Corpora Amylacea in Aging Brain and Age-Related Brain Disorders

Daniel Pirici[1,*] and Claudiu Margaritescu[2]

[1]*Research Center for Microscopic Morphology and Immunology, Department of Research Methodology, University of Medicine and Pharmacy of Craiova, Romania*

[2]*Department of Pathology, University of Medicine and Pharmacy Craiova, Romania*

Abstract: Corpora amylacea (CA) are glycoprotein-based hyaline-like bodies that accumulate in normal aging brain, and to an even greater extent, in the brains of patients suffering from a variety of neurodegenerative disorders.

Although many of the histochemical, tinctorial and structural properties of CAs have been described for more than a century and a half since their discovery, their pathogenic mechanisms, their subcellular origins and their functions are still debated. Two main theories have been advanced to explain the formation of CA, respectively the vascular and the metabolic theories, although pathogenically both mechanisms can be involved. The exact cellular source of CA in the nervous system is still under debate, although both a neuronal and glial origin has been suggested due to the presence of cell specific proteins. CAs contain around 90% glucose polymers (polyglucosan or polysaccharides), 3% phosphates and 5% proteins, most of them being aging or stress-related proteins. Ultrastructurally, CAs were described as masses of randomly oriented short linear electron-dense areas, situated in the cytoplasm of fibrous astrocytes, mainly in their distal processes. In transmission light microscopy they appear as circular bodies ranging from less than 2 µm to about 20 µm in diameter, with smooth surface or ragged appearance that typically have a concentric laminated or target-like patterns, with the cores staining rather more densely than the periphery.

Besides their presence in aging brain, in many neurodegenerative disorders some similar structures called polyglicosan bodies, are morphologically indistinguishable from normal CAs, and were described in: Anderson's disease, adult polyglucosan body disease, inflammatory demyelinating polyneuropathy, diabetic neuropathy, and in the neurons of patients with Lafora progressive myoclonus epilepsy. The differential diagnosis may include all the neuropathological diseases characterized by the production of peculiar materials with special morphology in the elderly.

All together, these data come to show that these "enigmatic bodies" are far from being completely understood, thus further investigations are needed to better explain the brain aging and the pathogenesis of different degenerative neurological diseases, and perhaps they could provide novel therapeutic targets to counteract age-related brain disorders.

Keywords: Corpora amylacea of brain, aging process, neurodegenerative disorders, inclusion.

Corpora amylacea (CA) are glycoprotein-based hyaline-like bodies that form in the normal aging brain [1, 2] and, and even in larger quantities, in the brains of patients suffering from a variety of neurodegenerative disorders [3-5].

Although they were first described by Purkinje in 1837 [6], the term of corpora amylacea (CA) was introduced by Virchow for the resemblance of these round bodies in the brain to starch, although they were staining brown instead of blue with iodine (the latin amylacea derived from Greek amylon, which means "starchy").

Since in their composition the polysaccharides and polyglucosans are prevalent, over the time several authors used as synonyms for these bodies terms such as: "amyloid or starch bodies", "Lafora-like Bodies", "Lafora-like Bodies", "Bielschowsky Bodies" and "Polyglucosan Bodies" [3]. However, because it seems to be some differences in what it regards their structure, topography, cellular position and the induced clinical phenotype, the most properly-used term for these structures continues to remain "Corpora amylacea".

1. ETIOPATHOGENY

Their pathogenic mechanisms, their subcellular origins and their functions are still under debate. Mainly two theories have been advanced to explain the formation of CA, respectively the vascular and the metabolic theories, although pathogenically both mechanisms can be involved (Figure **1**).

1.1. The Vascular Hypothesis of CA Formation

Generally it is considered that CA develops in the elderly, especially in chronic vascular diseases and diabetes mellitus [7]. It was suggested that in such conditions blood - brain barrier disturbances occur and consecutively CA develop mainly in the proximity of structures possessing a barrier function, as perivascular space, subpial and subependymal areas. Thus, it was assumed that one of the functions of CA is

*Address correspondence to this author at the Department of Research Methodology, University of Medicine and Pharmacy Craiova, Petru Rareş Street 2, 200349, Craiova–Dolj, România; Tel: +40 (0) 742758934; Fax: +40 (0) 251593077; E-mail: daniel.pirici@yahoo.com

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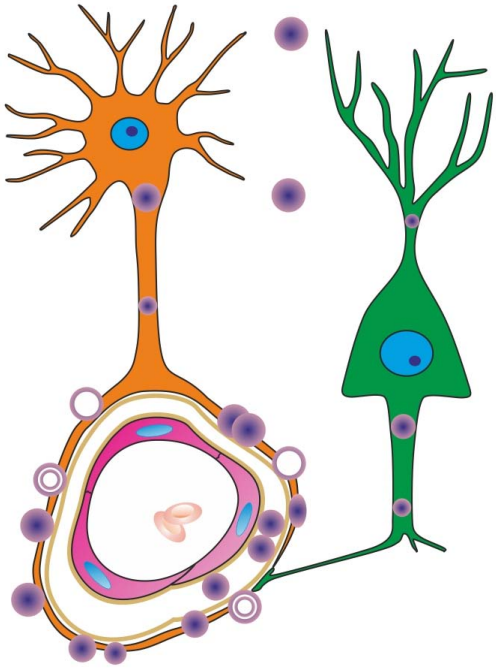

Figure 1: Schematic diagram presenting the main structures accumulating Corpora Amylacea (CA) in the brain, as well as their two main proposed origins (vascular and cellular). Besides being present in the neuritis and the glial feltwork, CAs are also deposited around the perivascular spaces (in the perivascular glia limitans), in the perivascular spaces, or even in the perivascular spaces. Subpial and subependimal glia limitans behave the same (image not shown). CAs can show a variety of compact, lamellar or doughnout-like patterns, without any predilection for their location.

directed towards sequestration of substances escaping the cellular metabolism [3]. Moreover, Maurizi *et al.* suggest that that the formation of CA cores may be associated with the cerebrospinal fluid substances [8]. In fact, Meng *et al*. assumed that CA could result from aggregation of a mix of interacting proteins, originating from extravasated blood cells released after transient increased permeability of the blood-brain barrier [9]. Also, Nam *et al*. showed that ependimar cells of the choroid plexuses, that normally bear tight junctions, are destroyed in the 90-year-old male, and suggested that the cerebrospinal fluid with extravasated blood cells contribute to brain CA formation [10]. Also the authors investigating the distribution of CAs found that many of these bodies are present in areas of stagnant cerebrospinal fluid (as are the horns of the lateral ventricles and other narrow anatomical spaces) and not in areas through which cerebrospinal fluid passes (such as on the surface of the cerebral cortex near the superior sagittal sinus). In these conditions it becomes obvious that diabetes may enhance the tendency for forming CA, given the hyperglycemia which increases the quantity of unused carbohydrate polymers, known to be the main component of CA [11]. An intriguing aspect suggesting a bidirectional dimension of this mechanism was the identification of freely CA in the cerebrospinal fluid [12].

1.2. The Metabolic Hypothesis of CA Formation

According to this theory, first it is a degenerative process, and later becomes accompanied by synthesis of stress proteins [13]. Thus, it has been suggested that CA play a role in cellular aging and cellular responses to oxidative stress [14,15], most probable by entrapping and sequestration of toxic products resulted from cellular metabolism during the process of aging [3]. In particular, the promotion of CA formation has been linked to oxidative stress and mitochondrial dysfunction [16]. In addition, although CA are frequently observed in the normal aged brain, they were also reported in neurodegenerative diseases, most probable due to the increased cellular stress that is a common denominator in such pathological conditions [17].

1.2.1. Neuropathological Evidence of Oxidative Stress Implication in CA Formation

In Alzheimer's disease and motor neurone disease, conditions associated with enhanced oxidative damage and mitochondrial dysfunction, large concentrations of CA have been reported [18-20]. Moreover, the tissue injuries associated with these conditions may be perpetuating by aggregation of advanced glycosylation end products within CA [21]. In the hippocampus of patients with mesial temporal sclerosis associated with chronic intractable epilepsy there have been reported increased numbers of CAs [22, 23], with some authors underlining the significant role played by free radicals in seizure-induced neuronal degeneration [24]. Taking into account that oxidative stress is responsible in hypoxic/ischemic encephalopathy for the perfusion-reperfusion and excitotoxic injuries, this could explain why were reported massive CA-like proliferations in these diseases [25]. The fact that glial cells in the brains of alcoholic subjects abound in CAs [26] could be explained by the alcohol augmentation of the oxidative stress pathways in the brains of these patients [27]. Also the increased number of CA in the brain of patients with multiple sclerosis plaques [17] could be explaining by the pathological involvement of iron deposition and oxidative stress [28]. Both these conditions could by incriminated for the excess CA formation described in a case of Friedreich's ataxia with isolated vitamin E deficiency [29, 30]. Huntington disease, also characterized by mitochondrial dysfunction and augmented oxidative stress [31] has been reported to accumulate CAs [32].

1.2.2. A Proposed Molecular Mechanism of CA Biogenesis During Oxidative Stress

In 2002, based on the data accumulated, Sahlas *et al*. proposed the following model for CA formation [33]:

- several studies proved that in the aging and degenerating brain, the oxidative stress induced by dopamine, amyloid or pro-inflammatory cytokines upregulate heme oxygenase-1 (HO-1) production in local astroglia [20, 28, 34-38].

- as a result of HO-1-mediated heme intracellular degradation, are released free ferrous iron and carbon monoxide ions which in turn expose the mitochondrial compartment to excessive oxidative stress [39].

- this in turn facilitates the opening of a nonspecific pore (the mitochondrial permeability transition pore), which favorises swelling of the organelle, disruption of the cristae and sequestration of nontransferrin-derived iron within the mitochondrial matrix [37, 40, 41].

- next, the distended mitochondria become autofluorescent (most probable due to the oxidized flavoproteins) and engage lysosomes enriched in cathepsin D in a complex autophagic process which results in the formation of Gomori-positive granules (that may represent the incipient CA forms) [42, 43]. As they contain redox-active ferrous iron which have non-enzymatic peroxidase activity were also referred to as peroxidase-positive inclusions [44, 45].

