Cajal-Retzius cells in acoustic cortex from patients in the early phase of Alzheimer’s disease: A Golgi and electron microscope study

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Key words: neurodegenerative disorders, reelin, synaptogenesis, central auditory functions
Słowa kluczowe: zaburzenia neurodegeneracyjne, reelin, synaptogeneza, ośrodkowe funkcje słuchowe

Summary

Background: Alzheimer’s disease, a neurodegenerative disorder of middle and older age, causes progressive decline of intellectual faculties, loss of employment skills, and gradual impairment of behavior and social performance, associated with early loss of insight, impaired communicative and linguistic skills, and neurological manifestations. Complex deficits of auditory functions are prominent in many patients. In previous studies we described the synaptic alterations in brains of early cases of Alzheimer’s disease, occurring in acoustic cortex, medial geniculate bodies, and inferior colliculi. In the present study we examined the morphological and morphometric alterations of Cajal-Retzius cells in layer I of acoustic cortex, since these cells, secreting reelin, play a crucial role in cellular development and neuronal circuit formation.

Material and Methods: We examined twenty brains from early cases of Alzheimer’s disease. For light microscopy we applied Golgi techniques, Golgi-Nissl, Holzer’s, Bielschowsky-Hirano methods, and the Bodian impregnation technique. For electron microscopy the specimens were fixed in Sotelo’s solution.

Results: The morphological study revealed alterations of the organelles of Cajal-Retzius cells, such as mitochondrial alterations, fragmentation of the cisternae of the Golgi apparatus, and synaptic alterations. The morphometric estimation revealed a dramatic decline in the number of Cajal-Retzius cells, in comparison with normal controls.

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Conclusions: Since Cajal-Retzius cells and reelin are important factors for synaptogenesis and the formation of local neuronal circuits, their loss may be implicated in synaptic pathology and the multifactorious pathogenetic pathways of Alzheimer’s disease, as well as in the impairment of central auditory function, which occurs frequently, even in early cases.

Introduction

Alzheimer’s disease is a progressive degenerative disorder of the central nervous system, characterized mainly by memory disturbances, loss of cognition, alterations of the personality and gradual decline of neurological functioning, leading eventually to vegetative state and death [1].

Despite the numerous existing hypotheses, the etiopathological background of the disease remains still unknown. Many studies and laboratory investigations in the fields of neurogenetics, neuropathology, neurochemistry and neuroimmunology substantially contributed in plotting the general orientation of approaching into etiological profile of the disease, leaving, however many questions unanswered.

From the morphological point of view the special sensory areas of the cortex seems to be particularly vulnerable among the isocortex in Alzheimer’s disease [2], since there is an area specific, lamina specific and cell type specific distribution pattern in the pathological alterations of Alzheimer’s disease [3,4].

All the numerous clinical findings concerning the central auditory function, although controversial in many aspects [5], plead in favour of a functional disorder of the acoustic cortex, presumably associated with neuropathological alterations at an extracellular and intraneuronal level.

From the clinical point of view, impairment of central auditory function has been described in Alzheimer’s disease [5], using parameters, such as list length, stimulus matching and order of recall on dichotic listening tasks, suggesting that Alzheimer’s disease is associated with an alteration of cortical function involved in selective allocation of speech attention [6]. The language impairment in Alzheimer’s disease, on the other hand, may take the form of progressive non fluent aphasia or that of semantic aphasia, associated with agnosia, less pronounced than in frontotemporal dementia or lobar atrophy [6]. However cases of preservation of musical memory in Alzheimer’s disease have been described [7], despite a coexistent severe impairment in declarative memory. In contrast to it, other cases demonstrated an impaired recognition of meaningful non verbal sounds, using a sound picture matching [8].

Substantial underlying histological alterations, such as neuronal loss, marked loss of dendritic spines in the second, third and fourth cortical layers, associated with obvious decrease of the axonic collaterals of the large triangular and round neurons, have been described in the acoustic cortex in a previous study [9], associated frequently with changes of structural organization of the layer I [10] as well as impressive alterations of the synapses [11].

