretion and organelle biogenesis [49].

The function of the Golgi complex consists mainly on vesicular transport, involving constant membrane fission and fusion, mediated by GTPases, coat proteins, Rabs, tethers and SNARE proteins, respectively, as it was documented from studies on glycosylation enzymes [50]. The

activity of  $\gamma$ -secretase, which consists of presenilins (PSs) [51], nicastrin [52,53], pen-2 [54] and the aph proteins [54,55], is closely related with the function of Golgi complex, since it requires the presenilin-dependent trafficking of nicastrin, through the Golgi apparatus [56].

It was hypothesized that the passage of nicastrin and other components of the  $\gamma$ -secretase complex through Golgi apparatus is essential for the molecular stabilization and the protease activity [56]. In addition APP, which is normally synthesized in the endoplasmic reticulum (ER), is transported to trans-Golgi network (TGN) for trafficking to the cell surface [57, 58], or to synaptic terminals. APP is transported by fast axonal transport, been recycled back for further trafficking or final storing within the lysosomal system [59], in view that TGN generates transport vesicles bound for distinct domains of the plasma membrane and early endosomes. Whereas a small proportion of APP molecules are delivered to the plasma membrane and then cleaved by  $\alpha$ -secretase into non-amyloidogenic fragments, the majority of APP molecules undergo degradation, following amyloidogenic pathway in the trans-Golgi network [59].

## 2. Material and methods

### 2.1. Patients

We studied the hippocampus, the acoustic and the visual cortices, the thalamus, the globus pallidus, the locus coeruleus, the red nucleus, the hypothalamus and many regions of the cerebellar cortex in ten brains of patients who suffered from AD, four men and six women, aged 62–87 years, who fulfilled the clinical, neuropsychological, and laboratory diagnostic criteria of AD.

The mean education of the patients was 15.2 years, and all of them spoke their native language fluently. Screening procedures were applied, which included medical history, medical examination, cardiological investigation, physical neurologic assessment, psychiatric and neuropsychological examinations. All the patients underwent EEG, carotid duplex Doppler, computerized tomography (CT) scanning and magnetic resonance imaging (MRI) of the brain, and single-photon emission computed tomography (SPECT).

The mental status of the patients was assessed by Mini mental State Examination (MMSE) and dementia rating scale (DRS) [60] and ADAS-COX test.

The cause of death of the patients was heart arrest, following to cardiac infarct one to seven months after the final neurological assessment. The postmortem examination of each one of the cases was performed within 6 h after death.

### 2.2. Electron microscopy

Small samples (2×2×2 mm) from the hippocampus, the acoustic and the visual cortices, the thalamus, the globus pallidus, the locus coeruleus, the red nucleus, the hypothalamus and from many regions of the cortex of the cerebellar hemispheres and the vermis were excised and immediately immersed in Sotelo's fixing solution, composed of 1% paraformaldehyde,



2.5% glutaraldehyde in cacodylate buffer 0.1 M, adjusted at pH 7.35. Then they were postfixed by immersion in 1% osmium tetroxide for 30 min at room temperature and dehydrated in graded alcohol solutions and propylene oxide.

Thin sections were cut in a Reichert ultratome, contrasted with uranyl acetate and lead citrate, and studied in a Zeiss 9aS electron microscope.

We studied the morphology of the mitochondria, the Golgi apparatus, and the synapses and we proceeded to morphometric estimations at electron microscope on micrographs of a standard magnification of 56.000 X.

### **2.3. Light microscope, Golgi staining, Golgi-Nissl method**

The remaining parts of the above-mentioned areas of the brain and the cerebellum were processed for silver impregnation techniques, according to rapid Golgi method.