- as a defense response to the mitochondrial dysfunction, in these cells manganese superoxide dismutase mRNA is upregulated, followed by increased enzyme levels and activity [46-48].

- in addition, in these cells are up-regulated several other redox-sensitive stress proteins. Thus, certain stress proteins (HO-1, HSP27 and ubiquitin) become incorporated within the nascent inclusions (*immature* CA) [15,20,33,49,50].

- subsequently, a proportion of these Gomori/ peroxidase-positive inclusions undergo progressive glycosylation resulting in quenching of their autofluorescence and culminating in the formation of *mature* CA [33]. Also, in some cases, degeneration of the host cell results in the deposition of inert CA within the extracellular space.

More recently, Song *et al*. suggested that glial CA are derived from dystrophic mitochondria engaged in a complex macroautophagic process which, in turn, is contingent on the antecedent overexpression of HO-1 [5]. Also the authors proved that CA formation is enhanced in the hippocampus of subjects with mild cognitive impairment and suggest that HO-1-related mitochondrial damage, mitophagy and CA formation are relatively early events in the pathogenesis of Alzheimer disease.

Also, in connection with the oxidative stress, Wilhelmus *et al*. proposed a novel mechanism in which transglutaminase (TG) 1-catalyzed cross-linking plays a key role in the age-related formation of CA [51]. It is well known that consecutive to cellular stress that occurs during normal aging or in neurodegenerative diseases, intracellular calcium levels increase, inducing the cross-linking activity of transglutaminases [52]. These effectors induce molecular cross-links, leading to polymerization of substrate proteins, leading to stable protein complexes which are resistant to proteolytic breakdown [53]. Thus, together with polymerized carbohydrates and other potentially damaging non-degradable by-pass products of the aging process [14, 15, 21], TG cross-linked proteins might form the core of CA [51].

2. SUBCELLULAR ORIGINS OF CAs

The exact nervous cellular source of CA is still debated, although both neuronal [54-57] and glial (in astrocytes or oligodendrocytes) origins have been suggested [37, 57-59], based on the presence of specific proteins. Also, several electron microscopy studies confirm this dual theory regarding the origins of CA. Therefore, while some authors conclude that the CA is a real inclusion developing in the processes of astrocytes [7, 60-63], others found CA in neurons [64-66]. However, related to the origin of CA in the glial cells it is still a matter of debate whether these polyglucosan bodies are the result of phagocytosis of residues of degenerated neurons/neuritis and vascular metabolites [9, 17], or their presence is related to glia pathophysiology/degeneration [67].

3. BIOLOGICAL ROLES OF CAs

Since in the early nineties it was suggested that one of the CA roles could be to prevent the recognition of immunogenic proteins by microglia and thus to protect the CNS from further injury [17]. In the same line, some authors talk about the relatively high affinity of CA to accumulate to some extent “protective” substances

(such as Bcl2, heat shock proteins, etc.) which could rescue neurons from the effects of ischemia or ageing [1].

In addition, more recently it was proved that apart from inducing stable and proteolytically more resistant protein complexes, TG1-catalyzed cross-linking of proteins also results in the formation of non-immunological forms of proteins and protein-complexes [51]. Thus, CA could prevent recognition of immunogenic proteins by the immune system, protecting thus the central nervous system from inflammatory responses and injury. However, it is unknown if theTG1-catalyzed cross-linked proteins observed in CA are indeed not recognized as neo-epitopes by the immune system, but it is certain that CA are not associated with reactive astrocytes and microglia [3], despite the various components of the complement system enclosed in their proteinaceous content [17].

In another study undertaken by Notter and Knuesel it was suggested that Reelin (an extracellular matrix glycoprotein that modulate synaptic plasticity) accumulation around CA precludes a microglia response and therefore they do not have major effects on the adjacent neurons although they are closely associated with aging-associated neurodegenerative changes leading to impairments in glucose metabolism, protein synthesis, transport and degradation [68].

Also, over time some authors have wondered if abundant CA deposition may cause a secondary disturbance of the function in the involved brain area [7, 61, 69]. It was suggested that on one hand the absorption of the cerebrospinal fluid changes, because the glia limitans may disappear in the event of the presence of dense CA, and the function of the blood-brain barrier may also be impaired, but on the other hand CA could have an improved protective effect, because the barrier is able to inhibit more efficiently the entry of toxic materials [7].

4. BIOCHEMICAL COMPOSITION

Chemically CAs are composed of glucose polymers 88% (polyglucosan or polysaccharides), protein 5% and phosphate 3% [3, 70]. Most of the proteins are aging or stress-related proteins such as advanced glycation end products, heat shock proteins, and ubiquitinated proteins [58, 71]. Moreover, nestin filaments [54], S-100 proteins [72] and mitochondrial epitopes have been showed in their structure [15].

Selmaj *et al.* after a proteomic analysis of collected CA from multiple sclerosis brains, suggested that they may represent remains of neuronal aggregates with highly polymerized cytoskeletal material [56]. In addition to major cytoskeletal proteins, the authors found a variety of proteins implicated specifically in cellular motility and plasticity, regulation of apoptosis and senescence, and enzymatic pathways. Also, the authors proved that the CA protein content derived from different cellular locations, including:(a) the cells' membranes (LDL receptor-related protein 1, LRP-1), (b) the cytosol (valosin-containing protein p97, 60S acidic ribosomal protein, alanyl-t-RNA synthetase, protein disulfide isomerase), (c) the endoplasmatic reticulum (thiol oxidoreductase ER60, heat shock protein gp96), (d) mitochondria (ATP synthase), and (e) the nucleus (RuvB-like 2 protein) [56].

Several other studies proved by immunohistochemistry that CA contain substances that have their origin in CNS cells, such as: tau [55, 73]; extracytoplasmic domain of the amyloid precursor protein [74]; hemoxygenase-1 [21]; serum carnosinase [75]; heat shock protein, like Hsp-60 [13], Hsp27 [2], and Hsp-72 [59]; S100 protein [72]; ubiquitin [58]; myelin basic protein; proteolipid protein; oligodendrocyte glycoproteins; ferritin [57]; c-Jun, bax and Bcl-2 [1].

Stam and Roukema reported small quantities of phosphates and sulphates in the CA and they concluded that glycogen can be the only polymer, to which these substances could be bound, since hexuronic acid and hexosamines, were present in low concentration (0.2% and 0.3% respectively) [76]. Investigation in infrared spectroscopy revealed an absorption pattern strongly resembling to other known glucose polymers and virtually identical to starch [70]. Histochemically, in the composition of CA were not detected mannose, fucose, lipids and nucleic acids [3]. However, regarding the nucleic acids content of CA, Balea *et al*. showed by in situ hybridization that they have an affinity for nucleic acids, and this affinity varies with the nucleotide sequence, the most relevant association being with an antisense probe for adenosine-2A receptor mRNA [77].

The X-ray techniques indicate the presence of a number of atomic species strongly bound within the CA, including Na, P, S, Ca, Fe, Cu and Cl [4, 78].

5. ELECTRON MICROSCOPY

Ultrastructurally, CAs were first described by Ramsey as masses of randomly oriented short linear densities, situated in the cytoplasm of fibrous astrocytes [79], mainly in their distal processes [80, 81].

These densities did not have a definite limiting border or space related to the cytoplasm and their central part, and sometimes contained an irregular dense core. Also there were described various amounts of glycogen granules both in CA and in the cytoplasm of astrocytes. Daems and Persijn investigating the CA polysaccharide content, in an electron microscopy study have noticed that the CA fibers consist of periodically bent lamina ordered helically ("crystalline") and randomly ("amorphous") [82].

The inner structure of the CA consists of a jumble of fibers 50-100nm in diameter, very similar to the normal glial fibrillary acidic protein cytoskeleton of astrocytes. Very often in the CA were found coarse amorphous granules without any regular arrangement or little finer granulations that usually were placed in the central part of the CA [7]. Typically, CA are not encapsulated but closely connected with myelin and other nervous structures and frequently surrounded by glycogen granules.

Leel-Ossy examined the progressive development of the CA by electron microscopy [7]. Thus, initially in the cytoplasm of the astrocyte are formed tiny irregular lamellar fiber masses. Later on, these masses increased in size and gradually the normal fiber pattern of the astrocyte disappears or persisted only at the edges of the cell. Finally, when the CA reaches a certain size, the astrocyte nucleus shrinks and disappears, in parallel with the destruction of other intracellular structures [7].

However, regardless of their neuronal or glial origin, various authors have failed to find major ultrastructural differences between these CAs. Moreover, considering the fact that peripheral polyglucosan bodies have the same characteristic to the central nervous system CA, more authors has begun to replace the original term CA with the polyglucosan bodies concept [3, 7, 83-85].

6. HISTOPATHOLOGICAL FEATURES OF CA IN BRAIN SPECIMENS

6.1. CA Topographic Peculiarities and their Variation with Age

Although the CAs in elderly subjects are found in almost all regions of the central nervous system, they seem to be concentrated in certain locations, but without knowing what drives this distribution [3]. In the brain, the CAs are seen congregated in the white matter where they are found in the glial feltwork beneath the ependymal lining of the ventricles (Figure **2a**, **b**), particularly beneath the corpus callosum, in the roof and to a less degree the floor of the third and fourth ventricles, and in the roof of the aqueduct, often in very large numbers. On the outer surfaces of the brain, they lie most usually in the glial feltwork beneath the pia mater (Figure **2c**, **d**), especially at the base of the brain, on the medial surfaces of the temporal lobes and over the hippocampal formations [3]. They are very common in the surface glial feltwork in the outer part of cortical layer I, but most usually they lie in the depths of sulci, and especially in the insula, rather than on the convexity of the surface. When present in the white matter they tend to congregate around vessels of medium and large size (Figure **2e**, **f**) and are often seen in the Virchow–Robin spaces [3]. Similar results were reported by other authors who underline that in elderly subjects the CAs are commonly seen in the subpial, perivascular, and subependymal regions [2, 22].