The present study was undertaken in order to analyze the morphological and morphometric parameters of Cajal-Retzius cells of the acoustic cortex in early cases of Alzheimer’s disease, using silver impregnation technique, Golgi-Nissl method and electron microscopy.

Cajal-Retzius cell [12, 13] is a prominent neuron of the layer I of the cortex of the brain hemispheres, which is implicated in the development of the neocortex, the establishment of cortical lamination and the neuronal circuit formation. This neuron acts mainly by secretion of reelin, a glycoprotein, which is the product of the reeler gene [14], playing a crucial role in the establishment of the inside-out pattern of neuronal migration [15], a prerequisite for the development of the cortical structures in the central nervous system [16,17].
Material and Methods

We describe the morphological alterations of the Cajal-Retzius cells in twenty cases of Alzheimer’s disease, seven men and thirteen women, aged 52-87 years, who fulfilled the clinical, neuropsychological and laboratory diagnostic criteria of Alzheimer’s disease.

The mean education of the patients was 15.1 years and all of them have spoken the native language fluently.

Screening procedures included medical history, medical examination, cardiological investigation, and physical neurological assessment, psychiatric and neuropsychological examinations. All the patients underwent EEG, carotid duplex Doppler, CT scanning, magnetic resonance imaging (MRI) of the brain, and SPECT. The mental status of the patients was assessed by Mini-Mental State Examination (MMSE) and dementia rating scale (DRS) [18]. The neurological, the neuropsychological estimation as well as the laboratory investigation of the patients was all evocative of Alzheimer’s disease.

After the patients expired, the post mortem examination was performed within two hours. The brains were excised at an environmental temperature of 4°C.

Ten additional brains macroscopically intact, derived from apparently healthy individuals, who died accidentally, were used as normal controls.

For electron microscopy multiple small samples from the acoustic area of the temporal cortex were excised bilaterally and immediately immersed in Sotelo’s fixing solution, composed of 1% Paraformaldehyde, 2.5% glutaraldehyde in cacodylate buffer 0.1M, adjusted at pH 7.35.

Then the specimens 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 estimated the dendritic branches and the dendritic spines of Cajal-Retzius cells, as well as the morphology of the organelles at electron microscope on micrographs of a standard magnification of 56.000X.

For light microscope, most of the remaining acoustic area of the cortex (Heschl gyri of the superior temporal gyrus) was processed for silver impregnation techniques, according to rapid Golgi staining. Thus, the acoustic cortex was cut in coronal sections, after a three-week fixation in formalin and immersed in potassium dichromate (7g potassium dichromate in 300 ml water) for ten days. Then the specimens were immersed in 1% silver nitrate for ten days, according the rapid Golgi method.

Following a rapid dehydration in graded alcohol solutions, the specimens were embedded in paraffin and cut, some of them at 100µ and some at 10µ, alternatively. In every two sections, one section was hydrated, stained with 1% methylene blue, dehydrated and was mounted in permount, between two cover slips. The same technique was applied to all sections, which were subsequently studied in a Zeiss Axiolab Photomicroscope.

Some paraffin embedded preparations of the acoustic cortex were stained with haematoxylin and eosin, Holzer’s method, Bielschowsky-Hirano method and Bodian impregnation technique.

Statistical analysis was based on the Student’s t test on the basis of 200 measured sections from twenty specimens (ten from Alzheimer’s brains and ten from normal controls). Significance is taken as p<0.005.
Results

Light microscopy
The study of the acoustic cortex of the brains, which were excised from patients suffered from Alzhei-
mer’s disease, revealed a marked decrease of the number of Cajal-Retzius in the layer I of the cortex
(Fig. 1) and an impressive abbreviation of dendritic arborization of the few Cajal-Retzius cells, which
were still in place, in comparison with the normal control brains. As a consequence, the thickness of
the cortical layer I was diminished.
The cell bodies of the Cajal-Retzius cells in Alzheimer’s brains were mainly round or elongated, in
contrast to the large polyhedral, pyriform or ovoid shape of that of the normal controls. Those cells in
Alzheimer’s brains extended short primary horizontal dendritic branches, which protruded short
triangular spines, in an obviously decreased number. The majority of the primary dendritic branches
gave rise to short secondary ones, which were extended into neuropil space mostly at right angles,
forming short three dimensional dendritic fields. In the contrary the normal control brains, exhibited
a wide three dimensional dendritic field of Cajal-Retzius cells, whose the prominent horizontally
oriented primary dendrite, generated numerous secondary and tertiary dendritic branches mostly
oriented horizontally.
The Golgi staining revealed that only few ascending axonic profiles came in contact with the Cajal-
Retzius cell’s primary dendritic branches.