Thus, after a four-week fixation in formalin they were immersed in potassium dichromate (7 g potassium dichromate in 300 mL water) for 10 days. Then they were immersed in 1% silver nitrate for 10 days. Following a rapid dehydration in graded alcohol solutions, the specimens were embedded in paraffin and cut, some of them at 100  $\mu$  and some at 25  $\mu$ , alternatively. Many sections of 25  $\mu$  were stained also with methylene blue, according to Golgi-Nissl method. All the sections were mounted in permount, between two cover slips and were studied in a Zeiss Axiolab Photomicroscope.

We estimated the dendritic arborization, the morphology and the number of the dendritic branches, and the morphology of the dendritic spines in light microscope on sections stained according to rapid Golgi and Golgi-Nissl methods

### **2.4. Statistical analysis**

Statistical analysis was based on the *t*-test on the basis of 5000 mitochondria and 600 Golgi apparatus from 30 specimens of AD brains and 30 specimens of normal control brains.

## **3. Results**

### **3.1. Silver impregnation techniques**

The silver impregnation technique or black reaction (*reazione nera*) according to Golgi [61] is a simple and easy histological procedure that enables the visualization of the three-dimensional morphology of neurons and glial cells. Santiago Ramón y Cajal has been applying Golgi technique extensively for the histological analysis of the CNS, defending successfully the "neuron doctrine" and sharing with Camillo Golgi the Nobel Prize in 1906. After 140 years from its first application, the Golgi technique continues to remain a very useful and valuable method in neuropathology for the morphological and morphometric estimation of neuronal circuits at the early stages of the degenerative processes of the brain [62].

The application of silver impregnation technique in our specimens revealed neuronal loss and marked abbreviation of the dendritic arborization in all the layers of the acoustic and the visual cortices, the hippocampus, the thalamus, the globus pallidus, the locus coeruleus, the red nucleus, the hypothalamus (Fig.1), the cerebellar cortex (Fig.2) and the vermis of the cerebellum (Fig.3). The layer I, of the acoustic and visual cortices, which includes Cajal-Retzius cells, which normally protrude very long horizontal axonal profiles with substantial number of collaterals [63. 64], was practically empty of neurons in patients who suffered from AD, in contrast to normal controls.

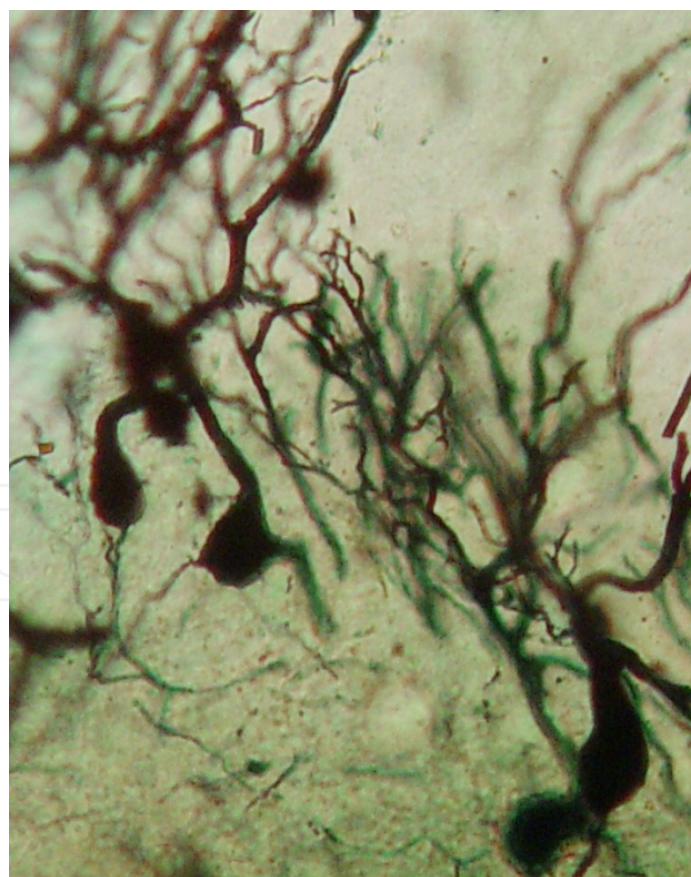


**Figure 1.** Neuron from the Hypothalamus of a case of AD, showing abbreviation of dendritic arborization and marked loss of dendritic spines (Golgi staining 2,400X)