Also some authors revealed that the deposition of CA is age related. Thus, Fawcett *et al*. stated that CA appeared initially between the third and fifth decades of life and become more frequent in older ages [86]. Busard *et al*. investigating the mean number of CA in frontal and temporal cortical grey matter found that, regardless of sex, after the age of 40 years they were much increased, but exceedingly variable [87]. On the other hand, Chung and Horoupian found a small number of CAs in hippocampal (Figure **2g**, **h**) and extrahippocampal tissues in a group of 20 control subjects aged 16 to 51 years with various diseases [22]. Mrak *et al*. reported that CA are rarely observed in childhood but are invariably present by 40 years of age with the highest density in globus pallidus, hippocampus and posterior columns of the spinal cord [88]. Also, Schipper *et al*. reported that CA present around the periventricular regions had affinity for PAS which increased with advancing age [89]. Moreover, Ayesha and Tahirshowed a significant increase of CA in frontal lobe and hippocampus with advancing age, and the authors suggested that this CA deposition interferes with the function of neurons and presumably affects the memory in elderly people [90]. They also found that initially the CA appeared as small, compact circular deeply basophilic structures, but with the advancement of age they changed to lightly stained, large concentric whorls.

6.2. Light Microscopic General Characteristics

Generally, they appear as circular bodies ranging from less than 2 μm to about 20 μm in diameter (Figure

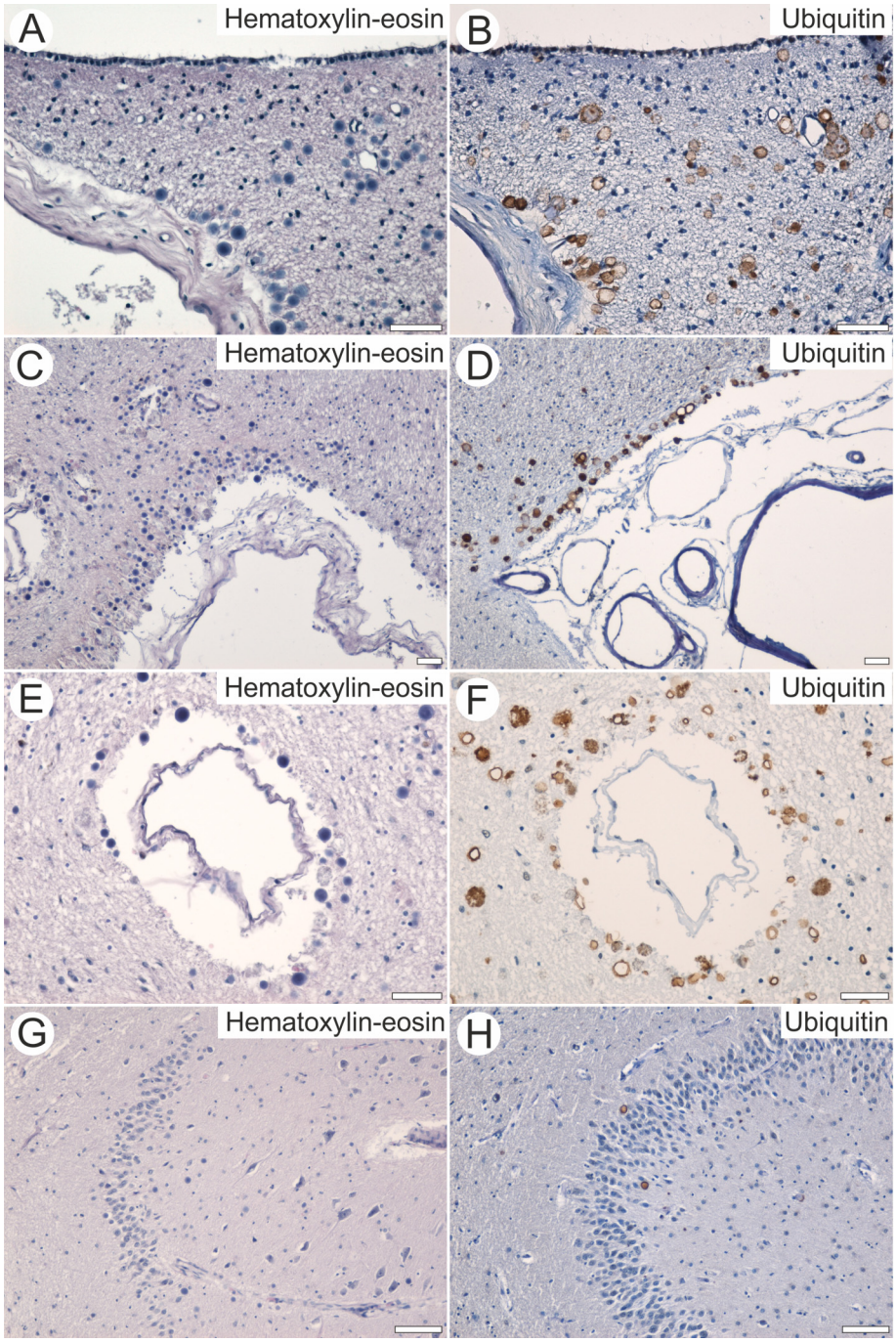

Figure 2: Corpora Amylacea (CA) typically accumulate beneath the ependymal lining of the ventricles (**A** and **B**), in the glial feltwork beneath the pia mater (**C** and **D**), around vessels of medium and large size (**E** and **F**), with a lower extend in the brain parenchyma, as for example the dentate gyrus of the hippocampus (**G** and **H**). CAs are classically identified as positive after immunodetection for ubiquitin. Human aged tissue collected from patients deceased from non-neurological causes. Scale bars represent 200 µm.

3a) [3]. They also may have oval or elongated forms (Figure **3b**). Rarely, were observed fusion of two or more CA (Figure **3c**). Frequently, they have smooth surface, but sometimes a ragged appearance was noticed. Typically, they were reported as concentric laminated or targetoid patterns, with the cores staining rather more densely than the periphery (Figure **3d-f**).

There were reported variations in their size by neurologic topography (Figure **3g**, **h**) and with increasing age. Thus, is was established that in the cortex and in the striatum, the CA are usually small, measuring 1.5 µm or less in diameter, and only occasionally reach 10 or 12 µm [32, 91]. In addition, Mizutani *et al*. found intra-axonal bodies in the ventral posterolateral nucleus of the thalamus with an average

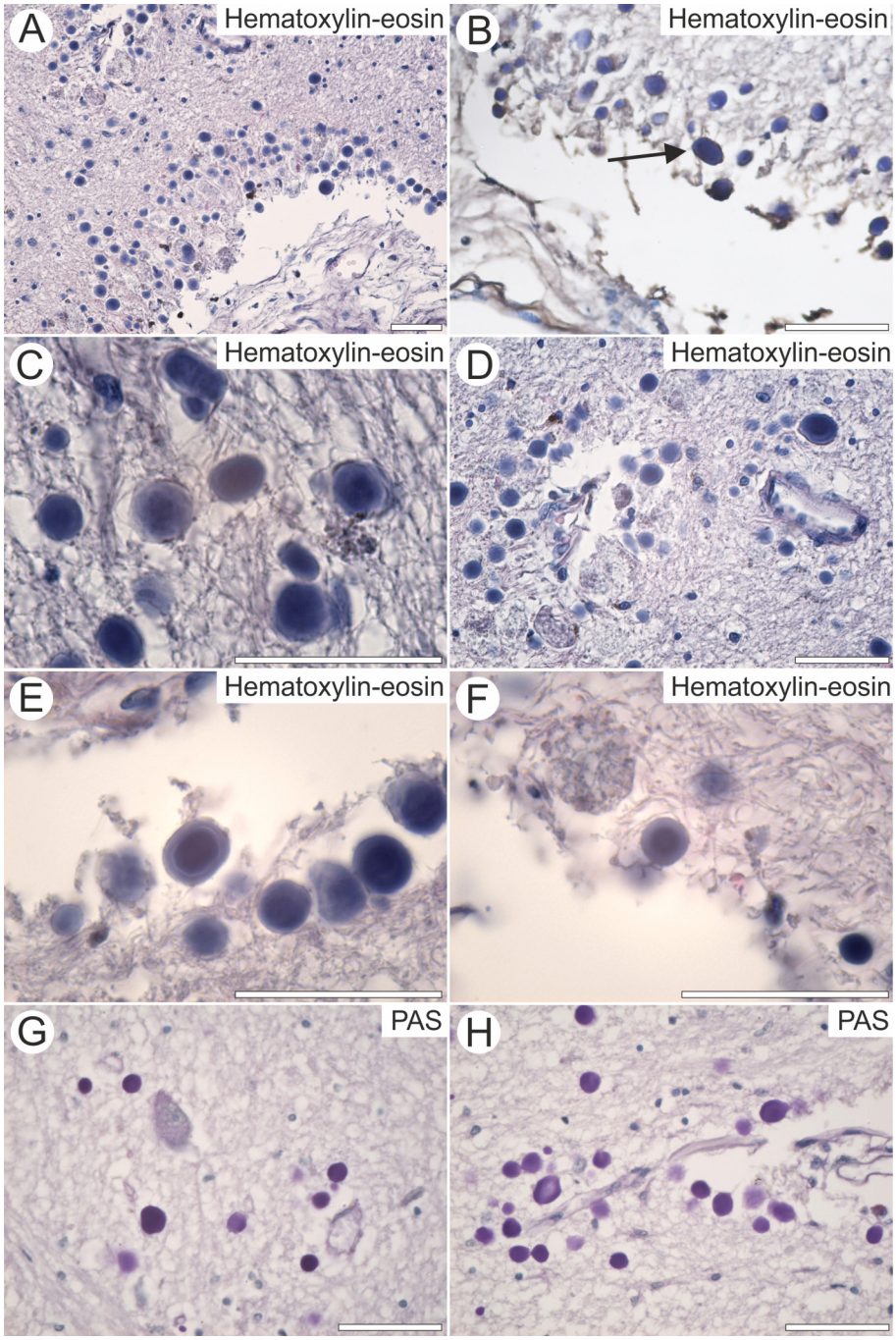

Figure 3: Corpora Amylacea (CA) range in shapes from circular (**A**) to elongated (**B**) silhouettes. Frequently fusions can be observed between two or more CAs (**C**), while their inner content can be described as concentric laminated or targetoid (**D**, **E**), and frequently the core seems to be picking up more stain than the periphery (**F**). Variantions in shapes and sizes can be observed in difefernt locations, as for example here in the striatum (**G**) or aroud small blood vessels (**H**). Human aged tissue collected from patients deceased from non-neurological causes. Scale bars represent 200 µm.

diameter between 4 and 18 µm and occasionally up to 24 µm [92].

