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Influence of age on the pathology of hippocampus and variation in the number of neurons of the hilar region in the brain of dogs#

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Abstract

Brain is a vital organ that always has high oxygen demands and is highly vulnerable to injury caused by reactive oxygen species (ROS). The hippocampus is a complex structure seen deep in the temporal lobe which is constituted by the dentate gyrus, hippocampus proper (Cornu Ammonis- CA1-CA4) and subiculum. The important functions of the hippocampus are learning and memory. Age-influenced pathological lesions in the hippocampus and variation in the number of neurons in the hilus are the main discussion of this study. The major gross lesions observed in the brain of both younger and older dogs were cerebral congestion, thickened meninges and cerebral edema. The major histopathological findings in the hippocampus were thickened blood vessels, accumulation of lipofuscin pigments in the neuronal cytoplasm, satellitosis, gliosis, , neurons with the vesicular nucleus, chromatolysis and neuronophagia. A statistically significant reduction in the number of neurons in the hilar region of the hippocampus was observed in aged dogs.

Keywords: *Dog, hippocampus, age, neuron loss, hilus*

Aging is an inevitable process that is characterised by various physical, biochemical, and molecular changes in the body. Better management practices, proper disease diagnosis and medication result in an increased life span of companion animals. The normal cognitive function in living beings is mainly controlled by the hippocampus and entorhinal cortex which are more susceptible to age (Tapp *et al.,* 2008). The important functions of the hilus of the hippocampus are visuospatial learning and memory (Cotman and Head, 2008). Simic *et al.* (2005) reported the age-associated loss of neurons in the hilus and subiculum of the hippocampus in humans. Head (2011)

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reported a significant reduction in the volume of the hippocampus due to its gradual atrophy with increased age. The present study was undertaken to describe the influence of age on the pathology of the hippocampus and variation in the number of neurons of the hilus.

Materials and methods

A total of 18 dog brain samples comprising of nine young (≤ 2 years) and nine aged (≥ 7 years) were obtained through the routine necropsies performed at the Department of Veterinary Pathology, Pookode during the period from January 2020 to September 2021. The clinical history and anamnesis were collected from the owners and confirmed that the animals are not having any systemic diseases. During necropsy, the gross lesions observed in the brain were documented and the brain samples were collected in 10 per cent neutral buffered formalin (NBF). The coronal sections of the hippocampus were fixed in 10 per cent neutral buffered formalin (NBF). Following the formalin fixation, samples were processed through ascending grades of alcohol (50 per cent to 100 per cent), cleared in xylene and then embedded in paraffin. Serial sections of the tissues were cut at six-micron thickness using a rotary microtome (Spencers, India) and stained with routine haematoxylin and eosin procedure (Suvarna *et al.,* 2013). The sections were examined under a light microscope (Zeiss) and the various histopathological lesions in the hippocampus were recorded. The number of neurons in the hilar region of the hippocampus was counted using ten high-power fields (400x). IBM SPSS statistics software, version 24.00 was used for the statistical analysis. An Independent t-test was done for the statistical analysis of variation in the number of neurons in the hilar region of the hippocampus related to age.

Results and discussion

The main clinical signs observed in the aged dogs (≥ 7 years) were reduced activity, difficulty in finding food dropped on the floor, urination or defecation in an already cleaned area and failure to recognise familiar people or animals. Among the younger animals (≤ 2 years), difficulty in finding dropped food on the floor and reduced activity were the reported clinical signs in two animals.

Gross lesions observed in the aged dog brain samples were thickened and cloudy meninges (33.33 %), engorged cerebral blood vessels with mild contraction of the gyri and widening of sulci (88.89 %) and cerebral edema (11.11 %). The two younger dogs showed engorged cerebral blood vessels (66.67 %) and thickened meninges (22.22 %). Maxie (2007) reported thickening of the meninges, cerebral oedema and engorgement of blood vessels in the brain of aged dogs. Borras *et al.* (1999) reported the gross lesions in aged dog brains as leptomeningeal thickening, gyrus contraction and widening of sulci.

Histopathological examination of the hippocampus in aged dogs revealed lipofuscin accumulation in the neuronal cytoplasm (Fig.1.A) (3/9), gliosis (6/9), neuronophagia (Fig.1.B) (4/9), satellitosis (Fig. 1.C) (5/9), vesicular nucleus (5/9), perivascular vacuolation (4/9), perineuronal vacuolation (3/9), chromatolysis (3/9), calcification (1/9), vasculitis (1/9) and pyknotic neuron (1/9). Histopathological analysis of hippocampus in younger dogs showed chromatolysis (Fig.1.D) (5/9), pyknotic nucleus (5/9), perivascular vacuolation (4/9), perineuronal vacuolation (4/9), satellitosis (4/9), gliosis (4/9), collapsed blood vessels (1/9), neuronophagia (1/9), condensation of the Nissl's granule towards the periphery (1/9) and vesicular nucleus (1/9).