Loss of tertiary dendritic branches was also noticed in the acoustic and visual cortices in all of the specimens. Abbreviation of the dendritic arborization was mostly prominent in neurons of layers III and V of the acoustic and visual cortices, the pyramidal neurons of the hippocampus, the polyhedral neurons of the locus coeruleus and in Purkinje cells of the cerebellar cortex, which demonstrated also a marked decrease of the number of dendritic spines (Fig.3) in comparison with the normal controls.



**Figure 2.** Purkinje cells from the cerebellar hemisphere of a case of AD, demonstrating marked abbreviation of the dendritic arborization and considerable loss of dendritic spines (Golgi staining 2,000X).

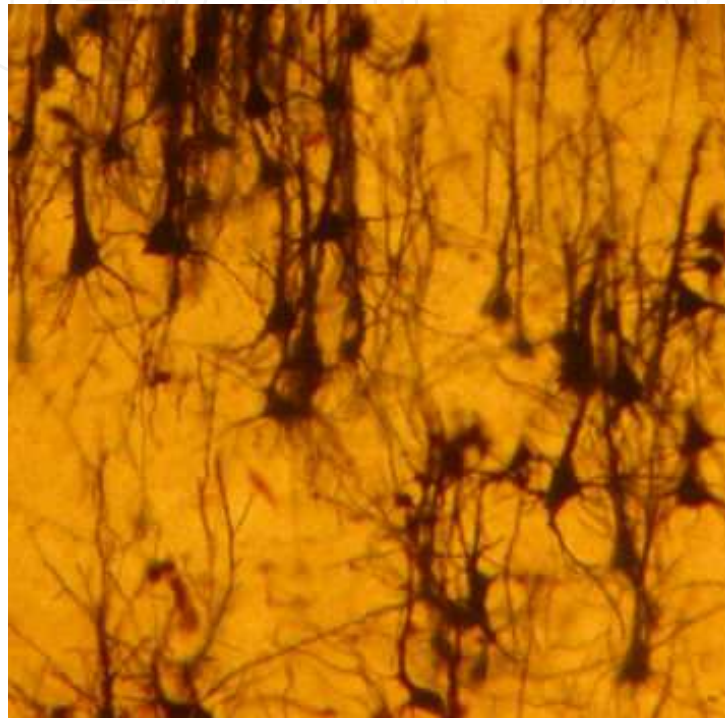


**Figure 3.** Purkinje cells from the vermis of the cerebellum of a case of AD, showing dramatic reduction of dendritic branches and loss of dendritic spines (Golgi staining 3,100X)



In addition, the axonal collaterals in layers III, IV, V, and VI of the acoustic and visual cortices were dramatically decreased in comparison with the normal controls.

The decrease of the branches of the apical dendrites of the cortical neurons as well as decrease in spine density was widespread phenomena seen in the large majority of neurons of the acoustic (Fig.4) and the visual cortices, in the hippocampus, the thalamus, the globus pallidus, the red nucleus, the locus coeruleus, the hypothalamus and the cerebellar cortex (Figs 1,2).



**Figure 4.** Neurons from the acoustic cortex of a case of AD, showing marked decrease of tertiary dendritic branches and tremendous loss of spines (Golgi staining 2,400X).

