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Review article

PRINCIPLES OF AGE-RELATED CHANGES IN THE CANINE AND FELINE BRAIN

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In the aged dog and cat, especially dog, a cognitive decline develops naturally in many different domains, but at the same time it also exhibits human-like individual variability in the aging process. In the aging dog and cat brain lesions develop spontaneously. Dogs share some morphological characteristics with those of Alzheimer's disease in man. The canine brain with its plaques and tangles which show oxidative changes, forms a spontaneous model for understanding the early changes and their interrelationships in Alzheimer's disease. Additionally, the aged dog represents a useful model for the development of preventive or therapeutic interventions to improve aged brain function. These interventions can then be translated into human clinical trials.

Key words: aged dogs, aged cats, amyloid, Alzheimer disease, oxidative damage

Principles of neuropathology embrace a variety of different aspects including the history of neuropathology, embryology, anatomy, cellular composition of the CNS, use of a tissue-specific terminology for pathological processes, techniques specific for the nervous system, and the knowledge about the occurrence of lesions of no significance and artifacts. Most of these principles have evolved over decades and represent the basis of our current concept on neuropathology. Some scientists have experienced revolutionary changes in the methodologies applied for the investigation of CNS disorders.

Histopathology, immunohistopathology and various molecular techniques including *in situ* hybridization, reverse transcriptase polymerase chain reaction, different blotting methods, though not developed for nervous disorders only, have been applied to further investigate CNS disorders on a cellular and molecular level. Recent advances in immunohistopathology and in molecular pathology do now allow a more detailed and sophisticated interpretation of neuropathological findings. The above mentioned techniques can be used to identify the cause of the observed pathology or to improve our understanding about the pathogenesis in various nervous disorders [1-3].

Neuropathology has been and still is set apart as an area for specialists. The expected

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lifespan of Western man is approaching 75 to 80 years. Over the course of this period, manifestations of degenerative changes appear in most organ systems: hair pigment loss, visual acuity falls, changes in the connective tissue matrix of the skin became evident etc. Thus, with advancing age, signs of deterioration can be anticipated in many organ systems.

With respect to the CNS, a statement can be made that applies equally to all diseases of humans and animals: some disorders are highly comparable, some share some points of similarity, and others are limited to humans or to individual animal species. Thus, neuronal lipofuscinosis in the aged human being and the enderly dog are probably analogous conditions, whereas the full neuropathological spectrum of Alzheimer's disease is not encountered spontaneously in any of the domestic animals.

In general, the effects of the aging process on the CNS have received scant attention in veterinary medicine. Fore example, geriatric cerebral atrophy is well recognized in humans, but in routine animal pathology it is not normal practice to weigh the brain at necropsy (or even always to remove the brain from the cranium). Accordingly, we have no universal data from which to evaluate whether a comparable state of age-related regression of the brain occurs in domestic animals. An impression that we have gained over many years in that, if cerebral atrophy occurs in old dogs and cats, it is apparently mild. Several authors comment that the brains of old dogs are mildly hydrocephalic. Only in general terms can we compare the brain of an aged human with an aged dog or cat.

In animals, there are notably few studies of changes in the CNS cell populations with aging. Furthermore, most researchers in these investigations have turned their attention to laboratory rodents rather than to domestic animals. Some studies employ the cat, but this animal is used widely in neurological investigations, particularly neurophysiology, more specifically, in studies of the brains of aged dogs and cats. A common theme emerging from these studies is that as animals age, degenerative changes and/or depletion are found in some neuronal populations. In contrast, neuroglia may be reduced in number, remain constant, or even increased in the aged.