6.3. Histochemical Staining that Aid to CA Identification

The presence of polysaccharides in the CA composition can be identified by a variety of reactions, namely:

- McManus–Hotchkiss PAS reagent (Figure **4a**, **b**) and with Best's Carmine that strongly stains the CAs [93, 94]; but more specific for polyglucosans and their acid esters being the PAS-dimedone method [70].
- Gomori's methenamine silver method [93, 94] that highlights their content of carbohydrates (Figure **4c**, **d**);

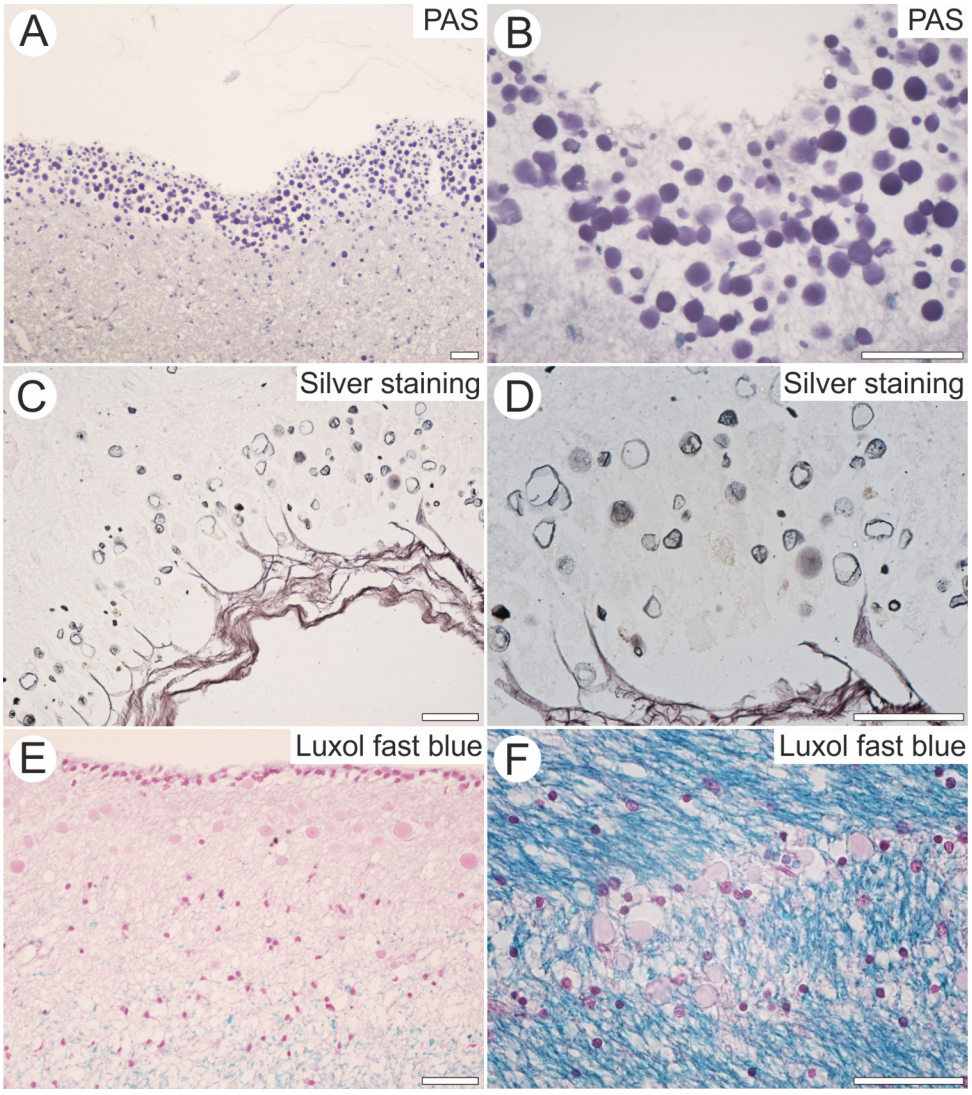

Figure 4: Periodic acid-Schiff staining reveals the presence of neutral polysaccharides in the structure of corpora amylacea (CA) (**A** and **B**), while Gomori's methenamine silver highlights the carbohydrates content (**C** and **D**). CAs are devided of myelin-deriving phospholipids as ascertained by Luxol fast blue/ Nuclear red stainings (**E** and **F**). Human aged tissue collected from patients deceased from non-neurological causes. Scale bars represent 200 μm.

- The starch-like qualities of CA can be reveled with Lugol's iodine that colors the CAs in dark brown and then by treating them with sulphuric acid the color turns in dark violet. The purple brown color is suggestive for an admixture of short and long linear polyglucosan chains that are present in the CAs;

- Hale's dialysed iron, Alcian blue at low pH and methylene blue at pH-2 reacts with CAs proving the acidic nature of some of their components;

None of these reactions are affected by prior exposure to diastase or hyaluronidase. However, exposure to α- and especially γ-amylase amyloglucosidase and also to trypsin digestion, greatly reduces their affinity for PAS and other dyes, while DNAase, RNAase and pepsin have no effect [70].

Presence of the acidic components could also explain the CA haematoxyphilia reaction that is more striking with Erhlich's stain than with that of either Harris or Mayer stains [95]. A metachromatic stain with toluidine blue, an equivocally staining for iron and weakly positive reactions for proteins were also reported [3]. The lack of myelin-deriving phospholipids has been ascertained by a negative staining for Luxol fast blue (Figure **4e**, **f**).

6.4. CA Immunoreactivity

Several immunohistochemical investigations proved that CAs contain substances derived from several sources. Thus, several authors identified in the composition of CA materials that most likely could have a neuronal origin such as: tau protein [55, 73],

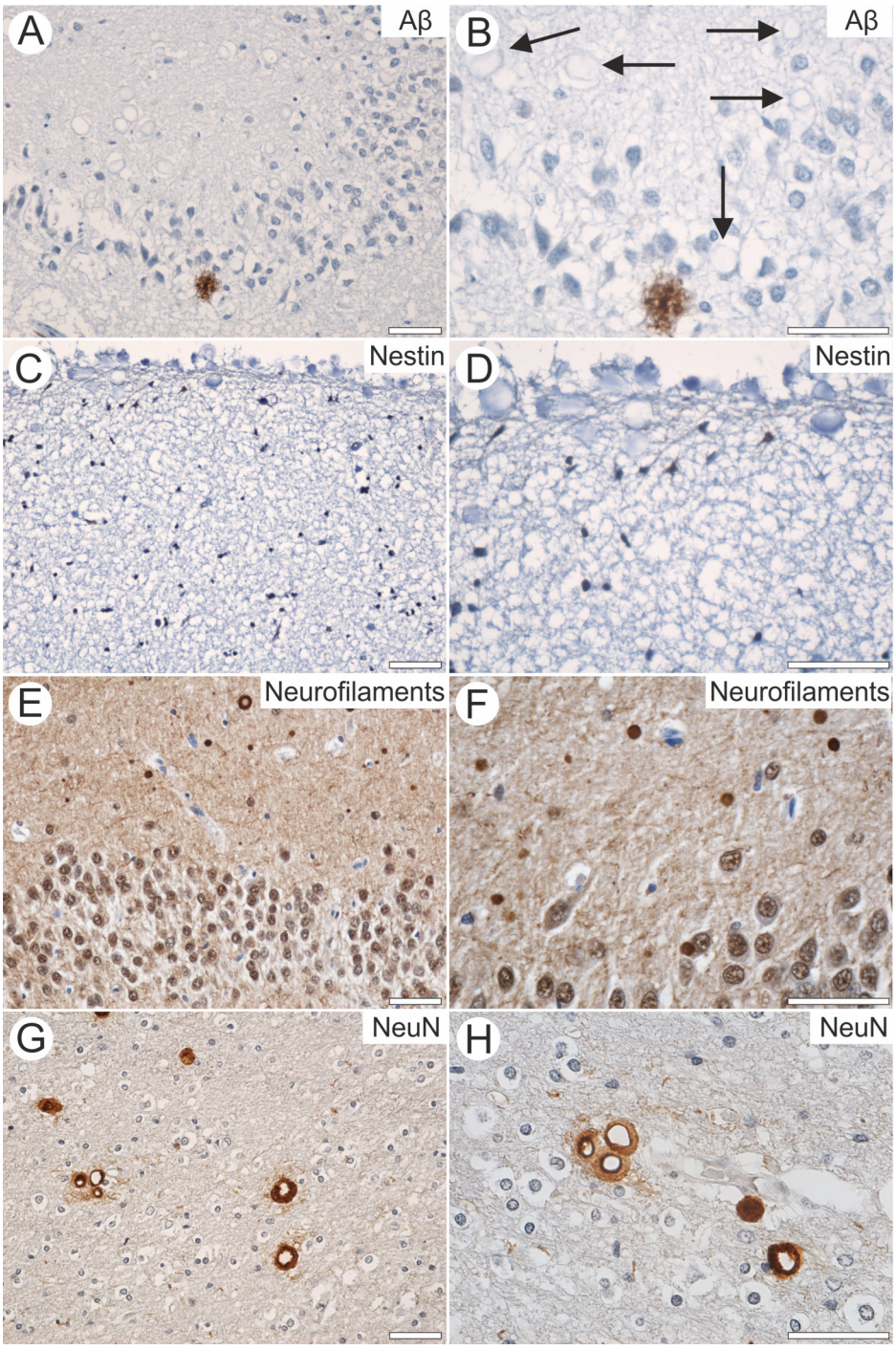

Figure 5: Immunohistochemistry profile of corpora amylacea. Amyloid Aβ fragment (as detected by the 4G8 clone) is not present in the structure of CA (**A**, **B**), the images include a a small senile Aβ – positive plaque. Nestin is also not expressed by the CAs (**C**, **D**), while their neuronal origin is underlined by positivity for anti-neurofilaments antibodies (**E**, **F**) and for the NeuN marker (**G**, **H**). Human aged tissue collected from patients deceased from non-neurological causes. Scale bars represent 200 μm.