Ozawa *et al.* (2016) found that increased gliosis was associated with neurodegenerative disease due to the imbalance between inflammatory and anti-inflammatory molecules. Borras *et al.* (1999) reported the presence of lipofuscin in the hippocampus of both aged and young dogs. They observed diffuse granular lipofuscin pigment in the older group and perinuclear accumulation in the younger dogs. A previous study by Gray and Woulfe (2005) found that lipofuscin will damage the proper functioning of cellular systems.

The dentate gyrus, hippocampus proper (CA-1 to CA-4) and subiculum are the important regions of the hippocampus (Vullo *et al.,* 1996).

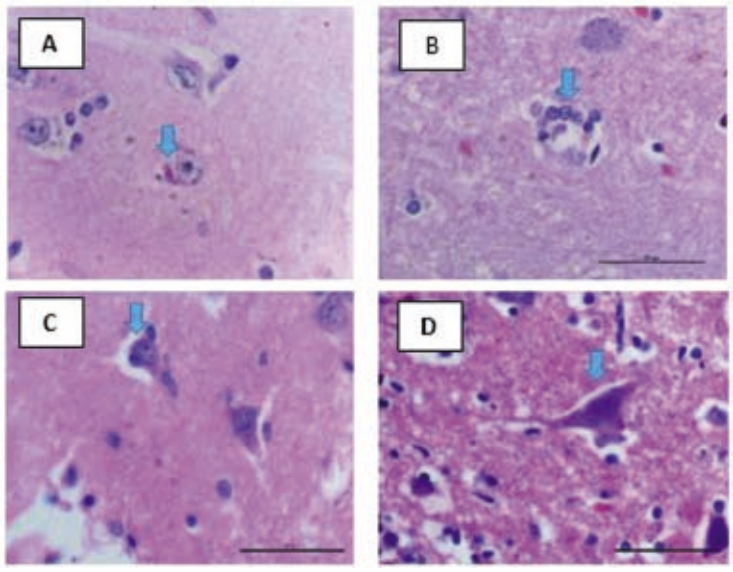

Figure 1. Histopathology of dog Hippocampus: A. Perinuclear lipofuscin accumulation in 8 years old dog (Case No. 287/21). B. Neuronophagia in 7 years old dog (Case No. 84/20). C. Satellitosis in 11 years old dog (Case No. 57//20). D. Chromatolysis of neuron in 4.5 months old dog (Case No. 59/21). (A. 400X., B, C & D. 1000X. H&E).

A study by Silva *et al. (*2006) reported that the damage to the neurons in the hilus of the hippocampus resulted in loss of memory and learning functions of the brain. It was based on cell width and cell density, the CA region was divided into four parts as CA-1 to CA-4 (Ragbetli *et al.,* 2010). Small pyramidal cells were observed in CA-1 region. A slender dense band of neurons in CA-2 and CA-3 consisted of broad large pyramidal cells with less density. The CA-4 or the hilar region is represented by the scattered arrangement of pyramidal cells which were enclosed by densely packed small granular type cells called the dentate gyrus (Fig. 2).

The average number of neurons counted from the hilar region of the hippocampus using ten high power fields (400x) are represented in Table 1. Statistical analysis showed a mean value of the number of neurons in the hilus as 73.11±18.90 and 42.44±21.72 in the younger and aged dogs respectively. Analysis with an independent t-test described a statistically significant difference (P=0.006) in the mean value of the number of neurons in the hilar region of the hippocampus in aged and younger dogs.

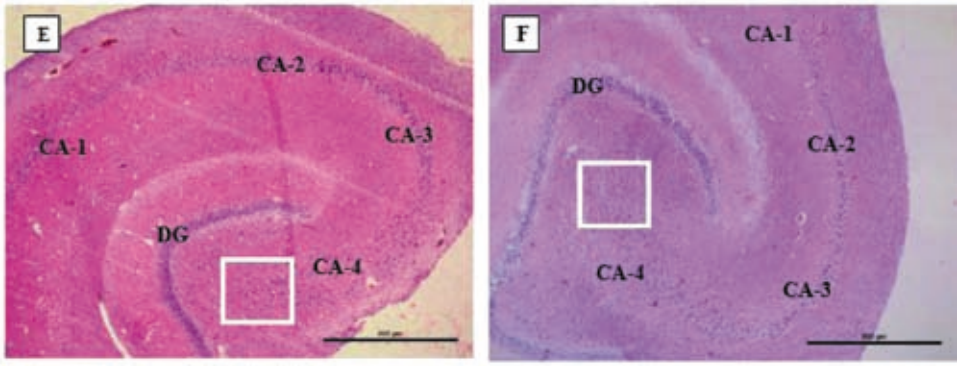

Figure 2. Hippocampus of a younger and older dog: E. Densely packed neurons, 4.5 months old dog, (Case No. 59/21). F. Reduced number of neurons, 11 years old dog, (Case No. 57/21**).** DG- Dentate Gyrus, CA- Cornu Ammonis, highlighted square- Hilus, where the number of neurons were counted. E & F . 100X.

