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Studies of Histology, Histochemistry and Micrometry of Hippocampus in Surti Buffalo (*Bubalus bubalis*)

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A B S T R A C T

The brain of six adult surti buffaloes was collected for the study of hippocampus. Harris' Haematoxylin and Eosin (H & E) Staining method was used for general microscopic structure of the hippocampus. Aldehyde-Thionin-PAS method was utilized for Nissl's substance and mucopolysaccharides. Vogt's method was used for nerve cells, Sevier-Munger method for neural tissues and the micrometry of hippocampus was performed by various layers of cornu ammonis and dentate gyrus. The mean value of thickness of different layers of cornu ammonis was measure by micrometry. Microscopic study revealed thatthe hippocampus was consist of six layers, viz.; alveus, stratum oriens, stratum pyramidale, stratum radiatum, stratum lacunosum and stratum moleculare. The hippocampus commissure and the dentate gyrus showed PAS positive reaction. Furthermore, these structures also showed a positive reaction with silver nitrate staining (Sevier-Munger staining). The present study revealed that almost all the fibres were myelinated fibers.

Introduction

The buffalo is known as 'black gold' of Indian subcontinent due to its colour and contribution to the economy through milk, meat, hide and bones. The buffalo alone contributes about 54 per cent of the total milk produced in India and help to maintain number one country in milk production (163.5 million tons) in world (DAHD report, 2017-18). Brain exerts centralized control over the body through its components. Hippocampus is a small organ located in temporal lobe which forms the important part of limbic system, supposed to regulate emotions. The hippocampus is very close to olfactory structure. Two grooves in brain, the rhinal sulcus and hippocampal fissure are key anatomical land mark. The name hippocampus arises from the resemblance of its cell laminae to the outline of the seahorse. The neuronal networks are plastic with properties of undergoing consistent changes. It is composed of different type of cortex, having only three neuronal cell layers instead of the six layers found in the cerebrum (Kumaravel *et al.*, 2015).

Various buffalo breeds have been the subject of a wide variety of scientific research, however, there are limited reports on studies

pertaining to anatomical features of the nervous system in general and the hippocampus in particular.

Materials and Methods

The fresh whole brain (without duramater), immediately after slaughter of six adult Surti buffalo, were procured aseptically from the local abattoirs of Anand town, Gujarat. The samples were preserved and fixed in the 10% neutral buffer formalin. Each brain was carefully dissected to expose the hippocampus in the laboratory, Department of Veterinary Anatomy and Histology, College of Veterinary Science & AH, AAU. The samples of hippocampus were used for the histology, histochemical and micrometrical studies. The samples were processed by paraffin embedded tissue sectioning method. Tissue sections of 4-6 µm thickness were obtained by a rotary microtome. The sections were then stained by following staining methods. Micrometry was performed under calibrated microscope.

Harris' Haematoxylin and Eosin (H & E) Staining method was used for general microscopic structure of the hippocampus (Luna, 1968). Aldehyde-Thionin-PAS method was utilized for Nissl's substance and mucopolysaccharides (Luna, 1968). Vogt's method was used for nerve cells (Luna, 1968) and Sevier-Munger method for neural tissues (Luna, 1968).

The micrometry of the hippocampus was performed by various layers of cornu ammonis and dentate gyrus. The different layers of the cornu ammonis (alveus, stratum oriens, stratum pyramidale, stratum radiatum, stratum lacunosum, stratum moleculare) and the dentate gyrus (stratum moleculare, stratum granulosum, polymorphic layer) were measured by micrometrical examination. The biometrical data obtained were analyzed statistically (Snedchor and Cochran, 1994).

