NEUROPATHOLOGY OF AGING. CORRELATIONS WITH ALZHEIMER DISEASE

Sorin RIGA[1], Dan RIGA[1]*, Aurel ARDELEAN[2], George PRIBAC[2], Anca HERMENEAN[2],
Daniela MOTOC[2], Francisc SCHNEIDER[2]
[1] Department of Stress Research and Prophylaxis,
"Al. Obregia" Clinical Hospital of Psychiatry, Bucharest, Romania
[2] "Vasile Goldis" Western University, Arad, Romania

ABSTRACT
Introduction.
The present study fills a gap in the Romanian bio-medical research domain through a global and unitary investigation of aging processes in the central nervous system (CNS) of both humans and animals, from macroscopic-regional-zonal levels to tissual-cellular-subcellular ones. In addition, the determination of correlations with Alzheimer disease (AD) has an important epistemological, biological, medical and therapeutic significance.
Materials and methods.
Human brains from elderly people (65 yrs. - 85 yrs.) and very elderly persons (85 yrs. and over), as well as aging CNS from mice, Wistar rats and guinea pigs were processed using macro- and microscopic morphological methods. Brain samples were investigated by light microscopy (histochemical stains and silver impregnation techniques), fluorescence and transmission electron microscopy. Neuropathological changes in aging brains were compared with brains of AD, prepared by the same methods and published in a previous paper.
Results.
By gross, imagistic and sectional anatomy, we evinced very gentle and/or mild to moderate macroscopic changes: cortical atrophy and ventricular dilatation, with gradual slow decline of brain weight and volume, modifications observed especially in elderly human CNS.
By microscopic anatomy, histology and cytology we identified the main changes and markers of brain aging at tissual-cellular-subcellular levels:
- mild reconfiguration of cyto- (neuron and glia), myelino- and lipopigmento- (LP - lipofuscin and ceroid) architectonics;
- constant and abundant presence LP (lipopigments) as hallmark of cellular aging in the majority of neurons and glia (microglia, oligodendrocyte and astroglia);
- moderate decrease of energetic system (mitochondria) and anabolic systems (hypoanabolism): polyribosomes
- and rough endoplasmic reticulum (Nissl bodies) and Golgi apparatus, as well as moderate increase of lysosomal hidrolytic activity (hypercatabolism);
- rare and diminished existence of specific AD neuropathological damages, seen in elderly and very elderly human brains, but much reduced in number, intensity and impairment;
- mild increase of apoptosis and rare and isolated zones of necrosis, especially in human brain aging.
Conclusions.
The present study is the first Romanian research, in which aging processes of mammal brains were investigated from anatomo-histologico-tissual levels up to cellular-subcellular and extracellular impairments and in close connection with AD neuropathology. Results allow and prefigure new directions in approaching human longevity, sanogenesis, aging and treatment of age-related pathology.

Keywords: cerebral senescence and Alzheimer disease, neuropathology and morphological correlations, selective and mild brain atrophy, reduction of neuron number and glial proliferation, increase of lipofuscin and ceroid pigments, decrease of Nissl bodies, diminished presence of Alzheimer neuropathology in aging brains

*Correspondence: *Dan Riga, Department of Stress Research and Prophylaxis, "Al. Obregia" Clinical Hospital of Psychiatry, 10 Berceni Rd., 041914 Bucharest 8, Romania, Tel. +40 21 334 3008, Fax +40 21 230 9579, email: D_S_Riga@yahoo.com*

… Last scene of all
That ends this strange eventful history,
Is second childishness *and mere oblivion,*
Sans teeth, sans eyes, sans taste, sans everything
                    William Shakespeare (1564-1616, **52 yrs.**),
                    English playwriter and poet,
                    As You Like It! (1600)

       It is our duty to resist old age; *to compensate for its defects by a watchful care; to fight against it as we would fight against disease. ...*
       Much greater care is due to the mind and soul; *for they, too, like lamps, grow dim with time, unless we keep them supplied with oil. ...*
       Intellectual activity gives buoyancy to the mind. *... Old men retain their mental faculties, provided their interests and application continue ... the aged remember everything that interests them.*
                    Marcus Tullius Cicero (106 BC - 43 BC, **63 yrs.**),
                    Roman philosopher, statesman, lawyer, political theorist
                    and Roman constitutionalist,
                    De senectute

       What wouldn't have Eminescu (1850-1889, 39 yrs.) given us, if he were to have reached old age, *since even in his early years he sat next to the two-three philosopher-poets of mankind ! ...*
       If he had had the royal old age of Goethe (1749-1832, 83 yrs.), *his human contribution to Man's culture would have been so great, that we could have glanced with serenity at any passing misfortune. ...*
       We say again: **it is true that the days of dusk enhance the shadows.**
       But ... **old age can be a gift,** *and sometimes -* **even the greatest gift of life, when it aids in attaining certain capital works of science or art. What would have become of Goethe (83 yrs.), Beethoven (57 yrs.), Michelangelo (89 yrs.), Leonardo da Vinci (67 yrs.) or Dante (56 yrs.), if they too had passed in their prime, or even in the midst of their path of life?**
                    Simion Mehedinți (1868-1962, **94 years**), philosopher, geographer, geopolitician,
                    professor and educator, trained to Titu Maiorescu's school,
                    De senectute. Aging within the framework of labour for education - Diminution and addition, Annals of the Romanian Academy, Series III, Tome XXIX, Mem. 12, Official Gazette and State Printing Office, Bucharest, 1947.

INTRODUCTION AND OBJECTIVES

Introduction

The human and animal ontogenesis, as well as aging phenomena are genetically controlled and also subject to environmental, natural and pathological factors and influences (Harman, 1993). In this context, systematic researches on aging neuropathology can elucidate important data and processes for cellular biology and pathophysiology of the central nervous system (CNS) and may result in efficient therapeutic interventions.

This investigation fills in a blank in Romanian bio-medical research and represents a complex study of brain aging in humans and animals, from macroscopic-regional-zonal levels to tissual-cellular-subcellular ones. Moreover, specific connexions with Alzheimer disease (AD) have considerable epistemological, biological, medical and therapeutic significances. The present research completes and expands the previous one (Riga et al., 2011 in press).

Objectives

The unitary exploration and description of cerebral senescence, containing macromorphological data (gross, imagistic and sectional anatomy), micromorphological researches (microscopic anatomy, histology, cytology, light, fluorescence and electron microscopy, cytochemistry and cellular biology) and patho-biological correlations with AD open new prospects in understanding aging processes in relation with age-associated brain pathology (AD) for further approaches and treatments.