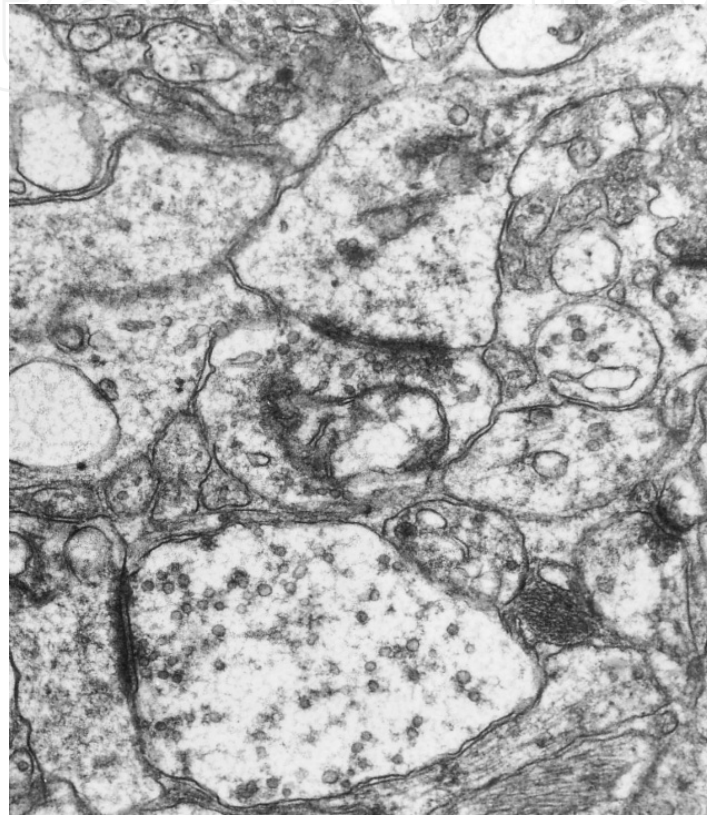
### 3.2. Electron microscopy

Electron microscopy is the most valuable and precise method for the morphological and morphometric study of the cell organelles, the synapses, the dendritic spines as well as the neuron- glial relationships in the CNS both in health and disease.

In our study, the electron microscopy revealed pathological alterations of the dendritic spines and impressive decrease in spine density in the secondary and tertiary dendritic branches in all of the layers of the acoustic and visual cortices.

The reduction in spine size was prominent in neurons of layers II, III, and V of the acoustic cortex. A substantial number of dendritic spines demonstrated large multivesicular bodies, abnormal spine apparatus, and mitochondria, which were characterized by marked morphological alterations. Morphological alterations of the dendritic spines were also seen in the pyramidal neurons of the hippocampus, the large polyhedral neurons of the thalamus, the

globus pallidus, the polyhedral neurons of the locus coeruleus as well as the Purkinje cells of the cerebellar hemispheres (Fig.5). Giant elongated spines were mostly seen in the hippocampus and in the Purkinje cells of the cerebellum. In a large number of presynaptic terminals of the acoustic and visual cortices of patients who suffered from AD, the ultrastructural study has revealed an considerable polymorphism and pleomorphism of the synaptic vesicles, which were dramatically decreased in number in comparison with normal controls.



**Figure 5.** Synaptic alterations in the cerebellar cortex in a case of AD. Impressive poverty of synaptic vesicles is noticed at the presynaptic terminals associated with mitochondrial alterations and disruption of the spinal apparatus (Electron micrograph 128,000X).

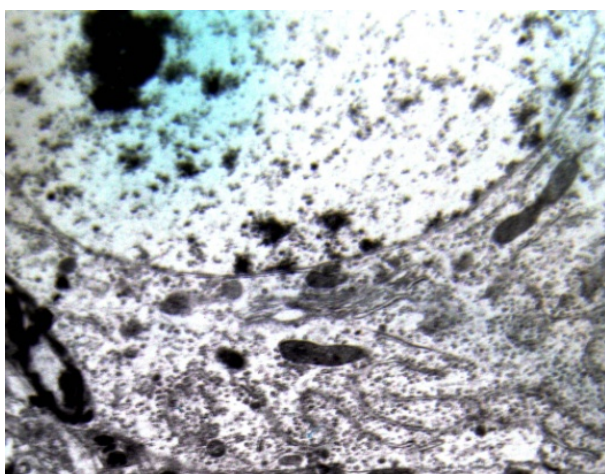
Substantial poverty of the synaptic vesicles was particularly seen in the presynaptic terminals in layers III, IV, and V of the acoustic and visual cortices, as well as in the mossy fibers of the cerebellar cortex. Decrease of the number of synaptic vesicles associated with polymorphism of the remained vesicles was noticed in the hippocampus, the thalamus, the locus coeruleus, and the parallel and climbing fibers of the cerebellar cortex.