fragments of amyloid precursor protein, but in our experience not Aβ (as detected by the clone 4G8) (Figure **5a**, **b**) [74], serum carnosinase [75], nestin (but not in our casuistry as detected with a polyclonal anti-nestin antibody) (Figure **5c**, **d**) [54], reactivity for NeuN [96], α-synuclein and parkin [51], and more recently MAP2 (dendritic marker) and Reelin [68]. Also, Renkawek and Bosman in line with the theory that CA could result by accumulation of altered neuronal membrane proteins, have argued that these bodies are intensly reactive for anion exchange proteins, that are normal constituents of neurons membranes [97]. Meng *et al*. reported the immunohistochemical localization of thrombospondin1 and ADAMTS13 in CA from vascular dementia and aged people and suggested that they could result from the aggregation of interacting proteins

from degenerating neurons and from extravasated blood cells released after transient increase in the blood–brain barrier permeability [9]. In our experience, some of the CAs were immunoreactive to neurofilaments (clone 2F11) (Figure **5e**, **f**) and NeuN (Figure **5g**, **h**) regardless of their anatomical localization. The patterns of expression were variate, ranging from a complete filling of the structure, to doughnut-like or targetoid expression patterns.

The investigations conducted by other authors pointed out toward a glial origin since the CAs were immunoreactive to: myelin basic protein, proteolipid protein, galactocerebroside, myelin/oligodendrocyte glycoprotein, ferritin [57], S100 protein (Figure **6a**, **b**) [72] and GFAP (Figure **6c**, **d**). CAs are also immunoreactive to ubiquitin (Figure **6e-h**) [58], heat shock proteins 28, 60, 70 and 72 [13, 58, 59], Hsp27 [2], Hsp32 and heme oxygenase-1 [20], suggesting their origin from both neurons and glia. As ubiquitin is one of the main markers capable of revealing material destined to be degraded, antibodies targeting this protein will also reveal most variate grow patterns of CAs: homogenous filled structures, doughnut-like or

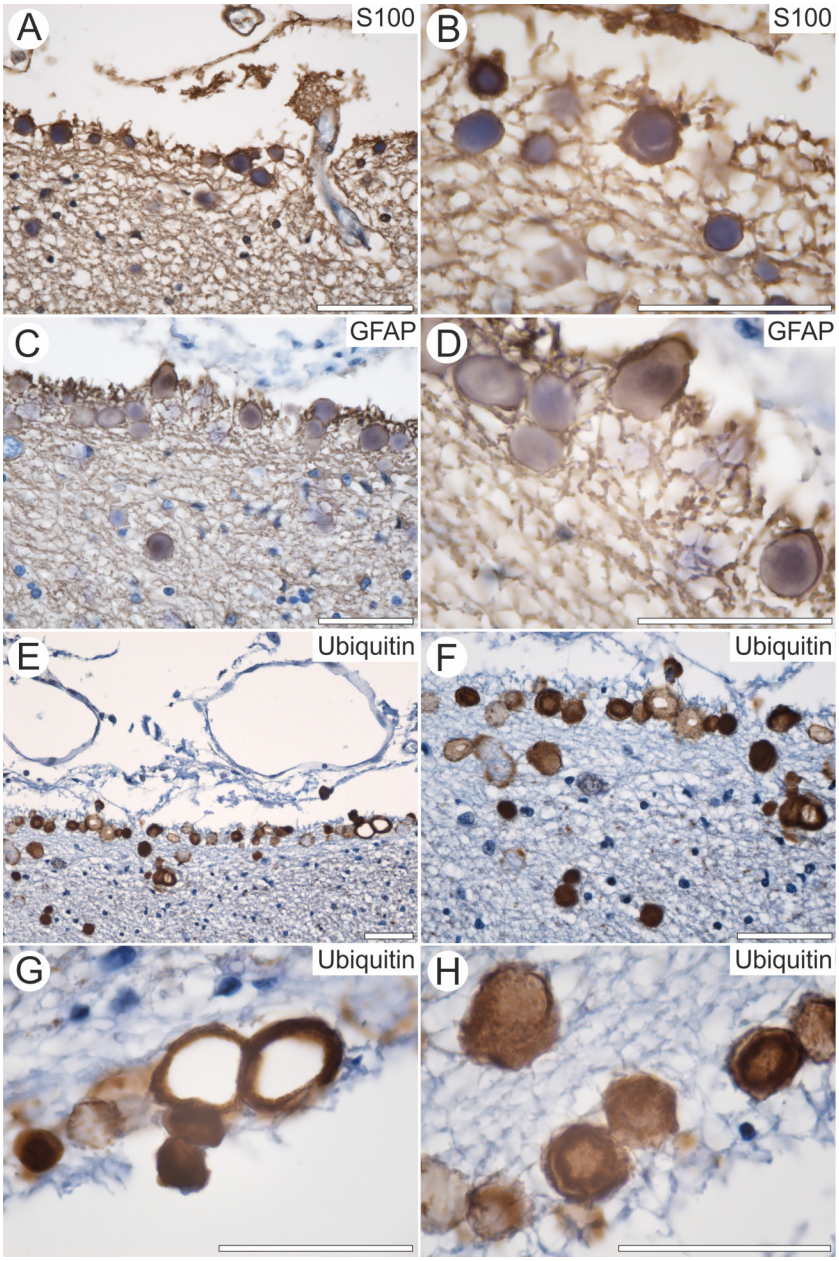

Figure 6: Immunohistochemistry profile of corpora amylacea (continuation). The glial origin of corpora amylacea (CA) is demonstrated here by a membranous-like reactivity for anti-S100 (**A**, **B**) and anti-GFAP antibodies (**C**, **D**). Anti-ubiquitin antibodies are, however, the most used immunostaining for detecting CAs of all morphological types and locations (**E-H**). Human aged tissue collected from patients deceased from non-neurological causes. Scale bars represent 200 μm.

ring-like entities, as well as targetoid expression pattrens. Our immunohistochemical investigation proved that CA were also reactive to AQP4 (Figure **7a**, **b**) and HIF-1α (Figure **7c**, **d**). A membranous-like increased expression for AQP4 in CA bearing or CA devoid astroglial feltwork around perivascular spaces, subpial and subependimal glia limitans is a strong evidence that these bodies accumulate in regions with intense metabolic buffering of the brain matter. Accumulation of HIF-1α is another reason to further support the hypoxic-oxidative stress pathogenic mechanisms implicated in genesis of CAs.

Also there was reported a CA reactivity to advanced glycation end-products (that represents insoluble and non-degradable products that result from the interaction between reducing sugars and long lived proteins) [14, 21]. Liu *et al*. have found a positive CA reaction for proteoglycans and suggested that these bodies may result from accumulation of glycoconjugates normally present in the brain tissue matrix as the result of aging [98].

Moreover, in the CA it was shown the consistent presence of mitochondrial epitopes [15, 49]. In the same direction, Botez *et al*. showed that CA are immunoreactive for the mitochondrial membrane associated protein Bcl-2, and for the major component of activator protein 1 transcriptional factor c-Jun, suggesting that their presence into CA composition is related to mitochondrial damage and/or a transient overload of proteolytic systems during cellular injury [1].

In addition, Singhrao *et al*. found positive reactions for both classical and terminal pathway-specific components of the complement cascade, the immunoreactivity being more intense in tissues from multiple sclerosis patients [17]. The authors suggested that some protein constituents of CA were derived from cells previously subjected to complement attack.

From a more technical point of view, regarding the interpretation of the variate morphology of CAs, we have observed that performing any heat-mediated antigen retrieval methods expels the cores of the CAs, leaving the ring-like or a doughnut-like pattern as the most prevalent variety.