MATERIALS AND METHODS

Aging human and animal CNS

15 human brains (without psycho-neurological diseases) from elderly people (65 yrs. - 85 yrs.) and very elderly persons (85 yrs. - 93 yrs.) were investigated and compared with aging brains from 15 mice (18 mos.), 30 Wistar rats (26.6 mos.) and 15 guinea pigs (48 mos.). CNS samples were prepared by macro- and microscopic morphological methods. Neuropathological modifications of aging were compared with brain

damage in Alzheimer disease (AD), processed by the same methods and published in a previous paper.

Clinical methods

ICD-10 (World Health Organization, 1992), NINCDS-ADRDA Alzheimer's Criteria (McKhann et al., 1984), DSM-IV-TR (American Psychiatric Association, 2000) and MMSE - Mini-Mental State Examination (Rush Jr. et al., 2008), CT and MRI diagnostic guidelines, clinical descriptions, tests and neuroimaging were applied to differentiate brain aging and exclude AD.

Gross, imagistic and sectional anatomy. Macroscopic investigations

Gross, imagistic and sectional types of anatomy were used for bring out the general, regional and zonal modifications. Elderly CNS were compared with AD brains regarding the differences in cerebral global configuration.

Microscopic anatomy, histology, cytology and cellular biology. Microscopic methods

Light microscopy (histochemical stains and silver impregnation techniques), fluorescence and transmission electron microscopy pointed out complex tissual, cellular-subcellular and extracellular damages of cerebral senescence.

Elderly human brains were removed post-mortem and fixed by immersion in formalin for 6-8 hrs. Afterwards brains were divided by frontal sectioning in coronal slices. Smaller pieces from different regions were formalin-fixed for 10-12 hrs. and then processed automatically (fixation, dehydration and paraffin-embedding) for light microscopy. Paraffin blocks were later cut at 6 μm. Silver impregnation techniques (Bielschowsky, Palmgreen, von Braunmuhl and Bodian) were used for amyloid plaques, neurofibrillary tangles and neuropil threads identification; standard stains (Haematoxylin-eosin and Congo red) for vascular amyloidosis; and specific staining methods (Oil red O, Sudan black B, periodic acid-Schiff-PAS, Nile blue, long Ziehl-Neelsen's acid fast and Schmorl's ferric-ferricyanide) were selected for lipopigment distribution and their histochemical characteristics. Also, 6 μm unstained sections were examined in fluorescence microscopy (Thompson and Hunt, 1966). For transmission electron microscopy, small formalin-fixed pieces were subsequently processed by postfixation in 2 % osmium tetraoxide, dehydration in graded acetone series, and embedding in Epon 812. Semithin sections were stained in toluidine blue O and ultrathin sections were double-stained with uranyl acetate and lead citrate (Hayat, 1970).

For animal brains, in order to avoid the interactions of blood constituents with the fixative and to eliminate the tissue damage caused by low aldehyde concentrations, the fixation by cardiac perfusion was started by a pre-washing with Tyrode solution containing 1 % gum acacia. Rapid fixation followed with phosphate buffered 19 % glutaraldehyde, then with a slightly hypertonic buffered 4 % glutaraldehyde for 20 min. Afterwards the fixed brains were removed and sectioned in frontal slices. The subsequent processing stages were the same with the above-mentioned ones for human brains.

RESULTS

Gross, imagistic and sectional anatomy. Macroscopic changes

Gross, imagistic and sectional anatomy of elderly human brains concomitantly evinced some macroscopic changes:

- gentle or mild to moderate cortical atrophy (gyral shrinking), (Fig. 1), especially in frontal lobes, basal ganglia, hippocampus and amygdala;
- mild or gentle to moderate widening of the sulci, particularly in frontal and temporal lobes;
- gradual slow decline of brain weight and volume with small ventricular dilatation.

In elderly animal brains, we found the same characteristics, but in a very mild degree comparatively with human CNS.

In AD, neuropathological impairments were much more intense to severe.

Visualization of mild cortical atrophy
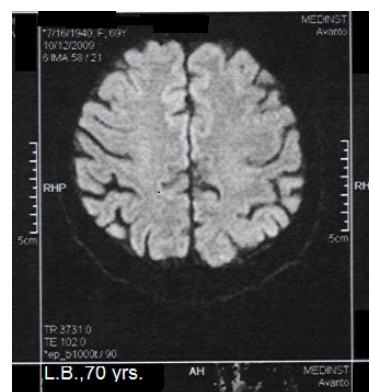
Fig. 1. Aging, Mild cortical atrophy, L. B. (female, 70 yrs.)

Microscopic anatomy, histology and cytology. Microscopic modifications

During ontogenesis, aging and longevity, as time dependent phenomena, nervous tissue architecture is changing. Loss and modification of some nervous structures and apparition of specific impairments, both within and outside of neurons are the main microscopic changes.

Therefore, the tissual architecture is mildly modified by neuronal loss and simultaneous increase of number and reactivity of neuroglial cells:

- neuronal cyto-, myelino- and synapso-architectonics; and
- glial (oligodendroglia, astrocyte and microglia) cyto-architectonics are changed by modifications in neuron/glia index and by apparition of
- characteristic neuronal and
- glial lipopigmento-architectonics, having regional and zonal specificity.

We observed that in aging:

- human CNS have more pregnant changes than animal brains, and in
- human CNS with AD, this tissual reconfiguration is more intensely altered.

Neurons and glia in aging processes

In elderly brains (human and animals) we observed gentle or mild to moderate loss of neurons, axons and synapses, and simultaneous increase of number and reactivity of neuroglial cells.

Moreover, cerebral aging also brings about, in a mild to moderate intensity:

- a decrease in the surface/volume of neurosoma (neuronal shrinkage), particularly in prefrontal layer III pyramidal cells;
- simplifications, destructions and aberrations of dendritic arborization, by losses of dendritic trunks (processes) and ramifications and of dendritic spines;
- axonal enlargements to meganeurites, reductions (sometimes considerable) of cortical myelin *(Gennari's and Baillarger's striae),* as well as of subcortical myelin *(corona radiata),* and distortion (often massive) of myelin sheaths, in transverse, oblique and longitudinal visualization;
- pathological activation of microglia and astroglia, with number increase.

We must note that in AD brain there are the same modifications, but much more intensified, especially in cerebral cortex (temporal, frontal, parietal, hippocampus) and certain subcortical regions (nucleus basalis of Meynert, corpus amygdaloideum, basal ganglia), (Riga et al., 2011 in press).

Lipopigment (LP) storages - lipofuscin and ceroid accumulations

LP - lipofuscin and ceroid - are the main markers of brain vulnerability, distress, aging and connected pathology. Lipofuscin *(age, senile, wear and tear pigment)* is the basic feature of cellular senescence, while ceroid is the cumulated product of aggressive external (environmental) and/or intrinsic (mainly genetic) factors, and both represent subcellular waste, garbage (tertiary lysosomes). During ontogenesis, neuronal and glial LP progressively accumulate, as a time dependent phenomenon, and lipofuscin and ceroid are present together, due to gradual aging and negative influence of the environment.