There is a **degenerative change** in the white matter of many aged dogs and cats. The degeneration affects densely myelinated tracts such as the corpus callosum, corona radiata, and hippocampal alveus and appears to be an incidental observation at necropsy. The alteration is a pallor and fine vacuolation of the myelinated fibers, the appearance of isomorphic gliosis, and the accumulation of perivascular macrophages containing a pale yellow pigment material. Gliosis takes the form of elongation and hypercellularity of the chains of interfascicular glia and is quite distinct from that associated with inflammatory disorders. Pigment in the perivascular histiocytes is probably derived from myelin degeneration and will stain with luxol fast blue. It has been designated variously as lipid and lipofuscin, but Jolly suggests the term ceroid, to be limited to lipopigments formed from lipid degeneration and/or peroxidation. Ubiquitin-and galactocerebroside-immunoreactive granules and globules have been demonstrated in these degenerated white matter tracts. Focal axonal swellings –spheroids- are a nonspecific response to many diverse insults, including trauma, hypoxia, intoxications, nutritional deficiencies, and storage disorders. Axonal spheroids are also encountered

in the CNS and the autonomic ganglia with increasing frequency with progressive age. Spheroids in low numbers may be found at all ages and at all levels of the neuraxis, but are generally more common in the aged, in the gray matter more than the white matter, and particularly in the medulla (nucleus gracilis and also the nucleus cuneatus) and sacral spinal segments [4-6].

A novel **intracytoplasmic inclusion** occurring in neuronal perikarya of old dogs has been recorded by Suzuki et al [7]. Affected neurons include both central and peripheral populations. Within the neuraxis, neurons of the substantia nigra, pontine nuclei, and piriform lobe are most consistently affected. These structures are round, amphophilic, finely granular bodies and usually single. They are PAS and alcian blue positive and bind the lectin concanavalin A, indicating mannose or glucose residues [8].

Lafora bodies (polyglucosan bodies) are intracytoplasmic neuronal inclusions that occur in the CNS of humans and some animal species. Their presence has been associated with a neurological disorder in the dog that we have discussed elsewhere under the designation neuronal glycoproteinosis. In the dog and cat, these complex glycoprotein bodies are more commonly encountered as an incidental observation with advancing age. They occur throughout the CNS, particularly in the thalamus, tectum of the midbrain, cerebellum and medulla, and the caudal lumbar, sacral, and caudal spinal cord segments. They are found within neuronal perikarya, while those within axons may appear to be free in the neuropil. Lafora bodies are ovoid in shape, basophilic, and strongly PAS-positive and bind the lectin concanavalin A. The feline examples may contain galactose, as they are also reactive with peanut agglutinin. Both canine and feline bodies are labeled by a monoclonal antibody to human polyglucosan. Lipofuscin storage, meningeal thickening as a result of fibrosis, meningeal calcification, vascular alterations (atherosclerotic vascular degeneration, mineralization) have been reported as a result of the aging process [9-11]. Additionally, the examination of the brain sections of aged dogs stained with Congo-red showed senile plaques and the presence of amyloid in the wall of the brain vessels [14] (fig. 1,2,3).

Age-related amyloidosis is a common disorder in aged humans. Amyloid deposition of several chemical types, can be found in different organs: for example in the brain: amyloid of beta protein type (Aβ); in the heart and aorta: amyloid of transthyretin type (ATTR); in the pancreas: amyloid of islet amyloid polypeptide type (AIAPP); etc. In the brain of dementing patients, Aβ is found in senile plaques (SP) and in blood vessels with cerebral amyloid angiopathy (CAA). The Aβ-protein is a 4kDa polypeptide, which is comprised of 39-44 amino-acids and is produced by proteolysis of the β precursor protein (AβPP). Deposition of the Aβ occurs in the extracellular compartment of the brain (in the plaques) and of arterial walls (CAA). Secondly, brains of aged dementing people contain neurofibrillary tangles (NFTs) which are prominent after repeated trauma, in patients with Alzheimer's disease (AD) and in aged persons with Down's syndrome. A third hallmark of the brain of dementing patients is loss of synaptic interneuronal connections.