Mitochondrial pathology was demonstrated in the majority of the dendritic spines in all of the specimens. That consisted of substantial change of mitochondrial shape and size, fragmentation of cristae, and accumulation of osmiophilic material in a considerable number of mitochondrial profiles (Fig.6). Many dendritic branches included mitochondria, which showed an unusual polymorphic arrangement of the cristae, which either showed a concentric configuration or they were arranged in a parallel way to the long axis of the organelle. Some Purkinje cell dendrites

(Fig.6) and a substantial number of climbing fibers included very large elongated mitochondria. Small round mitochondria intermixed with dense bodies were seen in association with fragmentation or dilatation of the cisternae of the Golgi apparatus in the soma of a considerable number of neurons of the visual cortex (Fig.7), the hippocampus, the locus coeruleus, the red nucleus, the polyhedral neurons of globus pallidus, and the Purkinje cells in AD brains.



**Figure 6.** Abnormal mitochondria in a dendritic profile of Purkinje cell in a case of AD. The disruption of the cristae is a prominent phenomenon (Electron micrograph 128,000X).

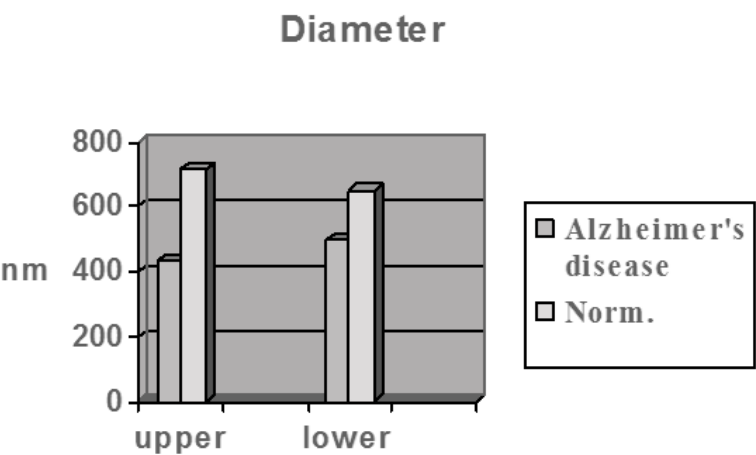


**Figure 7.** Elongated mitochondria and mitochondria in fusion, intermixed with dense bodies and dilated cisternae of Golgi apparatus in a case of AD (Electron micrograph 25,000X).



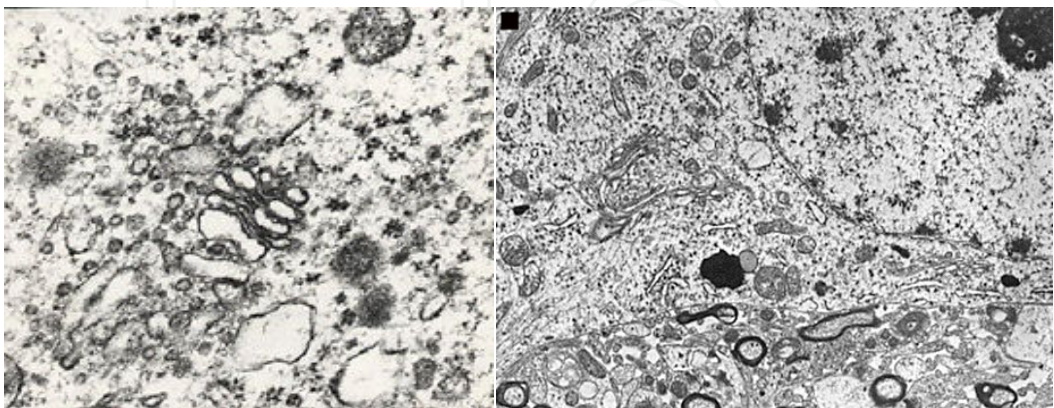
It is worth to emphasize that morphological alterations of the mitochondria were also seen in the soma of astrocytes, the perivascular processes, and the astrocytic sheaths in AD brains, in contrast to normal controls.