LP accumulations in the CNS (Figs. 2 - 11) have some important features:

- brain ubiquity: in all the CNS regions and zones, from cerebrum to spinal cord;
- presence in all nervous tissue: in whole cellular types, from different types of neurons - post-mitotic cells (Figs. 2-6), to glia (astrocytes, oligodendrocytes, but especially microglia) - mitotic cells (Figs. 7-10), and to pericytes (Fig. 11) and endothelial cells;
- specific patterns of LP architectonics, in close relation with senescence and age-related pathology;
- LP evolution in two-stages: stage I - LP increase in number, surface, volume, complexity, in both neurons and glial cells; stage II - LP become a constancy in chronic inflammatory-degenerative nervous pathologies (Alzheimer's disease, Parkinson's disease etc.).

Objectification of lipopigment accumulations
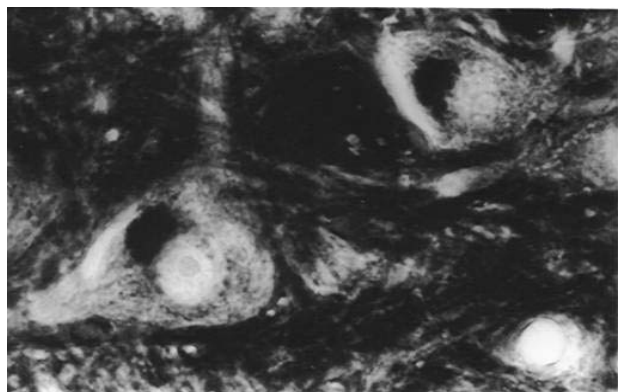
Fig. 2. Elderly **rat** (26.6 months). Brain. Pontine reticular formation. Light microscopy (Sudan black B). Extensive masses of **neuronal LP**, gathered into perinuclear-unipolar clusters. X 900.
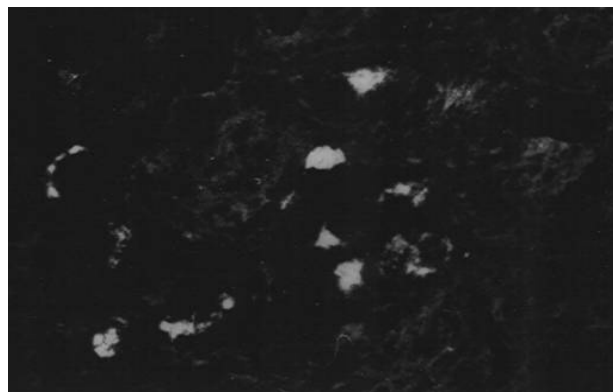

Fig. 3. Elderly **rat** (26.6 months). Brain. Pontine reticular formation. Fluorescence microscopy (Autofluorescence). Large perinuclear, uni- and bipolar accumulations of **neuronal and glial LP.** X 650.
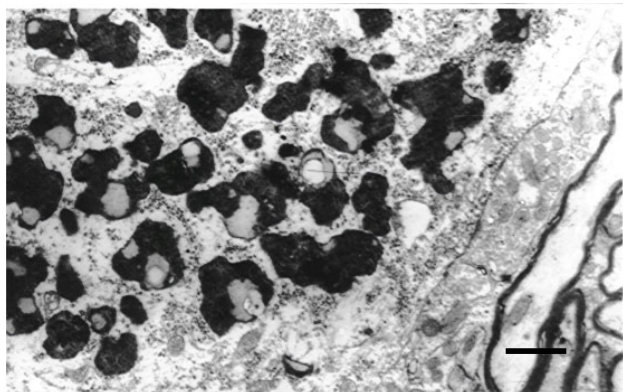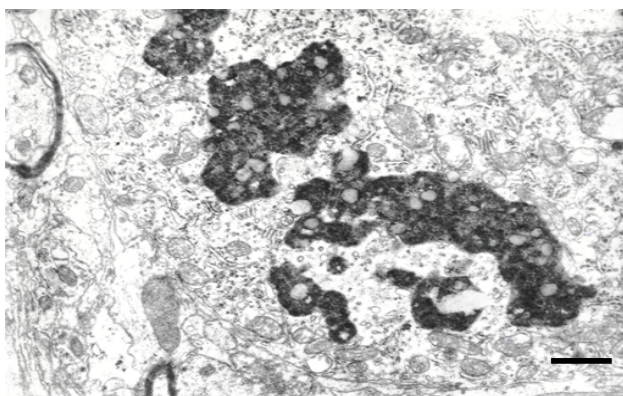
Fig. 4. Elderly **rat** (26.6 months). Brain. Cerebral cortex. **Pyramidal neuron.** Electron microscopy. Numerous polycyclic LP storages,tend to cluster and occupy a wide neuroplasm surface Bar: 0.5 µm.
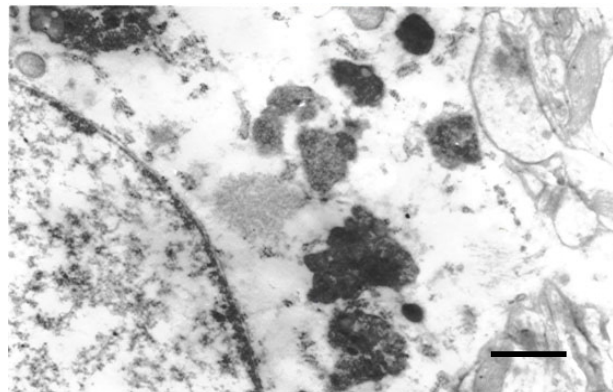

Fig. 5. Elderly **rat** (26.6 months). Brain. Cerebral cortex. **Pyramidal neuron.** Electron microscopy. Correlation in neurosoma between extension of LP conglomerates and intense decrease (down to dis-appeareance) of the components of anabolic system. Bar: 0.5 µm.