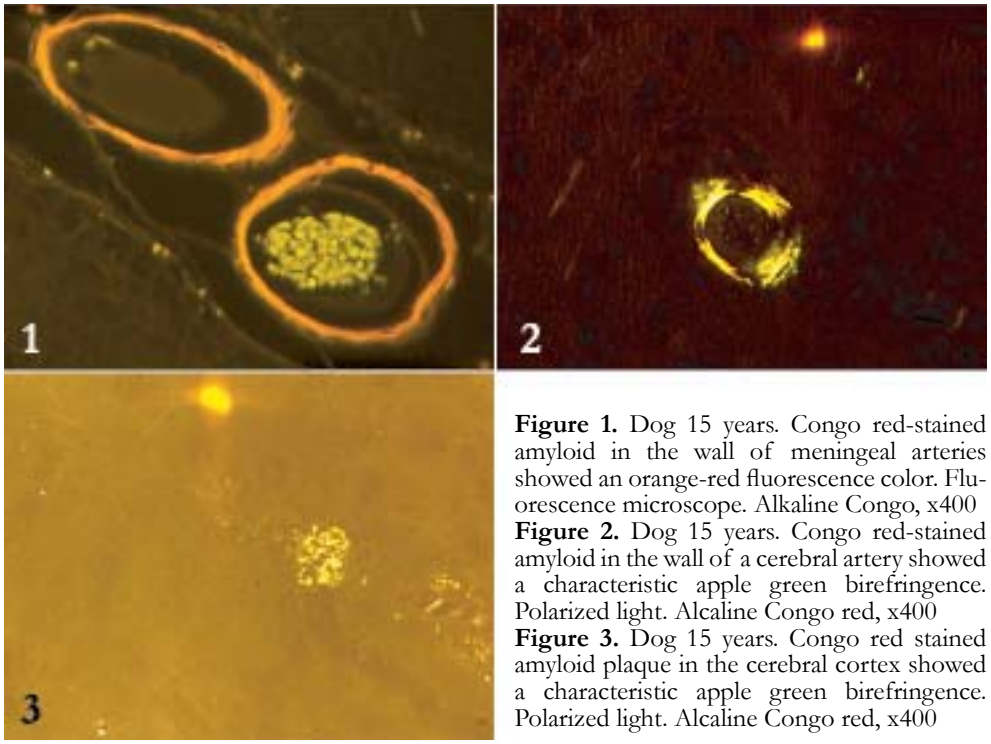

Figure 1. Dog 15 years. Congo red-stained amyloid in the wall of meningeal arteries showed an orange-red fluorescence color. Fluorescence microscope. Alkaline Congo, x400
Figure 2. Dog 15 years. Congo red-stained amyloid in the wall of a cerebral artery showed a characteristic apple green birefringence. Polarized light. Alcaline Congo red, x400
Figure 3. Dog 15 years. Congo red stained amyloid plaque in the cerebral cortex showed a characteristic apple green birefringence. Polarized light. Alcaline Congo red, x400

SPs and CAA have been detected in the brain of some other species, such as non-human primates, dogs, bears, cats and camels. To generate animal models of amyloidogenesis and Aβ-associated abnormalities, some research groups have created transgenic (TG) mice that express AβPP, AβPP fragments, or related proteins and which show Aβ deposits and neuritic plaques. However, there is no consensus about the similarity of these murine pathophysiological processes with significant features of AD. They are hampered by the lack of spontaneous development and variability as it occurs in man. Dogs, however, share the same environment and may provide a unique model for comparative studies on the early changes in their human counterparts. Up to now diffuse and primitive plaques are well known, whereas neuritic plaques rarely develop in the aged canine brain [14] (fig.4,5). CAA is also observed [12-15]. Recently, the occurrence of neurofibrillary tangles in the aged canine brain [14] has been reported (fig. 6). Furthermore, the presence of oxidative damage in association with plaque formation in the brain of aged dogs was also detected [14]. The presence of 4-hydroxynonenal (HNE) as a consequence of lipid peroxidation is well known in the human brain and visceral organs [17-20]. HNE is positive in the amyloid deposits and the antibody reacted with neurons and perivascular macrophages and microglial cells (fig. 7,8). It has been demonstrated that amyloid proteins (Aβ and Aβ2M) when formed, generate free radicals. According to them is suggested that free radical injury is involved in the pathogenesis of amyloid depositions in canine brain [14]. Aging dogs have been used to test a number of different therapeutics that have also been tested in human

clinical trials [21,22]. A diet rich in a broad spectrum of antioxidants and mitochondrial co-factors improved congition and reduced neuropathology in aging dogs over a 2.8 years period of time [23,24].