From the morphometric point of view the ellipsoid mitochondria of the dendritic spines in normal control brains appear to have an average diameter of  $650 \pm 250$  nm and a mean axial ratio of  $1.9 \pm 0.2$ . The round or global mitochondria appeared to have in normal controls a mean radius of 350 nm. In AD brains, the ellipsoid mitochondria of the neurons in acoustic and visual cortices appear to have an average diameter of  $480 \pm 250$  nm and a mean axial ratio of  $1.7 \pm 0.2$ . The round mitochondria appear to have a mean radius of 280 nm (Chart.1).



**Chart 1.** Decrease of the diameter of mitochondria in the visual cortex in Alzheimer’s cases in comparison with normal controls.

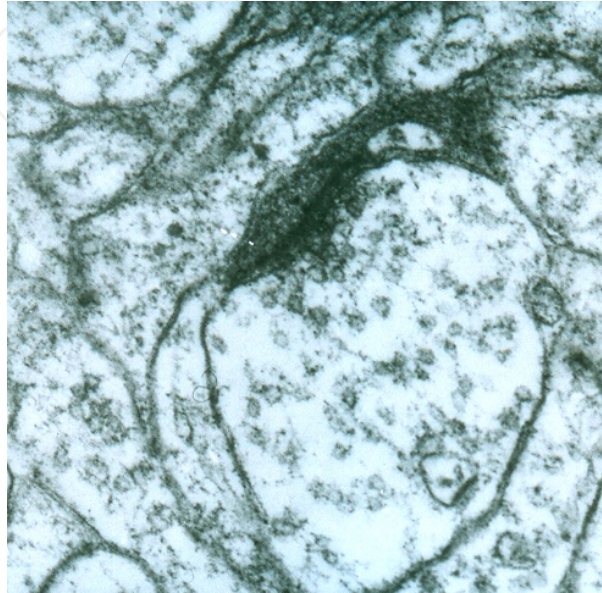
In the majority of Purkinje cells of the cerebellum, in the hippocampal neurons and in neurons of the parietal, frontal, acoustic and visual cortices the Golgi apparatus was mostly fragmented and atrophic (Fig.8) in comparison with normal controls.



**Figure 9.** Fragmentation of the cisternae of the Golgi apparatus in a Purkinje cell of the cerebellar cortex of a case of AD (left) (Electron micrograph 128,000). Normal control (right) (Electron micrograph 65,000X)



It is important to underline that the fragmentation of the Golgi apparatus was seen in neurons which did not show any tau pathology, such as accumulation of intracellular NFTs, and were located in areas with minimal deposits of A $\beta$  peptide. However the atrophy and the fragmentation of Golgi apparatus coexisted with mitochondrial alterations and dendritic and spinal pathology in the large majority of neurons (Fig.9).