Fig. 6. Elderly **guinea pig** (48.0 months). Brain. Cerebral cortex. **Pyramidal neuron.** Electron microscopy. Correlation in neurosoma between extended polycyclic aggregations of LP and impaired, defective and damaged mitochondria. Bar: 0.5 µm.
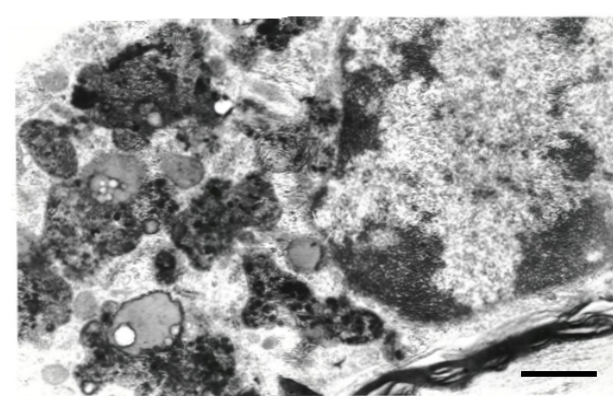

Fig. 7. Elderly **human** (80 years). Brain. Cerebral cortex. Vb pyramidal layer. Electron microscopy. Correlation in gliosoma of parenchymal **microglia** between aggregated LP deposits and reduced representation of free ribosomes and rough endoplasmic reticulum. Bar: 0.5 µm.
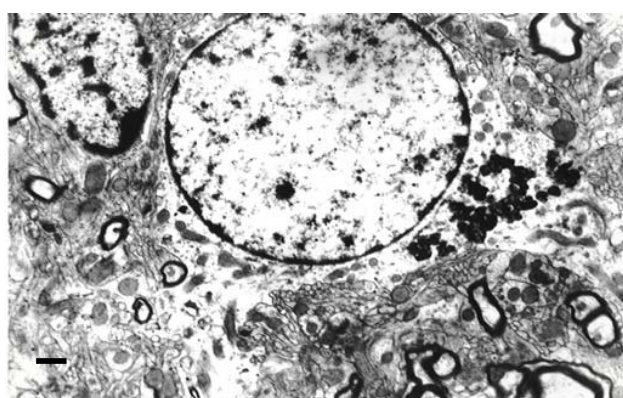
Fig. 8. Elderly **rat** (26.6 months). Brain. Lentiform nucleus. Putamen. Electron microscopy. **Astrocyte** with extended surface (volume) of LP. Large polycyclic masses of LP have heterogeneous structure. Bar: 0.5 µm.
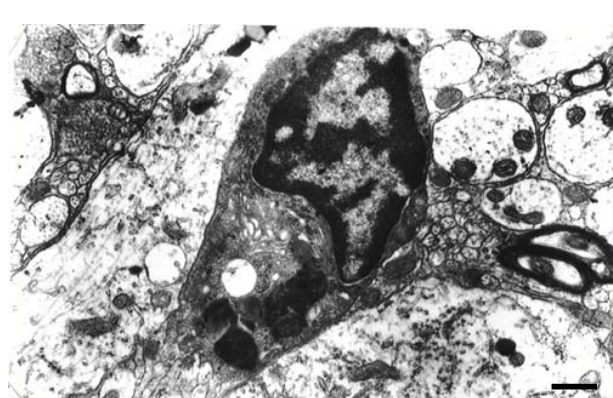
Fig. 9. Elderly **rat** (26.6 months). Brain. Hypothalamus. Area hypothalamica rostralis. Electron microscopy. **Oligodendrocyte** with LP having large surface (volume). LP dense structures are prevalent. Bar: 0.5 µm.
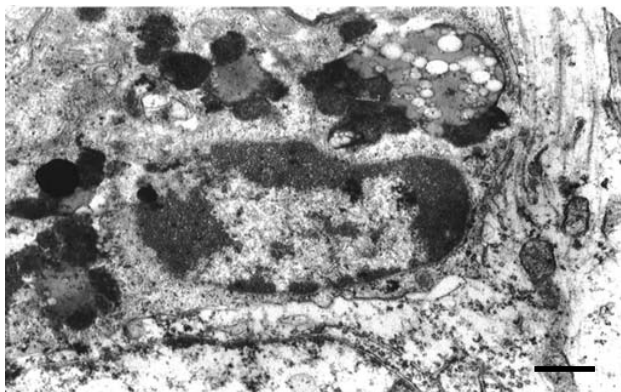
Fig. 10. Elderly **rat** (26.6 months). Brain. Tectum mesencephali. Inferior colliculi. Electron microscopy. Parenchimal **microglia** near to the neuron. Microglia filled with three vast LP accumulations, having heterogeneous internal structure. Bar: 0.5 μm.
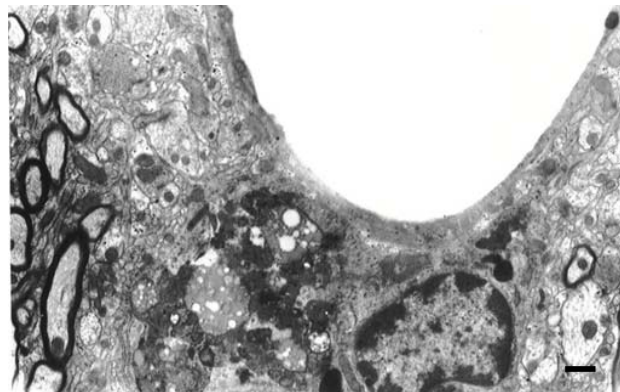
Fig. 11. Elderly **rat** (26.6 months). Brain. Hypothalamus. Nucleus dorsomedialis. Electron microscopy.The **pericyte (peri-vascular microglia)** has the cytoplasm filled with two extensive accumulations of LP. One of them has polycyclic shape and very heterogeneous internal structure. Bar: 0.5 μm.

In the aged neurons, LP are present in all cellular compartments: massively in all the perikaryon areas and dendrites, also in axons, and even in terminal buttons. They constantly coexist and are significantly correlated with important negative changes in nerve cell biochemistry and morphology.

Moreover, neuronal LP accumulations (Figs. 2 - 6) coexist with glial LP storages, in all types of glia (astrocytes, oligodendrocytes, but especially in microglia), (Figs. 7 - 11) and in all glial cellular partitions (gliosoma, glial dendrites and arborizations, and capillary end-feet).

Glial systems play an important role in collecting neuronal LP. Owing to their transporting properties, and migration capacity of microglia, glial cells deposit the LP clusters in pericapillary areas.

Thus, LP conglomerates appear in the whole nervous tissue, from neurons to perineuronal glia, neuropil, pericapillary glia and endothelial cells, realizing specific patterns of LP architectonics.

AD neuropathology, previously analysed, always coexists with large LP deposits, because in AD the LP storages become a permanence of nerve tissue.

Critical LP concentrations and direct, causal interrelations generate cascades of negative subcellular events, and indirect, associative impairment correlations, which together determine characteristic neuropathological aging profiles. These specific and associated negative neuro-pathologic consequences of LP accumulations have multiple and detrimental impacts on neuron and glia homeostasis, from neuronal function to CNS physiology.

LP morphological properties

Four topographic types of LP intracellular distribution have been identified in the neurons and glia from different brain regions (Riga and Riga, 1995a; and the present paper on elderly brains):

- randomly scattered in the cytoplasm; this diffuse pattern and non-pigmented cells characterize the young subjects;
- perinuclear aggregations with crescent aspects are present both in young and older animals and humans (Fig. 2);
- unipolar clusters, which gradually increase in number and size with age (Figs. 3, 4);
- bipolar conglomerates, which vary in size and shape; the percentage of nerve and glial cells with perinuclear, uni- and/or bipolar masses of LP dramatically increases in elderly and very elderly individuals; thus, LP accumulation becomes one of the most consistent cytological types of alteration correlated with the aging processes.