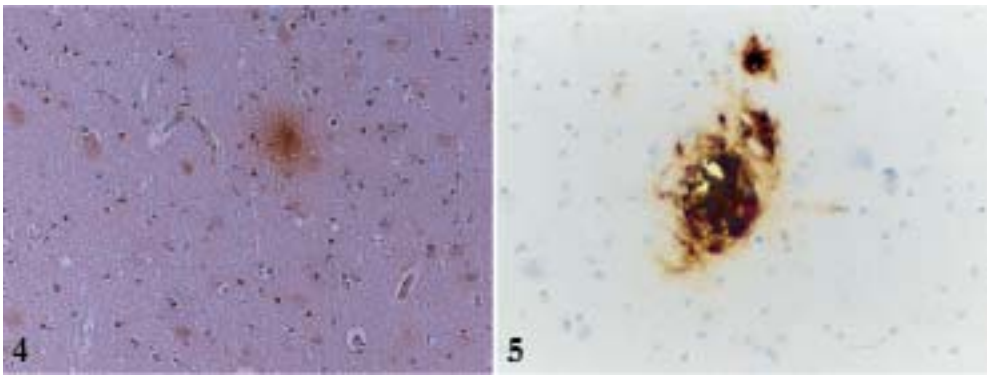

Figure 4. Dog 16 years. Aβ-positive antigenic material in the cortical white matter, frontal lobe. ABC method, anti-Aβ42 protein, x320; **Figure 5.** Dog 17 years. Typical amyloid plaque (mature) in the neuropil of cerebral cortex, parietal lobe. ABC method, anti-Aβ43 protein, x320

On the contrary, in the aged feline brain, two types of plaque are described: the first type represents Aβ antigenic material and the second type represents diffuse "preamyloid" plaques. The first type was characterized as non-well circumscribed Aβ positive antigenic material and was detected in all examined brains. Especially in the younger cats this type of Aβ deposits presented primarily in the deeper layers of the cortical gray matter, while the older ones presented this material all over the cortical gray matter layer. The Aβ positive antigenic material was distributed in the cellular layers of the frontal and parietal lobes around neurons and capillaries and was evident in all the samples of examined cats [16,21,25]. The second type was characterized by the diffuse accumulation of beta amyloid antigenic material without compact congophilic amyloid deposition. These plaques did not have obvious degenerative neurites and were not detectable either by HE or alkaline Congo-red staining. Morphologically, they were characterized by an immunoreactive core and less well outlined crown. They can be considered as diffuse plaques in comparison to the human senile plaque types. Diffuse plaques were evident only in the brain of the very aged cats (17-21 years old) and they were distributed throughout the cortical layers, especially the cellular layers of the parietal lobes (fig.9). It is interesting to note that a limited number of diffuse plaques were detected within the brain sections of a 12 year old Siamese cat. Some positive immune staining, representing both types of deposits, was seen dispersed in the cortical white matter of the very aged cats (17-21 years old). No reaction was detected in the sections of cerebellum, midbrain and pons. CAA was also observed [16,25]. Primitive plaques are not encountered, and neurofibrillary tangles are not detected. This finding is not in agreement with the findings of others [26], who found them only in one 20 year old cat. Typical primitive and neuritic plaques were not encountered in all published studies. This type of morphological pattern seen in the aged feline brain may indicate an

early stage of plaque formation. Since human diffuse plaques are known via primitive plaques finally to progress to neuritic plaques, the current findings in cat brains suggest that the presence of longer Aβ peptides is not the only prerequisite for seeding of amyloid *in vivo*. Therefore, the lack of feline primitive plaques must be related to other factors (i.e. apolopoprotein E, a_1-antichymotrypsin, metal ions etc).