Bodian method revealed neurofibrillary tangles, seen in large proportion in the large triangular neu-
rons of the second and third cortical layer. Only very limited number of interneurons demonstrated
neurofibrillary tangles. Senile plaques were found all over the acoustic cortex, been particularly promi-
nent in the depth of the sulci of Heschl’s transverse convolutions. Holzer’s staining revealed increased
gliosis in all the layers of the acoustic cortex.

Electron microscopy
Normal controls
The electron microscopy revealed, in normal controls, that Cajal-Retzius cells are large ovoid or
polyhedral neurons, surrounded by thick network of astrocytic processes. The Cajal-Retzius cells are
characterized by a prominent cytoplasm filled with free ribosomes, perinuclear Golgi apparatus,
several rows of cisternae of smooth endoplasmic reticulum, abundant rough endoplasmic reticulum,
several multivesicular bodies, substantial number of lipofuscin granules, dense bodies and large number
of polymorphous mitochondria.

From the morphometric point of view ellipsoid mitochondria appeared to have an average diameter of
650 ± 250 nm and a mean axial ratio of 1.9 ± 0.2. The round or global mitochondria appeared having
a mean mitochondrial radius of 350 nm.

The nucleus, situated usually in an eccentric position, is mostly round or elliptic in shape with several
indentations, demonstrating a homogeneous distribution of chromatin. In the majority of the neu-
rons, the longer axis of the nucleus is oriented in a parallel way to the surface of the cortex.

The majority of the neuronal processes of Cajal-Retzius cells demonstrated a typical orientation
parallel to the surface of the cortex, displaying a thick perpendicular network with their secondary
and tertiary dendritic branches.

The axons of Cajal-Retzius neurons are mostly characterized by a uniform diameter and a consider-
able number of microtubules. They are directed either downward or parallel to the surface for
a considerable distance.

Axonal collateral show mostly a three-dimensional arrangement in the layer I.
The dendrites develop a three dimensional dendritic field with their extensive arborization, showing
a remarkable perpendicular orientation in a plane parallel to the surface.
**Fig. 1.** Number of Cajal-Retzius cells per mm$^3$. RAC: Right acoustic cortex; LAC: Left acoustic cortex. P<0.005

**Fig. 2.** Cajal-Retzius cell of the layer I (plexiform) of the acoustic cortex of an early case of Alzheimer’s disease. (Electron micrograph. Mag. 7,000 X).
Fig. 3. An area of the perikaryon of a Cajal Retzius cell of the layer I of acoustic cortex of an early case of Alzheimer’s disease, aged 68 y. The fragmentation of the Golgi apparatus and the alteration of the mitochondria are obviously seen (Electron micrograph; Mag. 168,000 X).

Fig. 4. Dendritic profile of a Cajal Retzius cell of the layer I of the acoustic cortex of an early case of Alzheimer’s disease, aged 72 y. Mitochondrial alterations, dense bodies, myelin like bodies are seen (Electron micrograph. Mag. 46,000 X).
Brains from patients suffered from Alzheimer’s disease

An impressive poverty in Cajal-Retzius cells was noticed in all of the specimens of the acoustic cortex derived from brains of patients suffered from Alzheimer’s disease. The few cells, which remained still in the layer I of the acoustic cortex, demonstrated a dense pycnotic nucleus (Fig. 2). The perikaryon included few free ribosomes and limited number of cisternae of rough endoplasmic reticulum. The cisternae of the Golgi apparatus were mostly fragmented and the morphometric analysis revealed marked abbreviation of their surface in comparison with the normal controls (Fig. 3). Thick astrocytic processes, plenty of glycogen granules ensheathed the soma and the main dendritic branches of the neurons in all the layers of the acoustic cortex.