Internal structure of LP is generally polymorphic and increases in complexity with advancement of age (from homogeneous and very simple to highly heterogeneous and very complicated). In addition, the same nerve cell may evince different stages of lysosomal and LP evolution:

- primary lysosomes (L1), with homogeneous matrix, round shape and smaller in size on ultrathin sections;
- intermediate entities: secondary lysosomes (L2) and LP in first (early) stages of formation (LP1), with homogeneous or granular substructure, oval shape and slightly bigger size;
- final (last) stages of LP maturation: old, tertiary lysosomes, residual bodies, or late type of LP formation (LP2), with many inner nonhomogeneities (more often vacuolated), polycyclic (lobulated) shape and much larger in size (Figs. 4, 8, 9).

Moreover, in the elderly, many neurons and neuroglia, especially microglia, contain extensive areas

of LP, with extremely heterogeneous internal structure (Figs. 7, 10, 11):

- granules (dense bodies) of varying size and with different degrees of electronodensity, embedded in a matrix of somewhat lower density;
- vesicles and vacuoles (with or without internal vesicles) of various sizes and with tendency to confluence; the increase in number and size of vacuoles was noted with increasing age;
- lamellated bands (substructures), myelin-like figures, fingerprints in connection with electronodense portions;
- all inner components of LP granule are surrounded by a simple unit membrane;
- LP masses with lobulated (polycyclic) configuration tend to cluster and to occupy a large cytoplasmic zone.

Two functional types are present and characterize the intracellular life (genesis, evolution, maturation and aging) of LP granules. The early and late types differ in their fluorescence properties, composition and biochemical features, solubility, stainability, enzymatic activity, intracellular distribution, internal structure and their impact on cell physiology. In addition, in old individuals some neurons and glia contain early type of pigments and other cells have LP granules with late type characteristics. This fact signifies the presence of the nerve and glial cells in different life stages and aging in the same brain region.

LP intracellular locations and their interrelations with other subcellular organelles indicate interesting facts of cellular pathology:

- sometimes pigment granules are seen within damaged mitochondria; morphological similarity and spatial proximity of LP conglomerates to the mitochondria prompted the theory of mitochondrial genesis (Glees and Hasan, 1976); in addition, the demonstration of the oxidative enzymes in the LP masses (Nandy, 1971) evinces the taking over of mitochondrial fragments or swollen (altered) mitochondria by the lysosomal system;
- occasionally, the LP granules are present in close connection with components of Golgi apparatus for the enzyme transfer (e.g. acid phosphatase);
- moreover, lipofuscin pigments "have a long lysosomal life" (Samorajski et al., 1964), as old, tertiary lysosomes due to the similarity in fine structure with the lysosomes, to the mutual possession of specific hydrolytic enzymes and to the sequentiality of formation from lysosomal system;
- LP have inverse spatial and temporal relationships with the Nissl bodies (free

polyribosomes and rough endoplasmic reticulum): in old individuals the percent occupancy of cell volume by LP increases in an inverse relation with Nissl substance which decreases (Fig. 5);
- frequently, LP aggregations may be located in the external areas of the neurosoma (mostly in peripheral portions of the perikarya) or in dendrites, with rapid access to processing by nootropic or neuroactive drugs and with the possibility to be taken over by perineuronal glia (Riga and Riga, 1974; Riga and Riga, 1994a; Riga and Riga, 1994b);
- the appearance of pigment within the axon hillock and then in axon is a pathological change and this is frequently recognizable in the old human brain; further accumulations of pigment in proximal axon finally leads to voluminous spindle-shaped enlargements denoted as meganeurites;
- progressive LP accumulations are directly dependant on metabolic rate, being an indicator of repetitive long functional activity of neuronal networks and thus explaining the various regional brain patterns; moreover they represent the hallmark of cellular aging.

Neuronal LP and anabolic subcellular systems

Neuronal LP and anabolic subcellular systems are in inverse correlation. Extension of LP clusters is in connection with: decrease of ribosomal RNA, total RNA and water-soluble proteins, and consecutive diminution (Fig. 5) in number and surface/volume of polyribosomes and rough endoplasmic reticulum (Nissl bodies), and Golgi apparatus.

Neuronal LP and mitochondrial medicine

In addition, progressive neuronal LP storages are associated with: increase of the oxidative stress attack (Beckman and Ames, 1998; Fosslien, 2001; Harman, 2003); decrease of the antioxidative defence (Ames et al., 1993); cumulation of mtDNA mutations (Brunk and Terman, 2002; Terman and Brunk, 2004); increase in the number of damaged, impaired, defective, and giant mitochondria with a low rate of their degradation; and decreased number and area of normal and healthy mitochondria (Fig. 6).

The mitochondrial-lysosomal axis theory of aging (Brunk and Terman, 2002) demonstrates that mitochondria and lysosomes of postmitotic cells (such as neurons and cardiac myocytes) suffer the most remarkable age-related alterations among all cellular organelles. Moreover, by continuous oxidative stress and ROS (reactive oxygen species) production, the damaged oxidated mitochondrial components and structures

become the main sources in LP accumulations and storages via old, tertiary lysosomes.

Specific AD cellular and extracellular changes

Specific AD cellular and extracellular brain changes are described in our previous paper (Riga et al., 2011).

Briefly, they are represented by:

- cytoskeleton abnormalities: amyloid *(senile, argyrophil or neuritic)* plaques, neurofibrillary tangles, neuropil threads and dystrophic neurites; and
- other deteriorations: vascular amyloidosis *(congophilic angiopathy),* neuronal granulo-vacuolar degeneration, Hirano bodies and Lewy bodies.

In aging brains (preferably humans) these damages exist, but they have a rare or moderate presence. Amyloid (senile) plaques were found especially as simplified or incomplete structures.

DISCUSSIONS

Relevance of correlated animal and human studies

Investigation of brain aging on animal models is relevant, very useful for human senescence and easy to perform. Laboratory animals (especially rodents) have short period of ontogenesis and simplified CNS structure and function. But the most important fact is the similarity of aging mechanisms and processes in living systems. Thus, structure-function information, analysis and specificity, as general concept, are easier to translate from animals to humans.

In this way, translational science is an innovative approach in which knowledge is seamlessly brought from the laboratory to the patient's bedside and back again. In addition, translational research, medicine and science become novel concepts and tools for efficient and personalized human therapeutics in prolongevity. Correlation between animal and human researches in brain aging opens up a new way in epistemology and intervention in human longevity, sanogenesis, aging and management of age-related pathology.