**Figure 9.** Degeneration of dendritic spine in the visual cortex of a case of AD (Electron micrograph 130,000X)

## 4. Discussion

The mitochondria, which are the only nonnuclear constituents of the cell with their own DNA (mtDNA), having machinery for synthesizing RNA and proteins, are critical to homeostasis of the cell, by virtue of providing most of the energy for cellular processes, since energy, realized by oxidative phosphorylation, comes through the mitochondria, which generate most of the cell's supply of ATP. Mitochondria are also critical regulators of cell apoptosis, as being involved in a considerable number of neurodegenerative diseases [65, 66],

From the morphological point of view the shape and size of the mitochondria are highly variable [67], depending on fission and fusion [68]. Their morphology is occasionally controlled by cytoskeletal elements, namely the neurofilaments and the microtubules [69]. The change of the mitochondrial shape occurs mostly through their move to axons, dendrites, and synaptic terminals via anterograde transport [70]. During the various neuronal processes approximately one-third of the mitochondria are in motion along microtubules and actin filaments [71–73]. Mitochondrial motility and accumulation are coordinated, since mitochondria are transported to regions where ATP consumption and necessity for energy are particularly high, as it occurs in the synapses, which have high energy demand reasonably, for serving neuronal communication [74].

Mitochondrial alterations and dysfunction have been reported in several neurodegenerative diseases [75] mostly associated with oxidative damage [76-78] and vascular lesions [79]. Oxidative stress is mostly related with the accumulation of A $\beta$  peptide in the neocortex [80, 81], playing an important role in the pathogenesis of AD [82], since it is not only involved in the formation of senile plaques and in damage to the proteins of NFT [83], but also induces extensive damage to the cytoplasm of neuronal populations vulnerable to death [84].

It is also well documented that A $\beta$  peptide may increase mitochondrial reactive oxygen species (ROS) production [85, 86], causing further impairment of mitochondrial function [87], since the lack of histones in mitochondrial DNA renders them a vulnerable target to oxidative stress. In major examples of neurodegenerative diseases, there is strong evidence that mitochondrial dysfunction occurs early and acts causally in disease pathogenesis. Mutations in mitochondrial DNA and oxidative stress, on the other hand, may contribute to ageing [88, 89], which is the substantial biological background for the majority of the neurodegenerative diseases [65].

Mitochondrial dysfunction has been associated with energy crisis of the cell and excitotoxic cell death and is considered to be of substantial importance in the cascade of phenomena, which eventually lead to apoptosis. Some observations in early cases of AD [90] indicate that morphological alterations of the mitochondria and oxidative damage may be one of the earliest events.

The morphological alteration of the mitochondria seen in subcortical centers, such as in the thalamus, the globus pallidus, the red nucleus, the hypothalamus and the locus coeruleus, pleads in favor of a generalized mitochondrial dysfunction in AD, which may be associated with wide neuronal loss and synaptic alterations, seriously affecting the mental faculties, which are basically related to extensive neural networks [91] and synaptic activity. Moreover, an impressive number of disease-specific proteins interact with mitochondria. Well-documented studies [92] demonstrate that a significant amount of the N-terminal domain of APP targeted the mitochondria of cortical neurons and select regions of the brain of a transgenic mouse model for AD. The accumulation of trans membrane-arrested APP blocked protein translocation, disrupted mitochondrial function, and impaired brain energy metabolism.

In AD it may be considered that mitochondria-associated A $\beta$  peptide may directly cause neurotoxicity [93, 94]. Mitochondrial dysfunction, therefore, might be a hallmark of amyloid-beta-induced neuronal toxicity in Alzheimer's disease [95]. The binding site for amyloid beta peptide has been identified as alcohol dehydrogenase in the matrix space of the organelle. Recent evidence also suggest that PS1, PS2, APP, and  $\gamma$ -secretase activity are not homogeneously distributed in the ER, but rather are enriched in mitochondria-associated ER membranes (ER-MAMs or MAMs), which is a dynamic sub-compartment of the ER, which is connected with mitochondria [96]. Mitochondria and ER are closely connected in several functions such as transfer of Calcium, lipid metabolism, the control of apoptosis and autophagy [96]