Paired helical filaments (PHF) were seen in a few of the Cajal-Retzius cells of the layer I of the acoustic cortex, though they were rather abundant in the perikaryon of the neurons of the second and third layers. Paired helical filaments (PHF) were also seen in the initial part of the axon in numerous neurons located in the second and third cortical layer.

The mitochondria were decreased in number, in comparison with the normal control brains, demonstrating also a wide variation of size and shape, though most of them were small round or elongated (Fig. 3). A substantial number of mitochondria showed disruption of the cristae, though others included osmiophilic material in a granular or linear arrangement. Some mitochondria showed an impressive polymorphism in the arrangement of the cristae, which sometimes showed a concentric configuration or they were arranged in a parallel way to the long axis of the organelle.

Morphological alterations of the mitochondria were also seen in the dendritic profiles as well as in the primary dendritic branches of Cajal-Retzius cells (Fig. 4). Most of the dendritic profiles in all of the layers of the acoustic cortex demonstrated a large accumulation of microtubules, dense bodies and numerous dilated cisternae of the smooth endoplasmic reticulum.

From the morphometric point of view, the ellipsoid mitochondria of the neurons appear to have an average diameter of $495 \pm 250$ nm and a mean axial ratio of $1.4 \pm 0.2$. The round mitochondria have a mean radius of 265 nm.

An impressive poverty of the synaptic contacts of Cajal-Retzius cells with the neurons of the other layers of the acoustic cortex was noticed. The synapses were mostly axodendritic or axoaxonic, whereas axosomatic ones were rarely seen. The synaptic poverty was due to the minimal number of the dendritic spines as well as the limited number of the pre-synaptic terminals. An obvious polymorphism of the synaptic vesicles was noticed in the presynaptic component.

Discussion

In the present study we decided to apply for light microscopy the rapid Golgi and the Golgi-Nissl methods in order to proceed in morphological and morphometric estimation of Cajal-Retzius cells, since they are readily recognized by their large size and tangentially oriented large processes.

Most of the authors [19,20] have applied recently the histochemical method of reelin, in order to identify those cells and to study their properties. We have the feeling that reelin alone cannot be taken as a unique cell class-specific marker for the Cajal-Retzius cells, since reelin is expressed by different neuronal populations, some of which appear differently than the cells described initially by Cajal and Retzius [21]. In addition, a substantial number of reelin positive cells are GABA-ergic interneurons [22], and some of Cajal-Retzius cells show strong immunoreactivity for calcium binding proteins [23,24], such as calretinin, which is also expressed by other subpopulations of the local circuit neurons of the cortex [25].

In silver impregnation techniques, which have been used extensively for years for a three dimensional visualization of neurons and for precise morphological characterization of variable neuronal types, Cajal-Retzius cells can easily be distinguished from the other cells, which compose the layer I.
of the cortex, by their mostly large pyriform, ovoid or polyhedral size, the orientation of their processes, namely their typical dendritic arborization and their axons, which do not leave the marginal zone [26].

In electron microscopy Cajal-Retzius cells could be also easily recognized by the glial sheath, which surrounds their somas, except at the sites of synaptic junctions, as well as by the signs of degeneration particularly seen in the subplate and in the cortical plate [27].

The several existing data on Cajal-Retzius cells [28,29,30] of different mammalian species, including humans in different developmental stages, conclude that, in spite of their impressive variety of neuronal shape and orientation and the dynamic changes during corticogenesis, those cells give rise to a rich axonal arborization oriented horizontally, as well as to numerous horizontal dendrites, which extend ascending processes, contacting the pial surface [31,32]. Recent observations suggest also that human Cajal–Retzius cell are not restricted to the period of cortical migration, but they persist also into adult life [33].