CNS aging

During ontogenesis, the time-dependent processes and especially aging transform the morpho-functional systems from homogeneous and harmonious into heterogeneous and disharmonious. This transformation is determined by causal relationships, accumulations of unbalances, interdependences and covariates - expressed also by correlations (positive, negative or zero).

Brain senescence in mammals is a complicated and heterogeneous process with high regional specificity and individuality. Therefore, important CNS aging connections can be pointed out between:

- neuronal density and different types of glia presence and reactivity (Landfield et al., 1981; Morgan et al., 1999);
- brain lipopigments (LP) in neurons and glia (Cervós-Navarro and Sarkander, 1983; Riga and Riga, 1994a; Riga and Riga, 1994b; Riga and Riga, 1995a);
- LP in relation with mitochondria pathology - mtDNA mutations and giant mitochondria (Brunk and Terman, 2002), with anabolic organelles (Riga and Riga, 1995b) and with lysosomal dysfunction (Lynch and Bi, 2003; Riga and Riga, 1994a); and
- LP and Alzheimer pathology - meganeurites, neurofibrillary tangles and amyloid plaques (Abraham, 2000; Abraham and Slot, 2001; Bi et al., 1999; Braak, 1984; Braak and Braak, 1988).

Macroscopic changes

Gross, imagistic and sectional anatomy allowed us to differentiate aging (mild changes) from AD (moderate and sometimes severe modifications) in elderly people and also to investigate the general and global morphological aspects of mammal brain aging.

Microscopic modifications

Our researches performed by microscopic anatomy, histology and cytology certify and complete other anterior neuropathological data (Marinesco, 1909; Braak and Braak, 1988; Riga and Riga, 1974; Riga and Riga, 1994a; Riga and Riga, 1994b; Riga et al., 2009a; Riga et al., 2009b).

Mild reconfiguration (mainly simplification) of nervous tissue architecture (especially due to changed neuron/glia index) is accompanied by hallmark structures (neuronal and glial LP) and specific AD alterations (intra- and extracellular).

Neurons and glia in aging processes

Moderate neuron loss and glia increase, with modification of neuron/glia index were confirmed also by other authors and by our previous studies (Finch et al., 1999; Gonzáles-Scarano and Baltuch, 1999; Morgan et al., 1999; Riga et al., 2006).

In the brain, glial activations, the canonical features of mammalian aging and mediators of inflammatory and degenerative diseases, basically consist of:

- astrocyte hyperactivity, fibrous phenotype (increased levels of glial fibrillary acidic protein - GFAP); and
- microglia activation (increased expression of major histocompatibility complex MHC class II antigens and increased levels of transforming growth factor-beta-1 mRNA - TGFβ-1).

They are attenuated by food and caloric restriction (Finch et al., 1999; Morgan et al., 1999).

Lipopigment (LP) storages - lipofuscin and ceroid accumulations

LP are represented by lipofuscin and ceroid. Lipofuscin, progressively accumulated in ontogenesis, is the hallmark of cellular senescence. Ceroid, pathologically formed, is the stamp of external (environmental) aggressions and of internal factors (cellular distresses, also including genetic factors). At some time in their evolution, LP display almost identical biophysical, biochemical and morphological characteristics and properties (Porta, 1991).

By their implication and negative consequences on neuronal and glial physiology, LP represent the main marker of brain vulnerability, distress, normal and pathological aging, and associated diseases (Riga and Riga, 1995a).

Neuronal LP and catabolic subcellular systems

Neuronal LP and catabolic subcellular systems have shown interesting correlations. For example, progressive LP accumulations and aggregations interact and are associated with:

- proteasome (multicatalytic proteinase complexes) instability and inhibition (Grune et al., 2004; Keck et al., 2003) ;
- lysosome (center of main hydrolases) dysfunction (Evans, 1993; Lynch and Bi, 2003), i.e. decreased activity of cathepsin L [EC3.4.22.15], a thiol proteinase, with advancement of age;
- augmented amount of some lysosomal hydrolytic enzymes (Porta, 1991), i.e. increased activity and concentration of cathepsin D [EC3.4.23.5], a carboxy proteinase;
- deficient and poor function of cellular recycling systems; and finally with
- accumulations of water-insoluble proteins, oxidized proteins, advanced protein glycation/ glycooxidation end products, advanced lipid peroxidation end products, as pluri-metabolic sources and compounds of subcellular garbage (Riga et al., 2004; Terman and Brunk, 1998).

Therefore, aging can be explained as a catabolic malfunction (Terman and Brunk, 2004). In addition, the "garbage" accumulation theory of aging (Terman, 2001) considers the agglomeration of intracellular waste materials, resulting from imperfect intracellular degradations as fundamental feature of senescence.

Specific AD cellular and extracellular changes

LP storages negatively interact with neuron structure, and are constantly presented and correlated with the appearance and development of cytoskeleton damage, as well as with amyloid deposits, and amyloid-related pathology (Braak, 1984; Grune et al., 2004).

Cytoskeleton abnormalities are represented by pathological filaments, which contribute to the formation of three different lesions, referred to as:

- amyloid *(senile, argyrophil or neuritic)* plaques;
- neurofibrillary tangles; and
- neuropil threads.

Together with dystrofic neurites (meganeurites), microgliosis and astrocytosis, they form the neuropathological picture of Alzheimer's disease.

Amyloid plaques, located extracellularly, within the neuropil, vary in diameter from 15 to 200 μm. We must to notice similar denomination - senile plaques, which signify that this tissual structure (typical for AD, senility, pathological process) is also present in aging brain (senescence, normal process). They are comprised of an intricate feltwork of pathologicaly changed and often ballooned processes of nerve cells (dendrites, as well as axons), reactive astrocytes, activated microglia, and frequently, a core of extracellularly deposited amyloid. Amyloid represents an extracellular protein storage, composed by the Amyloid β peptide - Aβ, a proteolytic fragment of the amyloid precursor protein - APP. In plaques, Aβ is associated with several other molecules: complement components, serine protease inhibitor α1-antichymotrypsin, heparan sulfate proteoglycans and apolipoprotein E (Abraham, 2000). In addition, these Aβ-associated compounds contribute to the aggregation of Aβ and its resistance to proteolysis. Moreover, Aβ induces an inflammatory reaction by stimulating microglia. Activated microglia secrete proinflammatory cytokines and ROS (reactive oxygen species), that are detrimental to the nervous tissue.

Almost always LP clusters are connected (associated) to amyloid (senile) plaques. We must remark the large variations of amyloid (senile) plaques in aging brain (without AD): from simple (incomplete) to complex structures (complete, typical plaques).