Many morphological alterations of AD could be well linked to mitochondria changes, since blockage of mitochondrial energy production shifts APP metabolism to the production of more amyloidogenic forms of amyloid [97]. In addition amyloid beta peptide promotes permeability transition pore in brain mitochondria [85, 98]. It is important to mention that many protein

systems are also essential in mitochondrial function and morphological integrity as well as in binding to the cytoskeleton [99]. Mitochondrial porin is an outer-membrane protein that forms regulated channels (Voltage-Dependent Anionic Channels) between the mitochondrial inter-membrane space and the cytosol [100]. Porin may play an important role in binding to neurofilaments and microtubules, since porin-rich domains contain most of the binding sites for MAP2 [101, 102]. In addition, preselinin-2 modulates endoplasmic reticulum-mitochondrial interactions [103], a fact that would plead in favor of the crucial role that mitochondria play in the pathogenetic cascade of AD.

Recent studies reported increased mitochondrial fission and decreased fusion, due to increased amyloid beta ( $A\beta$ ) interaction with the mitochondrial fission protein Drp 1, inducing increased mitochondrial fragmentation, impaired axonal transport of mitochondria and synaptic degeneration in AD [104]. In addition, the interaction of the voltage-dependent anion channel 1 protein (VDAC1) with  $A\beta$  peptide and phosphorylated tau block mitochondrial pores, leading to mitochondrial dysfunction in AD [105].

The number of the mitochondria varies, according to energy state of the cell. Some evidence suggests that the mitochondria redistribute towards the dendritic profiles, in response to stimulation as a manifestation of synaptic plasticity [106]. Normally, a limited number of dendritic spines contain mitochondria, which are mostly small and round, which are increased in number inside the dendritic branches during the synaptogenesis. Decrease in energy metabolism and altered cytochrome c oxidase (CytOX) activity are among the earliest detectable defects in AD [107], affecting presumably neuronal plasticity and synaptogenesis. Some observations suggest that mitochondrial cytochrome c oxidase may be inhibited by a dimeric conformer of  $A\beta_{35}$ , a phenomenon which further emphasizes the role of the  $A\beta$  peptide on the mitochondrial dysfunction in AD [108].

Among the ongoing therapeutic efforts [109], those targeting basic mitochondrial processes, such as energy metabolism, free-radical generation, or specific interactions of disease-related proteins with mitochondria, hold great promise. On the basis of the mitochondrial pathology, in the pathogenetic spectrum in AD, new strategies inducing protection to mitochondria by the administration of efficient antioxidant factors could be introduced in the treatment of early cases of AD.

Golgi apparatus, on the other hand plays a very important role in posttranslational modifications, transport, and targeting of large number of proteins, which participate in axoplasmic transport or are transported to plasma membrane, to lysosomes, to synaptic terminals and dendritic spines. A substantial body of evidence suggest that Golgi complex is involved in the pathogenesis of amyotrophic lateral sclerosis (ALS) [110-112] and AD, as it is well documented by highly specific immunocytochemistry techniques [47, 113].

The size of the Golgi apparatus may be an index of neuronal activity. Thus the fragmentation of the cisternae of Golgi apparatus may be associated with impaired trafficking of proteins to synapses and dendritic spines resulting in synaptic degeneration and cognitive impairment eventually. It is well documented that the trans-Golgi network (TGN) is the major sorting structure of the secretory pathway and the main site of intersection with the endo-lysosomal

system [48, 49]. Current data clearly suggest that perturbations to the endosomal retrograde sorting pathway promote the production of A $\beta$ . The recent discovery of an integral membrane protein, Gamma-secretase activating protein (GSAP) [114,115], which associates with  $\gamma$ -secretase, APP and promotes the production A $\beta$  peptide may provide an important link for understanding how  $\gamma$ -secretase is directed to APP and help to clarify the site(s) of A $\beta$  production. GSAP might be also an ideal target for designing  $\gamma$ -secretase modulators in an attempt for treating AD [116,117].

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