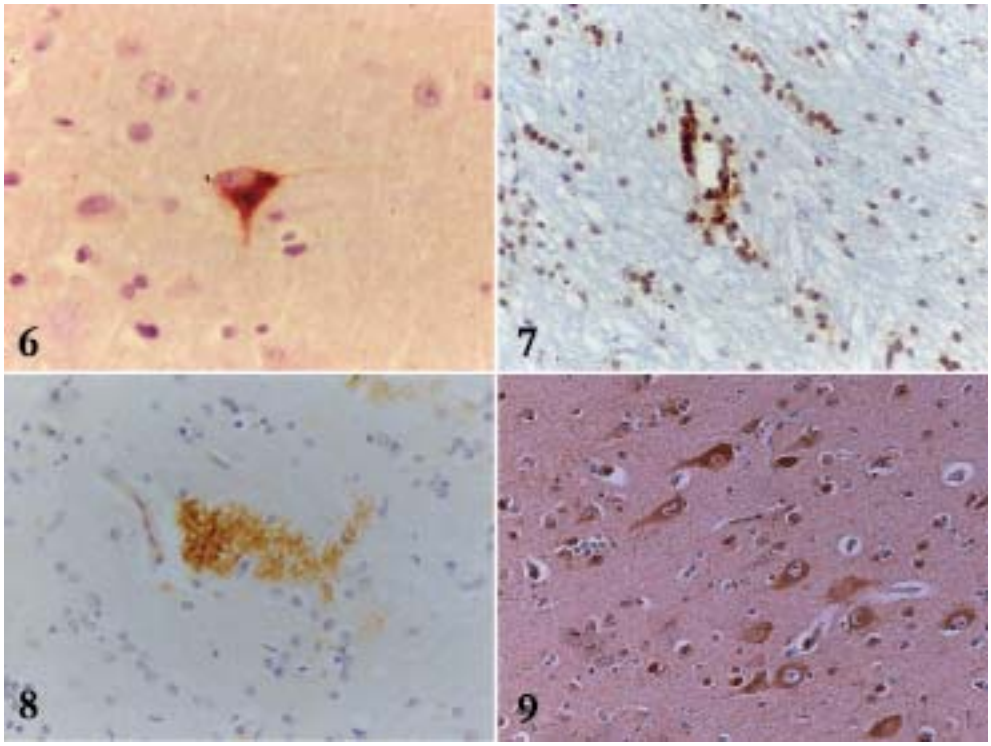

Figure 6. Dog 23 years. Immunohistochemical staining with mAb tau AT-8 in aged canine brain. Positive neurofibrillary tangle in the cerebral cortex. APAAP method, x400; **Figure 7.** Dog 15 years. HNE-staining of perivascular inflammatory cells in the brain parenchyma of the aged canine brain. ABC method, x320; **Figure 8.** Dog 23 years. HNE-staining of neuronal cells. ABC method, x320; **Figure 9.** Cat, 17 years old. Aβ-positive antigenic material in the cortical white matter, frontal lobe. ABC method, anti-Aβ42 protein, x320

Additionally, it has been suggested that Siamese cats are prone to develop AA amyloidosis. From the current results it appears that the Siamese cats were also more sensitive for developing Aβ-positive deposits than the Domestic Shorthair (DSH) cats [25]. It is interesting to note that diffuse plaques were presented in one 12-year old Siamese cat while the majority of the diffuse plaques were detected in over 17-years old cats [25]. The latter finding compared with the sensitivity to the susceptibility for AA amyloidosis supports the view that some general factors, especially genetic, concerning amyloid other than SAA or Aβ might occur in this breed of cat.

Summarized, in order to understand the disease mechanisms in the CNS the knowledge of the principles of neuropathology is still compulsory while the canine and feline

are a useful and complementary model system to transgenic mice to help develop therapeutics or approaches that may slow or halt AD in clinical trials.

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PROMENE NA MOZGU PASA I MAČAKA TOKOM STARENJA

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Kod ostarelih pasa i mačaka, posebno pasa, gubitak kognitivnih sposobnosti se prirodno razvija na različitim poljima, ali istovremeno ispoljava slično kao i kod ljudi sa individualnim razlikama u procesu starenja. U toku starenja kod pasa i mačaka promene u mozgu se razvijaju spontano. Kod pasa se javljaju neke morfološke promene, slične promenama kod ljudi koji su oboleli od Alchajmerove bolesti. Pored toga, ostareli pas predstavlja koristan model za razvoj preventivnih ili terapeutskih intervencija radi poboljšanja funkcije ostarelog mozga. Ovakve intervencije se mogu preneti na klinička ispitivanja na ljudima.