Also there is an interesting relation between neurofibrillary tangles (tightly packed bundles of paired helical filaments located within neurosoma, whence they may extend into proximal portions of the dendrites) and LP (lipofuscin and ceroid) storages. In general, the central bundle of the tangle forms a dense and intricate feltwork around the accumulation of LP granules within the neurosoma.

Neuropil threads, inconspicuous structures loosely scattered through the neuropil, are formed of small bundles of paired helical filaments contained in slender thread-like profiles. They do not cluster or accumulate in patches or columns.

An important conclusion for human beings appears. The regressive neuropathological changes, as seen in the senescent brain, mimic, to a certain extent, the morphological characteristics observed in Alzhemer's

disease and in neuronal ceroid-lipofuscinoses (Braak and Braak, 1988). The main difference is that in aging the neurodegeneration process progresses slowly or very slowly, and it is far less marked than that seen in the diseased CNS.

Neuronal LP and apoptosis

Programmed cell death via apoptosis signifies a morphologic pattern of cell death affecting single cells. Apoptosis is induced by the activation of a family of proteases (cysteine endo-peptidases), called caspases (cysteine aspartate-specific proteases). Caspase activation (Delhalle et al., 2003) can be initiated by two molecular pathways:

- extrinsic pathway - the DR (death receptor) pathway, which is induced by ligand binding to TNFR (tumor necrosis factor receptor) superfamily members; and
- intrinsic pathway - the mitochondrial pathway, which is triggered by mitochondria in response to intracellular injuries, such as DNA damage.

Morphological and biochemical changes are represented by cell shrinkage, condensation of chromatin, formation of cytoplasmic blebs, release of mitochondrial cytochrome c, fragmentation of cell DNA into multiples of 180 bp (base pairs), and cell fragmentation into membrane-bound small apoptotic bodies, that are eliminated (cleared) through phagocytosis by neighboring cells. Being encoded in the cell genetic programme, apoptosis becomes the main mechanism for cellular deletion in the regulation of cell population, a key factor of tissual homeostasis, in embrio- and morphogenesis, an arbiter of cellular growing and differentiation.

The other major mechanism of cell death is necrosis, pathological cell death. Necrosis affects groups of cells or parts of an organ structure. Necrosis is caused by external noxious factors, is not genetically controlled, and is accompanied by inflammation.

In biological wear and tear, aging and neurodegenerative pathology, the apoptosis can be prematurely started, activated and/or accelerated. In this way, there is a close correlation between senescent mitochondria, old lysosomes filled with LP (biological garbage) and the induction of apoptosis. Old mitochondria generate elevated quantities of superoxide and hydrogen peroxide. If large amounts of hydrogen peroxide diffuse into lysosomes (by autophagocytosis of giant and senescent mitochondria), they disturb the stability of lysosomal membrane, with subsequent leakage into cytosol of the lysosomal lytic enzymes. Moderate release of lysosomal enzimes can induce apoptosis (Brunk and Terman, 2002), while marked discharge of these enzymes causes cell death by necrosis.

CONCLUSIONS

The present study is the first Romanian research, in which aging processes of mammal brains were investigated from anatomo-histologico-tissual levels to cellular-subcellular and extracellular pathology. In addition, the authors achieved (together with the previous paper, recently published in *Studia Universitatis Vasile Goldis. Life Sciences Series,* 2011) a comparative and correlative investigation of cerebral senescence in different animal species, humans with normal aging - senescence and with pathological aging - senility, AD.

The conclusions and findings of our study are very important for future investigations and approaches of cerebral senescence:

1. Animal models for brain aging research are very useful as well as for simplified patterns in investigation of human cerebral senescence.
2. In biological wear and tear, aging and neurodegenerative diseases, we found similar intra- and extracellular damage, but in very different number and degrees of intensity. In animal brains, aging processes evolve very slowly, in human CNS they develop slowly (normal senescence), and in AD the aging processes aggravate rapidly (pathological senescence, senility, accelerated aging).
3. The present research has strong applicability in human sanogenesis, prophylaxis, health care systems and longevity sciences.

Relevance of correlated animal and human studies in aging neurosciences is very important. Aging processes in living systems, brain structure-function specificity, unique cellular architectonics and its ontogenetic dynamics, information analysis and synthesis, general concepts, subcellular hallmarks of normal and pathologic cerebral aging, effects and consequences on CNS integrity can be integrated in epistemological and gnoseological directions and entities for understanding their mechanisms of display, manifestation and management. In this way, translational research, medicine and science open up a new frontier in knowledge and intervention in human longevity, sanogenesis, aging and control of age-related pathology.

REFERENCES

Abraham CR. Amiloid β peptide: a century of discoveries. *Amyloid: Int J Exp Clin Invest.* 2000; 7:7-9.

Abraham CR, Slot F. Metalloendopeptidase EC 3.4.24.15 in neurodegeneration. In: A. Lajtha, N.L. Benik (eds). *Role of Proteases in the Pathophysiology of Neurodegenerative Diseases.* Kluwer/Plenum, New York, NY, 2001: 101-116.

American Psychiatric Association. *DSM-IV-TR. Diagnostic and Statistical Manual*

of Mental Disorders. Forth Edition Text Revision. American Psychiatric Association, Washington, DC, 2000.

Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and degenerative diseases of aging. *Proc Natl Acad Sci USA.* 1993; 90:7915-7922.

Beckman KB, Ames BN. The free radical theory of aging matures. *Physiol Rev.* 1998; 78:547-581.

Bi X, Zhou J, Lynch G. Lysosomal protease inhibitors induce meganeurites and tanglelike structures in entorhinohippocampal regions vulnerable to Alzheimer's disease. *Exp Neurol.* 1999; 158: 312-327.

Braak H. Architectonics as seen by lipofuscin stains. In: A. Peters, E.G. Jones eds. *Cerebral Cortex,* vol. 1. Plenum, New York, NY, 1984: 59-104.

Braak H, Braak E, Morphology of the human isocortex in young and aged individuals: qualitative and quantitative *findings*, In: J. Ulrich (ed.), *Histology and Histopathology of the Aging Brain* (H. P. von Hahn, ed., *Interdisciplinary Topics in Gerontology,* vol. 25), Karger, Basel, CH, 1988: 1-15.

Brunk UT, Terman A. The mitochondrial-lysosomal axis theory of aging. Accumulation of damaged as a result of imperfect autophagocytosis. *Eur J Biochem.* 2002; 269:1996-2002.

Cervós-Navarro J, Sarkander H-I (eds). *Brain Aging: Neuropathology and Neuropharmacology.* Raven Press, New York, NY, 1983.

Delhalle S, Duvoix A, Schnekenburger M, Morceau F, Dicato M, Diederich M. An introduction to the molecular mechanisms of apoptosis. *Ann NY Acad Sci.* 2003; 1010:1-8.