7. BRAIN NEUROPATHOLOGICAL CONDITIONS THAT ASSOCIATE WITH CA

7.1. Neurodegenerative Diseases of the CNS

Age is the single most important risk factors for degenerative diseases of the central nervous system and with the continuously increasing lifespan of humans, the incidence of neurodegenerative diseases

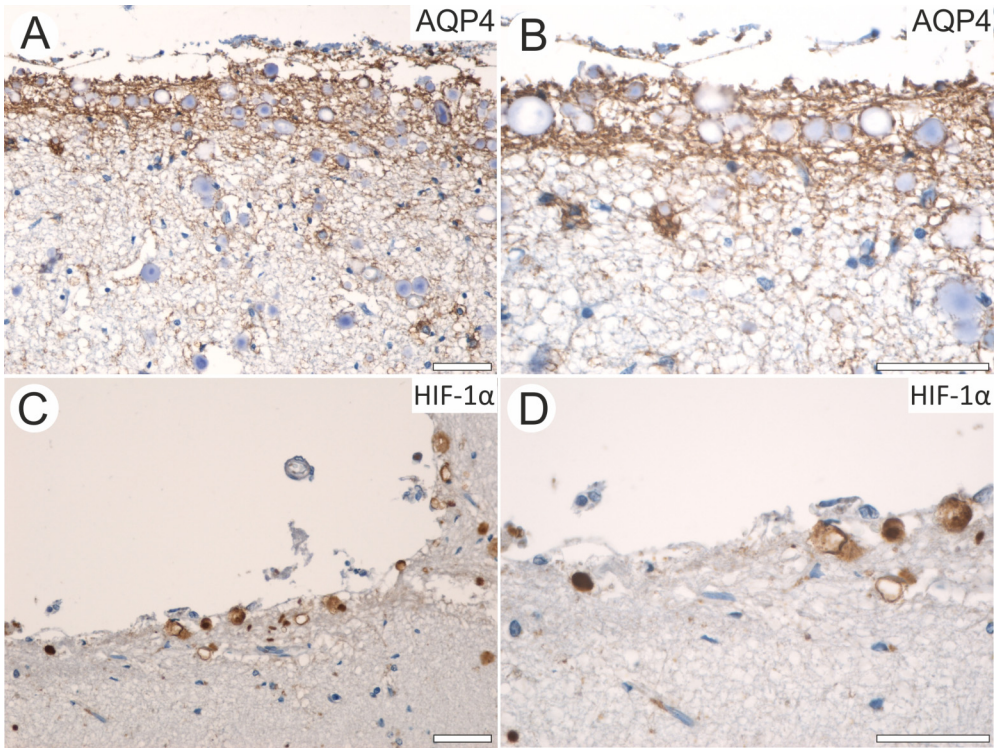

Figure 7: Immunohistochemistry profile of corpora amylacea (continuation). Corpora amylacea (CAs) are also marginally positive for anti-Aquaporin 4 (**A**, **B**) and HIF-1α (**C**, **D**) antibodies. Human aged tissue collected from patients deceased from non-neurological causes. Scale bars represent 200 μm.

will also raise. Some of these entities were classified as proteopathies as they are associated with the aggregation of altered proteins (Figure **8**).

7.1.1. CA in Alzheimer's disease

One of the first observations regarding CA deposition in Alzheimer's disease was made by Cisse *et al*., who reported a much higher density of these bodies than in normal aged subjects [58]. Renkawek and Bosmanfound abundant CA in the brains of Alzheimer disease patients, and suggested that accumulation of altered neuronal membrane proteins may be involved in their pathogenesis [97]. Singhrao *et al*. have drawn attention on the CA importance as repositories of the products of neuronal cell death and myelin breakdown, both in aging individuals and in diseases, such as Multiple Sclerosis, Alzheimer's disease and Parkinson's disease [17]. It seems that between the CA and Alzheimer's disease there is a strong etiopathogenic connection since advanced glycation end-products were both detected in neurons and in CA, as a result of proteins glycation such is the case of tau protein [99]. These non-degradable proteins could generate oxygen intermediates that lead to oxidative stress, that it is a pathogenic mechanism in the cell damage in Alzheimer's disease [100].

7.1.2. CA in Multiple Sclerosis

CAs have been frequently described in multiple sclerosis brain tissue, but without knowing its pathological significance [101]. Selmaj *et al*. investigating brain tissue from patients with multiple sclerosis, detected numerous CAs in the lesion areas (with an average density of 6 per mm^2), while the adjacent tissue was entirely unaffected [56]. Also the authors provided evidence supporting that biogenesis of CAs involves degeneration and aggregation of cells of neuronal origin.

7.1.3. CA in Hippocampal Sclerosis and Temporal Lobe Epilepsy

Hippocampal sclerosis is the commonest histopathological substrate in epileptic patients undergoing temporal lobe epilepsy surgery [102]. Several authors reported CA presence with different frequencies (6 to 63%) in the resected hippocampus of patients with temporal lobe epilepsy undergoing surgery [3, 22, 103-107]. Also it was established that the extent of CA accumulation could be correlated with seizure duration and interictal psychosis in patients with mesial temporal lobe epilepsy [106]. It was suggested that this premature CA accumulation in the hippocampus might be a reflection of a tissue reaction to buffer the free radicals and other toxic metabolites generated as a result of repeated seizures. Ribeiro *et al*. also found that epilepsy duration was significantly related to the presence of CA [107], but Van Paesscchen *et al*. reported a lack of correlation between the presence of CA and most epileptic variables, like age of seizure or age at operation [108]. Moreover, Chung and Horoupian [22] and Van Paesschen *et al*. [108] pointed out that the distribution of CA in the hippocampus parallels the neuronal loss, with the highest density in the CA1 and CA2 sectors,

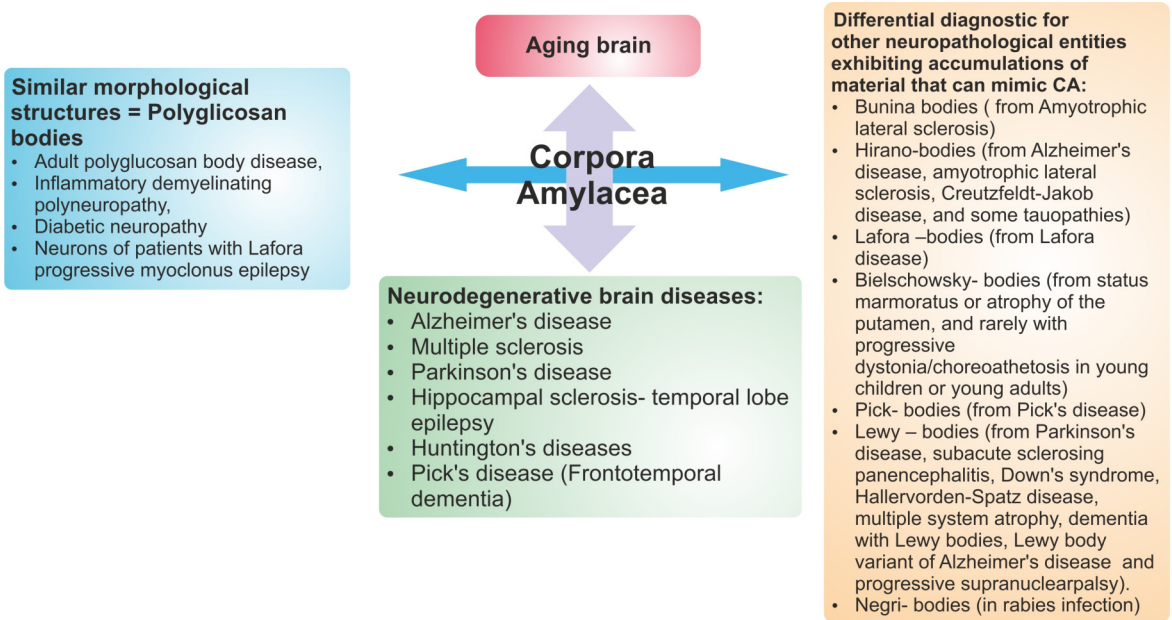

Figure 8: Integrative schematic diagram of different brain conditions that associate corpora amylacea, as well as the main differential diagnostics to be considered in their evaluation.

which were also the most severely affected regions for neuronal loss and gliosis [104]. It was demonstrated that CA tended to be more frequent in hippocampal sclerosis dementia than in frontotemporal lobar degeneration with ubiquitin-positive inclusions, but there was no difference in the frequency of argyrophilic grains [109]. Some authors have proposed CA as a possible marker for hypoxic-ischemic hippocampal sclerosis, since they may be more numerous in brains of patients exposed to repetitive hypoxic episodes [1, 25].

7.1.4. CA in Parkinson's disease

Although, Parkinson's disease is characterized at the tissue level by an intracytoplasmatic neuronal accumulation of aggregated α-synuclein protein under the name of Lewy bodies [110], CA deposits were also described. Thus, Mizutani *et al*. reported an increased number of CA in the ventral posterolateral nucleus of the thalamus in subjects older than the 50 years; they have been found more numerous especially in cases with Parkinson's disease [92]. In addition, Buervenich *et al*. identified clusters of CA under the ependyma of the lateral and fourth ventricles in post-mortem brain material from Parkinson patients [54]. Furthermore, it was demonstrated that parkin and α-synuclein, both PD-pathology related proteins [111, 112], were present in CA in both healthy aged and Parkinson patients' brains [51]. Thus to discrimination between Lewy bodies and CA, PAS staining was used, since LBs are PAS negative [113, 114].

7.1.5. CA in other Neurodegenerative Diseases

Averback reported the presence of CA within synaptic processes in the striatum from subjects with *Huntington's diseases* [32]. They compressed and distorted the synaptic vesicles and it seems that their number increases before synaptic elements are destroyed.

The presence of CAs was also found in post-mortem brains of patients with different neurological diseases, including *Pick's disease* [17].

In subjects with alcoholic encephalopathy there were recorded increased numbers of CAs arranged around blood vessels, pial and ependymal surfaces compared to controls [26].

7.2. CA in Type IV Glycogenosis (Andersen's disease)

Glycogen storage disease type IV (GSD-IV), also known as Andersen's disease or amylopectinosis, is a rare autosomal recessive disease caused by a deficiency of glycogen branching enzyme leading to the accumulation of amylopectin-like structures in altered tissues [115]. Subjects with this enzyme deficiency will store intracellularly very long unbranched glucose chains, and since they have a low solubility they will precipitate and subsequently form pathological deposits referred as polyglucosan bodies (PGB) responsible for cellular damage.

The disease is extremely heterogeneous in terms of tissue involvement, age of onset and clinical manifestations. GSD IV can manifest as several different subtypes, with variable ages of onset, severity, and clinical features, including the following: classic (progressive) hepatic subtype, non-progressive hepatic subtype, fatal perinatal neuromuscular subtype, congenital neuromuscular subtype, and childhood neuromuscular subtype [115, 116]. However, it seems that GSD IV phenotype is a continuum that ranges from mild to severe, thus pinpointing one of the aforementioned subtypes is difficult [117]. The diagnosis of GSD IV is suspected based on the clinical presentation, by the demonstration of glycogen branching enzyme deficiency in liver, skin fibroblasts, or muscle [118], pathological evidence of PGB accumulation in muscle or liver tissue, and/or the identification of biallelic mutations in GBE1.