Table 1. Average number of neurons in the hilar region of hippocampus

Sl. No.	Case No.	Age	Gender	Breed	Size	Average number of neurons in the hilus of the hippocampus
1	84/20	7 y	Male	Rottweiler	Large	13
2	11/21	7 y	Male	Crossbreed	Medium	61
3	12/21	7y	Male	Crossbreed	Medium	66
4	48/21	7y	Male	Crossbreed	Medium	56
5	57/21	11y	Female	Crossbreed	Medium	36
6	96/21	7y	Female	Spitz	Small	27
7	188/21	7y	Male	Labrador	Large	58
8	218/21	10y	Male	Nondescript	Small	56
9	287/21	8y	Female	Lhasa Apso	Small	9
10	59/21	4.5m	Male	Bully Kutta	Large	80
11	69/21	2y	Female	Spitz	Small	52
12	105/21	9m	Female	Spitz	Small	78
13	93/21	42 d	Male	Crossbreed	Small	63
14	140/21	2y	Male	Nondescript	Small	53
15	193/21	35 d	Male	Labrador	Small	107
16	207/21	1.8y	Male	Dobermann	Large	83
17	260/21	1.5y	Male	Nondescript	Small	54
18	267/21	3m	Female	Nondescript	Small	88

There was significant reduction in the number of neurons in the hippocampus in the aged animals (Fig. 3). The significant decrease in the number of neurons in the hilar region is due to increased neuronophagia, oxidative damage caused by free radicals and depletion in the neurotransmitter producing neurons like gamma amino butyric acid (GABA). Head (2011) stated that the free radicals formed in older age caused oxidative damage to lipids, nucleotides, and proteins. This can lead to the loss of function of neurons followed by neuronal demise which may be manifested as altered cognitive status. Pugliese *et al.* (2007) and

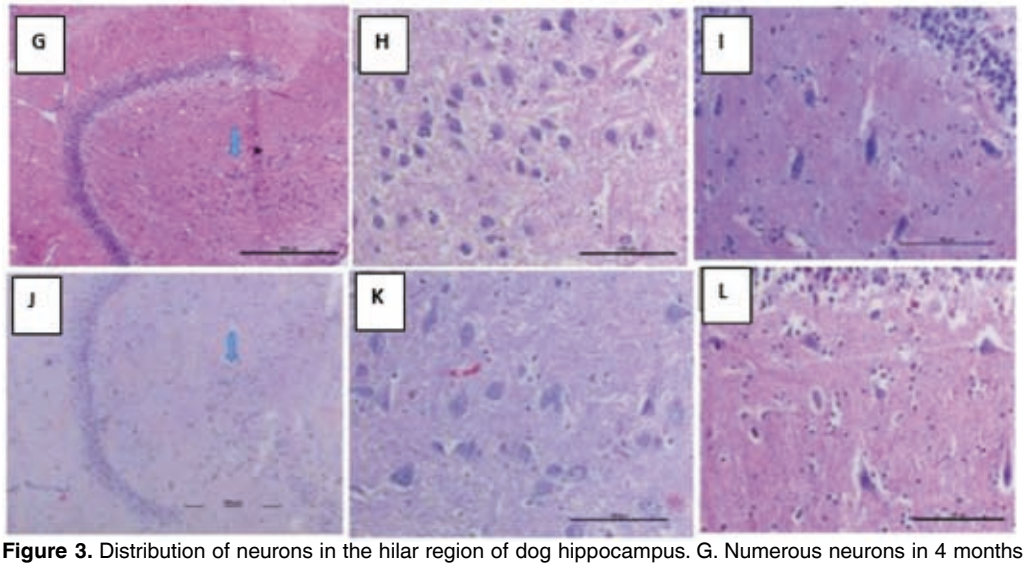

Figure 3. Distribution of neurons in the hilar region of dog hippocampus. G. Numerous neurons in 4 months old (Case No. 59/21). H. More neurons in 1 year old (Case No. 287/21. I. Pyknotic neurons in 2 years old (Case No. 140/21). J. Reduction in the number of neurons in 8 years old (Case No. 287/21). K. Lesser neurons in 10 years old (Case No. 218/21). L. Marked reduction in neurons in 7 years old (Case No. 11/21). (G & J :100X, H, I, K&L: 400X).

Tapp *et al.* (2008) reported loss of neurons in the hilus of the hippocampus in an aged dog model for Alzheimer's disease development.

Conclusion

Eighteen dog brain samples (9 younger and 9 aged) were examined in the present study. Engorgement of the cerebral blood vessels, thickened meninges, and cerebral edema were the common gross lesions observed in the brain samples. Chromatolysis, satellitosis, neuronophagia, gliosis and accumulation of lipofuscin pigments in neuronal cytoplasm were the common histopathological observations in the hippocampus of the aged dog brain samples. Analysis of the number of neurons in the hilar region of the hippocampus revealed that there was a statistically significant reduction in the number of neurons in the hilus of the hippocampus as age advances. Hence, the current study emphasises the influence of age on the reduction of the number of neurons in the hilus of hippocampus.

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Conflict of interest

The authors declare that they have no conflict of interest

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