Evans PH. Free radicals in brain metabolism and pathology. *Br Med Bull.* 1993; 49:577-587.

Finch CE, Rozovsky I, Stone D, Morgan TE. Glial activitation during aging in the rat brain: gene expression and proliferative potential. In: VA Bohr, BFC Clark, T. Stevnsner (eds), *Molecular Biology of Aging,* Munksgaard, Copenhagen, DK. Alfred Benzon Symposium; 1999; 44:304-315.

Fosslien E. Mitochondrial medicine-molecular pathology of defective oxidative phosphorylation. *Ann Clin Lab Sci.* 2001; 31:25-67.

Glees P, Hasan M. *Lipofuscin in Neuronal Aging and Diseases.* Georg Thieme, Stuttgart, DE, 1976.

Gonzáles-Scarano F, Baltuch G. Microglia as mediators of inflammatory and degenerative diseases. *Annu Rev Neurosci,* 1999; 22: 219-240.

Grune T, Jung T, Merker K, Davies KJA. Decreased proteolysis caused by protein aggregates, inclusion bodies, plaques, lipofuscin, ceroid, and "aggresomes" during oxidative stress, aging, and disease. *Int J Biochem Cell Biol.* 2004; 36: 2519-2530.

Harman D. Free radical involvement in aging. Pathophysiology and therapeutic implications. *Drugs and Aging,* 1993; 3: 60-80.

Harman D. Free radical theory of aging. In: Klatz R, Goldman R, eds. *The Science of Anti-Aging Medicine, 2003 Update.* American Academy of Anti-Aging Medicine (A4M), Chicago, IL, 2003: 15-31.

Hayat MA. *Principles and Techniques of Electron Microscopy,* vol. 1. Van Nostrand Reinhold, New York, NY, 1970.

Keck S, Nitsch R, Grune T, Ullrich O. Proteasome inhibition by paired helical filament-tau in brains of patients with Alzheimer's disease. *J Neurochem,* 2003; 85:115-122.

Landfield PW, Baskin RK, Pitler TA. Brain aging correlates: retardation by hormonal-pharmacological treatments. *Science.* 1981; 214:581-584.

Lynch G, BI X. Lysosomes and brain aging in mammals. *Neurochem Res.* 2003; 28:1725-1734.

Marinesco G, *La cellule nerveuse,* vol. 1 et vol. 2, Octave Doin et Fils Éd., Paris, FR, 1909.

McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology,* 1984; 34: 939-944.

Morgan TE, Xie Z, Goldsmith S, Yoshida T, Lanzrein A-S, Stone D, Rozovsky I, Perry G, Smith MA, Finch CE. The mozaic of brain glial hyperactivity during normal ageing and its attenuation by food restriction. *Neuroscience,* 1999; 3: 687-699.

Nandy, K. Properties of neuronal lipofuscin pigment in mice. *Acta Neuropathol. (Berl.),* 1971; 19: 25-32.

Porta EA. Advances in age pigment research. *Arch Gerontol Geriatr,* 1991; 12:303-320.

Riga S, Riga D. Effects of centrophenoxine on the lipofuscin pigments in the nervous system of old rats. *Brain Res,* 1974; 72: 265-275.

Riga S, Riga D. Antagonic-Stress: a therapeutic composition for deceleration of aging. I. Brain lipofuscinolytic activity demonstrated by light and fluorescence microscopy. *Arch. Gerontol. Geriatr,* 1994a; 19(S4): 217-226.

Riga D, Riga S. Antagonic-Stress: a therapeutic composition for deceleration of aging. II. Brain lipofuscinolytic activity demonstrated by electron microscopy. *Arch. Gerontol. Geriatr,* 1994b; 19(S4): 227-234.

Riga D, Riga S. Lipofuscin and ceroid pigments in aging and brain pathology. A review. I. Biochemical and morphological properties. *Rom J Neurol Psychiat,* 1995a; 33:121-136.

Riga S, Riga D. An antistress and antiaging neuro-metabolic therapy: accelerated lipofuscinolysis and stimulated anabolic regeneration by the Antagonic-Stress synergistic formula. *Ann NY Acad Sci.* 1995b; 771: 535-550.

Riga D, Riga S, Schneider F. Regenerative medicine: Antagonic-Stress® therapy in distress and aging. I. Preclinical synthesis - 2003. *Ann NY Acad Sci,* 2004; 1019: 396-400.

Riga D, Riga S, Hălălău F, Schneider F. Lipofuscin and ceroid pigments - markers of normal and pathological brain aging, pp. 213-221. In: R. Klatz, R. Goldman (eds), *Anti-Aging Therapeutics,* vol. 8, American Academy of Anti-Aging Medicine (A4M), Chicago, IL, 2006.

Riga D, Riga S, Ardelean A, Hermenean A, Schneider F. Leziuni neuro-degenerative în boala Alzheimer şi în patologia asociată îmbătrânirii. *A 27-a Sesiune Ştiinţifică Anuală a Societăţii Române de Biologie Celulară,* Bistriţa, 11-14 iunie, 2009; *Buletinul Societăţii Române de Biologie Celulară,* 37: 25, 2009a.

Riga S, Riga D, Hermenean A, Ardelean A, Schneider F. Modificări neuro-degenerative în îmbătrânire. *A 27-a Sesiune Ştiinţifică Anuală a Societăţii Române de Biologie Celulară,* Bistriţa, 11-14 iunie, 2009; *Buletinul Societăţii Române de Biologie Celulară,* 37: 26, 2009b.

Riga D, Riga S, Ardelean A, Pribac G, Schneider F. Neuropathology of Alzheimer disease. Connexions with cerebral senescence. *Studia Universitatis Vasile Goldis. Life Sciences Series,* 21(1), 2011, in press.

Rush Jr AJ, First MB, Blacker D (eds). *Handbook of Psychiatric Measures,* 2nd ed. American Psychiatric Publ, Washington, DC, 2008.

Samorajski T, Keefe JR, Ordy, JM. Intracellular localization of lipofuscin age pigments in the nervous system. *J Gerontol,* 1964; 19: 262-276.

Terman A, Brunk UT. Lipofuscin: Mechanisms of formation and increase with age. *APMIS,* 1998; 106: 265-276.

Terman A. Garbage catastrophe theory of aging: imperfect removal of oxidative damage? *Redox Rep,* 2001; 6: 15-26.

Terman A, Brunk UT. Aging as a catabolic malfunction. *Int J Biochem Cell Biol,* 2004; 36: 2365-2375.

Thompson SW, Hunt RD. *Selected Histochemical and Histopathological Methods.* Charles C. Thomas, Springfield, IL, 1966.

World Health Organization. *The ICD-10 Classification of Mental and Behavioural Disorders. Clinical Descriptions and Diagnostic Guidelines.* World Health Organization, Geneva, CH, 1992.