In brain the PGB CAs are present in great number throughout the CNS especially in subpial and subependymal regions and in the white matter clustering around blood vessels [3]. Also they have been reported in the innermost and outer layers of cortex, in the molecular layer of the cerebellum, in the dentate nuclei, and in the basal ganglia. Their size ranges from very small to rarely greater than 20 μm in diameter, and were reported some chemical and histochemical difference compared to classical CAs. Thus, it was demonstrated that PGB from GSD IV are not metachromatic with toluidine blue [119], suggesting they may have a lower phosphate content than the CAs [70]. Moreover, the PGB stain blue with iodine indicating the presence in their composition of long chains in higher proportion [119] then in CA which have an even admixture of short and long chains, thus the CAs stain in purplish brown with iodine [70].

7.3. CA in Adult Polyyglucosan Body Disease (APBD)

PBD is the adult-onset form of the glycogen storage type IV disease, an rare autosomal recessive

progressive neurological disorder due to a deficiency in glycogen branching enzyme [115, 120].

The disease affects predominantly Ashkenazi Jewish families, usually occurring between the fourth and fifth decade of life, with early onset bladder dysfunction followed by progressive spastic paraplegia, and to a lesser extent, peripheral neuropathy and cognitive impairment [121, 122]. Diagnosis is based on the combination of compatible clinical symptoms, with MRI of the brain and spinal cord, sural nerve biopsy showing characteristic polyglucosans within nerve tubes, assay of glycogen brancher enzyme (GBE) activity in skin fibroblasts or muscle tissue, and molecular genetic testing of GBE1. The main feature of the disease is the abnormal accumulation of PGB throughout the nervous system, predominantly within the myelinated nerve fibers (motor neurons) but also in other organs [3, 123-126].

In the brain, the PGBs has the same cellular distribution as classical CAs, being found in both neurites and astrocytes, but more often they are free in the neuropil. However, the PGB was also been observed in neurons [124, 126]. Their topography is the same as for the type IV glycogenosis, with the largest predominance in the white matter, especially around blood vessels, corpus callosum and beneath the ependyma [3]. In the cortex they are more numerous in the subpial layer, particularly in the depths of the sulci, but their maximum density was recorded in the IVth layer of the cortex, and in the striatum and thalamus.

Morphologically they are indistinguishable from classical CAs, mostly being round or oval, with unusual elongated forms extending to 150 μm in length being often seen, especially in the cortex [3]. Their size varies from 1-2 μm when they appear as small “dust-like” forms, and up to 25 μm in diameter. Also they have the same general histochemical and ultrastructural characters as normal CA.

In addition, the PGBs have also been described in inflammatory demyelinating polyneuropathy and diabetic neuropathy, or in the neurons of patients with Lafora progressive myoclonus epilepsy [127].

8. DIFFERENTIAL DIAGNOSIS WITH OTHER ENTITIES IN THE CNS

The differential diagnosis may include all the neuropathological diseases characterized by the production of peculiar materials with special morphology in the elderly such as: Bunina- bodies, Hirano- bodies, Lafora- bodies, Pick- bodies, Bielschowsky- bodies, Lewy- bodies, Negri- bodies, neurofibrillary tangles, senile plaques, Rosenthal fibers, etc.

8.1. Bunina-Bodies

Described for the first time in 1961 by Van Reeth *et al*. [128] and confirmed a year later by Tat’yana Bunina [129] is present in most cases of amyotrophic lateral sclerosis (ALS) [129-131].

They are most commonly found in the lower motor neurons, with predilection in lumbar spinal cord and in the brain stem nuclei of patients with an associated dementia [129, 132, 133]. In the brain, their presence has been reported rarely in Betz cells [134].

At the cellular level, they have been identified in the cytoplasm and dendrites [135] but not within the axoplasm [129]. Microscopically in H&E staining they appear as bright pink, oval eosinophilic inclusions, of 3–5 μm in diameter and that occasionally show clear areas in the centre and forms clusters [132, 136, 137]. Histochemically, they stains in purple with phosphotungstic acid-hematoxylin, in light blue with Kluver-Barrera stain, in red with Masson trichrome stain and do not react with silver, periodic acid Schiff, Sudan black or Congo red [129].

Ultrastructurally, they consist of an amorphous electron dense material surrounded by tubular and vesicular structures with a central area containing 10nm filaments and no limiting membrane [129]. Within these bodies were identified both constricted and unconstricted bundles of filaments, measuring 20-25nm in width [138].

Immunohistochemically these bodies react with transferrin [139] and cystatin C [135]. Latterly, these structures are recognized as markers of neurodegeneration with possible origin in the Golgi complex [140, 141].

8.2. Hirano-Bodies

Described since 1965 [142], they develop in the brain during normal aging, but preferentially occur in neurodegenerative diseases such as Alzheimer's disease, motor neurone disease, Creutzfeldt-Jakob disease, and some tauopathies [143-149].

In H&E stain they appear as intracellular, rod shaped eosinophilic structures most often encountered in neurons of the central nervous system, particularly in the hippocampal pyramidal cells. Ultrastructurally, they

are composed of filamentous actin arranged depending on the plane of section as either a herringbone or crosshatch pattern [150, 151].

Immunohistochemical studies indicate the presence within these bodies of actin; tropomyosin; vinculin; β-APP; tau, FAC1 (a nuclear protein), Hsp27; iNOS, MAP1,2; NF; and Smurf1 (Smad ubiquitination regulatory factor 1) [143, 152-159].

Although the physiological role of Hirano bodies is unknown, in Alzheimer's disease it was suggested that they could confer protection against cell death by sequestering c-terminal fragments of APP and possibly tau, preventing them to participate in signaling pathways which contribute to cell death [160].

8.3. Lafora-Bodies

Lafora disease (LD) is a progressive myoclonic epilepsy with an autosomal recessive hereditary pattern [161], characterized at the tissue level by the presence of inclusion bodies (Lafora bodies- LB), within neurons and the cells of the heart, muscle, liver, and skin. LD is caused by mutations in the EPM2A gene encoding laforin, or in the EPM2B gene encoding malin [162, 163].

In the brain, they are widespread in the cortex with the highest density in prefrontal motor cortex, affecting especially layers III and V, with emphasis on medium sized neurons [87, 164, 165]. The deep cerebral gray matter is less severely affected and areas of high density deposition include substantia nigra, dentate nuclei, superior olive, pontine reticular nuclei, and basal ganglia. Their presence appears to be restricted to neurons and ultrastructurally it seems to be almost entirely within dendrites and cortical neurites [87, 166-169]. Apparently, the smallest bodies (<5μm) lie randomly free in the neuropil as a dust-like, and with variable density [3]. LB vary in size from 1 to 30μm in diameter and usually are round or oval, although along a neurite they may be greatly elongated, sausage-shaped or thread-like forms and chains of smaller bodies [87].

Their morphology varies from tissue to tissue, but they generally contain a central core and have a peripheral cotton-like appearance. What differentiates the LB from all other polyglucosan bodies is the densely staining central core with striking outer radiating pattern of less densely staining material and their intraneuronal situation [3].

Biochemically they appear to have slightly increased percentage of proteins (9% compared to 4%) and lower phosphate content (1.26%) than ordinary CA [3, 70, 170-171]. Histochemically, they are basophilic in H&E stain, PAS positive, diastases resistant, Alcian blue positive, and variably metachromatic (with methyl violet or toluidine blue), but less intense than CA. Using PAS stain, Van Hoof and Hageman-Balidentified three types of LBs: (1) type I, which are the most numerous and consist of granular, polymorphic, "dust-like", uniformly stained particles; (2) type II characterized by a heavily stained core, surrounded by a less stained circular rim; and (3) type III, the rarest entity, that looks like type II inclusions but with "fissured" dark core, reminiscent to the letter "Y" [172].

Ultrastructurally, they are composed of 8–12nm filaments closely similar to those of CA, although the amount of electron dense amorphous and granular material within the central core is greater [169, 170]. Between the fibrils are dense granular structures about 15-50nm in diameter. Within the rim of LB type II were described 12 to 18 microspherules with a circular and regular arrangement [173, 174].

Immunohistochemically the LB presents reactivity to lectin, tau, ubiquitin, neurofilaments (160 and 200 KD) and desmin, but not vimentin or cytokeratin [175]. Machado-Salas *et al*., proved that the peripheral rim of LB type II are immunoreactive to neurofilaments suggesting an etiopathogenic connection between these inclusion bodies and neurocytoskeleton [174]. Also it seems that the brain LBs display different immunocytochemical reactions when compared to LBs from liver or muscle [176].

The consequence of brain LB deposition lies in onset and then inexorable worsening of epilepsy and neurodegeneration, leading to death by early adulthood [3, 162, 177, 178].

8.4. Bielschowsky-Bodies

This type of inclusions is often seen in association with status marmoratus or atrophy of the putamen, and rarely with progressive dystonia/choreoathetosis in young children or young adults [3, 85, 179-182]. It was speculated that Bielschowsky bodies might occur as a result of some as-yet-unknown genetic predisposition since they are not commonly seen in gliotic encephalopathy, the most frequent complication of perinatal ischemic/anoxic injury [183].

Typically, Bielschowsky bodies are restricted to the external (lateral) pallidum, rarely they have been reported in the adjacent inner pallidum and putamen and few cases have demonstrated additional

involvement of the substantia nigra and brain stem tegmentum.

They could be identified in the neuropil when they look similar to common CAs, but characteristically they have been identified within neuronal perikarya and neuritis, occasionally multicentric [85, 183, 184] and have the typical characters of Lafora bodies.

Morphologically, Bielschowsky bodies appear within neuronal perikarya as round or oval bodies, with variable size (up to 30–40mm), and in neuritis they are elongated and might form chains [3, 181]. Histochemically are very similar to the CAs, reacting with PAS, Best's carmine, Alcian blue, toluidine blue and iodine, but are partially diastase resistant [3, 183]. The neuronal perikarya Bielschowsky bodies tend to have a more densely staining inner core with radiating striations of typical Lafora bodies. Immunohisto-chemical investigations reported reactivity of some Bielschowsky bodies to ubiquitin and neurofilament proteins, but not to tau [183, 184].