Loss of activities of Cajal-Retzius cells is associated with neurological conditions, characterized mainly by malformations of the cerebral cortex as well as of the cerebellum [33]. Experimental studies, on the other hand, by inhibiting Reelin, demonstrated dramatic impairment in the development of entorhinal afferents of the hippocampus. Ablation also of Cajal–Retzius cells in organotypic cultures of hippocampus prevented the ingrowths of entorhinal afferents [34], suggesting that Cajal–Retzius cells and reelin are essential for the formation of layer-specific hippocampal connections. Absence of Cajal–Retzius cells in mutant animals is associated with defects of tangential cell migration from ganglionic eminance [35].

In addition Cajal-Retzius cells and reelin are important factors for the harmonious synaptogenesis in the hippocampus and the brain isocortex [36,37], a fact which underlines the crucial role of those cells in signalling pathway in the brain [38]. It is also important that the alleles of Apolipoprotein E (ApoE), E3 and E4 inhibits reelin binding to the lipoprotein receptors in vivo [39], considering that these alleles are closely related to late onset and sporadic Alzheimer’s disease [40].

The morphological alterations of Cajal-Retzius cells seen in the acoustic cortex of Alzheimer’s brains exceed those seen in the neurons of the other cortical layers in Alzheimer’s disease [41]. The fragmentation of the cisternae of Golgi apparatus was definitely more prominent than that seen in Purkinje cells of the cerebellum [42]. The mitochondrial alterations, on the other hand, were more dramatic in Cajal–Retzius cells than in other cortical and subcortical neurons [43], a fact pleading in favor of the vulnerability of Cajal-Retzius cell in Alzheimer’s disease.

The loss of Cajal-Retzius cells in Alzheimer’s disease may play a substantial role in the disruption of the microcolumnar ensembles of the association cortex [44], since they may be related to geometrical pattern of the laminar and columnar cortical organization of the cortex [45, 46] and the development of the thalamocortical connections [47].

In addition the loss of Cajal-Retzius cells may affect the balance between GABA-ergic and glutaminergic activity of the cerebral cortex, since the extracellular stimulation in the layer I could activate both of the above mentioned systems [48].

Some of the morphological and neurochemical phenomena in Alzheimer’s disease may also be associated with the loss of Cajal-Retzius cells. Thus, one of the morphological hallmarks of Alzheimer’s disease is the formation of paired helical filaments [1], a phenomenon which is associated with the hyperphosphorylation of tau protein [23,49]. In addition transgenic mice, who express human ApoE4, demonstrate increased levels of hyperphosphorylated tau protein [50]. On the other hand experimental studies revealed that hyperphosphorilation of tau protein may also be detected in mice deficient in reelin [51,52].
Although reelin immunoreactive Cajal-Retzius cells, have been described in the entorhinal cortex in patients suffered from Alzheimer’s disease [20], the dramatic decline of their density in the layer I of the acoustic cortex, in early cases of Alzheimer’s disease, estimated on the basis of Golgi and Golgi-Nissl methods as well as electron microscopy, pleads in favour of their probable implication in the pathogenetic pathways of Alzheimer’s disease, a concept which merits further investigation. We could hypothesize that the loss of Cajal-Retzius cells in the acoustic cortex in early cases of Alzheimer’s disease, may affect the integration of the auditory stimuli, a fact which is closely related with the impaired acoustic perception and the decline of verbal communication of the patients.

Conclusions

The morphological and morphometric evaluation of Cajal-Retzius cells in the acoustic cortex in early cases of Alzheimer’s disease revealed a tremendous decline of the number of those cells in comparison with normal controls. In addition, the few Cajal-Retzius cells, which remained still in place, demonstrated serious morphological alterations involving mostly the mitochondria and the Golgi apparatus.

Since the deficiency of reelin, a glycoprotein, which is secreted by Cajal-Retzius cells, may be associated with the hyperphosphorylation of tau protein, the loss of Cajal-Retzius cells in Alzheimer’s disease may be implicated in the multiple pathogenetic pathways of the disease.

In addition, the loss of Cajal-Retzius cells in the acoustic cortex may affect the cortical analysis of the auditory stimuli resulting in the impaired acoustic perception and the gradual decline of the aptitude of verbal communication.

References


Otrzymano / Received 02.08.2004
Zatwierdzono do druku / Accepted 17.08.2004