Ultrastructurally, the inclusions are not membrane bound and displayed fibrillar profiles radiating from a coarsely granular electron-dense core. The fibrils are of 4–10nm in diameter, apparently branched, an aspect that most probable is due to the superimposition of the individual somewhat flattened fibbers [181]. Frequently, within the filamentous bundles stand out remains of organelles [3].

8.5. Pick-Bodies

Pick's disease is a rare neurodegenerative disorder, classified as a tauopathy, consisting of neuronal accumulation of aggregates of hyperphosphorylated tau protein [185], known as "Pick bodies", important neuronal loss and swollen neurons (Pick cells); wich associate with frontotemporal lobe atrophy [186].

Pick bodies are usually found in the limbic system (most frequently in the amygdala and hippocampus), paralimbic, and ventral temporal lobe cortex, but they were also seen in anterior frontal and dorsal temporal lobes. In hippocampus the highest concentration of Pick bodies has been reported in pyramidial cells of the CA1 region, dentate granule cells and subiculum, while in the neocortex the highest density was observed in the neurons from the II and IV layers [187].

They are sharply demarcated circular intracyto-plasmic inclusions that are slightly basophilic on H&E stain, strongly argyrophilic and often inden-tated across the side towards the nucleus. The most selective silver impregnation stain aiming to identify the Pick bodies is the Bodian technique [186, 188].

Biochemically, one of the most important characteristics of Pick bodies is the abundant presence of the insoluble 3R isoform of tau protein [189-191]. However, it was demonstrated later on that 4R tau isoforms could be also present individually or as a mixed 3R/4R isoforms pattern [192, 193].

Ultrastructurally, these bodies appear as non-membrane bound structures with a loose arrangement of tau filaments, that mostly are straight filaments with a 15nm width, but occasional paired helical –like filaments with a long periodicity of 120 to 160nm were also reported [194-197]. Moreover, King *et al*. have reported that morphologically the Pick's filaments are straight filaments, paired filaments with long periodicities, and those with a period similar to that of Alzheimer's diseases -paired filaments, suggesting that a progression from straight to paired filaments could take place [192].

Immunohistochemically tau hyperphosphorelation can be recognized by antibodies targeting phosphorylation sites Ser202/Thr205 (AT8), Ser396/Ser404 (PHF-1), and the conformational shift for the Alz-50 epitope. Pick bodies are further reactive for ubiquitin, αβ crystallin, N-terminal segment of the amyloid precursor protein (APP), neurofilament proteins, neuronal surface glycoside (A2B5), clathrin, synaptophysin, and βII tubulin [193, 198, 199]. More recently, Rohn *et al*. proved the presence of amino-terminal fragment of apolipoprotein E within Pick bodies suggesting that this protein could contributes to Pick's disease pathogenesis [200].

8.6. Lewy-Bodies

Were first described by Friederich Heinrich Lewy in 1912 [201], and were later named after him by Tretiakoff [202]. Considered to be for a long time the pathological hallmark of Parkinson's disease [110, 203], they have been reported in other disorders such as subacute sclerosing panencephalitis [204], Down's syndrome [205], Hallervorden-Spatz disease [206], multiple system atrophy [207], dementia with Lewy bodies [207], Lewy body variant of Alzheimer's disease [208], and progressive supranuclear palsy [209]. However, Lewy bodies are typically absent in autosomal recessive juvenile-onset Parkinson's disease with parkin gene mutations [210-212], and even more than that they have also been found in the substantia nigra of elderly individuals without neurological disease [213, 214].

Topographically, they are more numerous in the surviving neurons of the substantia nigra in Parkinson's disease, but they may also be observed in the locus ceruleus, dorsal motor nucleus of vagus, nucleus basalis of Meynert, limbic and cortical structures [110]. The cortical Lewy bodies are the hallmark of dementia with Lewy bodies, but they also occur in ballooned neurons characteristic of Pick's disease and cortico-basal degeneration [215], in patients with other tauo-pathies [216], as well as in cases of multiple system atrophy, particularly the Parkinsonian variant [217].

Morphologically, Lewy bodies are divided into classical (brain stem) Lewy bodies (found in the brainstem nuclei and diencephalon) and cortical Lewy bodies (typically present in cerebral limbic cortex and amygdala). The classic type consist of circular intraneuronal cytoplasmic inclusions (about 8 to 30 μm in diameter), characterized by hyaline eosinophilic cores (that stain pink with H&E stain), concentric lamellar bands, narrow pale halos, and immunoreactivity for alpha-synuclein and ubiquitin [218, 219]. In contrast, cortical Lewy bodies lack a halo, but they are also positive to alpha-synuclein [220, 221].

Ultrastructurally, the classical Lewy body consist of a dense core that contain granular material surrounded by a halo of 10-nm-wide radiating fibrils, while the cortical Lewy body is less well defined, having a diffuse structure without a distinct core and halo, but containing alpha-synuclein fibrils [222].

Biochemically, Lewy bodies contain a mixture of lipids, proteins, and neurofilaments [223], but the main constituents are α-synuclein and ubiquitin [114, 224]. While in the classical Lewy body, ubiquitin tends to concentrate within the central core, whereas α-synuclein is located mainly in the periphery; in the cortical Lewy body such separation of ubiquitin and α-synuclein is not present [223]. Besides α-synuclein and ubiquitin, in Lewy bodies have been described so far more than 76 components [225]. They belong to ten different protein classes, including structural elements, cytoskelatal proteins, α-synuclein binding proteins, cytosolic proteins, synphilin-1-binding proteins, components of the ubiquitin-proteasome system, proteins implicated in cellular responses, proteins associated with phosphorylation and signal transduction, cell cycle proteins, and others [225].

Since, Lewy bodies are biochemically similar to aggresomes, a microtubule organizing centre that is integral to the regulation of abnormal proteins, some authors suggest that they could be dysfunctional aggresomes [226, 227], formed as a mechanism to protect the cell by the up-take of altered and un-functional proteins [226, 228]. However, the roles of Lewy bodies as toxic, protective, or just an epiphenomenon remain to be elucidated.

8.7. Negri-Bodies

Discovered by Adelchi Negri in 1903 [229], they represent pathognomonic inclusion bodies for rabies infection. They have a wide spread distribution in neurons in human rabies, but more frequently are observed in large neurons in some peculiar brain regions [230]. The largest Negri bodies are found in Purkinje cells and in the periaqueductal gray matter, while bodies of intermediate size are seen in pyramidal neurons of the hippocampus (CA1 region) and cortical neurons (third layer), and the relatively small bodies are present in trochlear nucleus. These intraneuronal inclusions were found only in infections caused by street virus [231], suggesting that the strains of fixed virus may exclude the formation of Negri bodies by causing a lytic processes of neuronal destruction [232].

Morphologically they appear as eosinophilic, sharply outlined, intracytoplasmic inclusion bodies of few microns in diameter (0.25-20μm) that commonly are round to oval, but sometimes could be triangular or elongated [233]. Their number per neurons ranges from 1 to 12. They could be selectively identified on histological samples by Mann's, giemsa, or Sellers stains. Thus, in Mann's technique it could be observed that their characteristic is in the presence of one to several dark-blue inner basophilic granules (reactive with methyl blue) with a diameter ranging from 0.2 to 0.5μm, that are placed into a magenta matrix.

Electron microscopy studies proved that they are composed by a matrix of granular or filamentous material, containing viral nucleocapsids, and viral particles which were also seen budding from the matrix into the endoplasmic reticulum [234, 235]. Viral particles were also seen budding from this structure [234-236]. Initially, it seems that these structures are devoid of membranes, but later on they becomes completely surrounded with a double membrane, with the cytoplasmic surface having a granular aspect suggesting a rough endoplasmic reticulum origin for these structures [237]. Finally, the shape of the bodies appeared to be altered, and viral particles were also seen budding from some of these structures.

The exact composition of the Negri bodies remains unknown. However, it was proved that all viral RNAs

(are located inside the Negri bodies [237]. Moreover, these authors proved that Negri bodies are the sites where viral transcription and replication takes place. Moreover, Menager *et al*. showed by confocal microscopy and 3-D imaging that Negri bodies have a highly organised structure, with a toll-like receptor 3-containing core surrounded by a halo of viral N and P proteins [238]. The authors suggested that by the sequestration of these receptors inside Negri body, the rabies virus could prevents the antiviral or apoptotic effect of this cellular protein. Lahaye *et al*. found some similarities to aggresome structures, Negri bodies being resistant to detergents, involve microtubules during their development and are surrounded by the cellular α-tubulin and chaperone network [237]. However, they were not associated with the microtubule organizing centre and were not surrounded by a vimentin network, thus they do not represent canonical aggresome structures. Based on these observations the authors suggested that Negri bodies might be the result of a cytoplasmic compartmentalization secondary to a defense mechanism involving the aggresome pathway.

9. CONCLUSIONS

Corpora amylacea are glycoproteinaceous, ubiquitinated, cytoplasmic inclusions that accumulate in subpial and periventricular regions of human brain in the course of normal aging, and to a greater extent in neurodegenerative conditions. Although many of the histochemical, tinctorial and structural properties of CA have been delineated a long time ago, their pathogenic mechanisms, their cellular origins and their functions are still under debate. It has been proposed that CA represent aggregates the of nervous system alterated products' that accumulate within astrocytic cytoplasm secondary to the oxidative stress and mitochondrial dysfunction. Thus, their formation may reflect a powerful neuroprotective mechanism by which they trap and sequestrate the potentially deleterious products of cellular metabolism, that are produced during normal aging, as well as in excessive amounts, during neurodegenerative disorders. Therefore, these "enigmatic bodies" are far from being completely understood, thus further investigations are needed to better explain the brain aging and the pathogenesis of different degenerative neurological diseases, and perhaps they could provide novel therapeutic targets to counteract age-related brain disorders.

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