Results and Discussion

The hippocampi consist of six layers, viz.; alveus, stratum oriens, stratum pyramidale, stratum radiatum, stratum lacunosum and stratum moleculare (Fig. 1). The stratum pyramidale formed the principal cellular component of the hippocampus proper (Fig. 2). The Hippocampus Commissure showing presence of blood vein, RBC, basket cells and nerve fiber in H&E stain (Fig. 3). The alveus faced the lateral ventricle. The cortical band of the hippocampus proper (Cornu of Ammonis) was divided into four fields according to its width, cell size, and cell density. The four fields of Cornu Ammonis (CA) were called as CA1, CA2, CA3 and CA4 (Fig. 6). The cortical band of the hippocampus proper (cornu of ammonis) was divided according to its width, cell size and cell density and CA1 contained small pyramidal cells (Fig. 7).

Field CA2 had a narrow, dense band of large pyramidal cells and field CA3 by a broad, loose band of large pyramidal neurons (Fig. 6). CA4 formed the loosely structured end field and was enclosed by the narrow, dark band of cells of the dentate gyrus (Fig. 2). The dentate gyrus was composed of three layers and was embedded into the hippocampus. The layers of dentate gyrus were moleculare layer, granular cells layer and polymorphic cells layer. The molecular layer of dentate gyrus was in contact with the moleculare layer of the hippocampus (Fig. 2 and 6).

The hippocampus, hippocampus commissure and the dentate gyrus showed PAS positive reaction (Fig. 4). Furthermore, these structures also showed a positive reaction with silver nitrate staining (Sevier-Munger staining). The study revealed that almost all of the fibres were myelinated in nature (Fig. 4 and 8). The alveus faced the lateral ventricle. The stratum pyramidale formed the principal cellular component of the hippocampus proper (Table 1).

Table.1 The micrometrical parameters of the hippocampus of the Surti buffalo (N=6)

Sr. No.	Measurements	Name of Stratum (Layer)		Mean ± SE (µm)
1	Thickness of different layers of Cornu Ammonis (µm)	Alveus		42.87±5.22
		Stratum oriens		392.98±72.84
		Stratum pyramidale		462.04±48.62
		Stratum radiatum		242.93±40.42
		Stratum lacunosum		233.40±38.64
		Stratum moleculare		257.22±32.17
2	Thickness of different layers of Dentate Gyrus (µm)	Stratum moleculare		347.72±21.41
		Stratum granulosum		73.83±8.59
		Polymorphic cells layer		245.31±43.57
3	Thickness of different fields of Stratum Pyramidale (µm)	CA1		338.21±21.13
		CA2		191.16±22.46
		CA3		245.08±14.54
		CA4		2112.61±170.71
4	Number of Neuron present in the different fields of stratum pyramidale (No./ mm^2)	CA1		33.50±0.76
		CA2		22.50±1.02
		CA3		16.66±1.11
		CA4		17.33±0.33
5	Length and width of neuron present In the different fields of stratum pyramidale (µm)	Length	CA1	14.43±0.70
			CA2	18.31±1.66
			CA3	22.2±1.11
			CA4	18.87±1.40
		Width	CA1	11.1±0.70
			CA2	12.21±0.70
			CA3	13.32±0.85
			CA4	18.87±2.22
6	Area of neuron present in the different fields of stratum pyramidale (μm^2)	CA1		124.76±6.61
		CA2		176.99±21.28
		CA3		230.67±14.87
		CA4		269.84±21.44

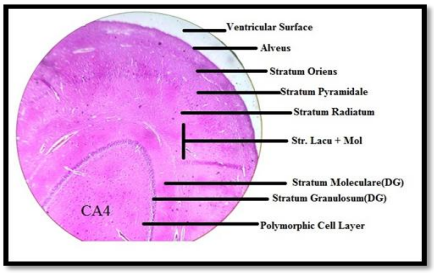

Figure-1: Photomicrograph of Section of Hippocampus and Dentate Gyrusshowing different layers, CA4 = CornuAmmonis field 4, Str. Lac + Mol = stratum lacunosum& stratum molecure, H & E staining, 4x.

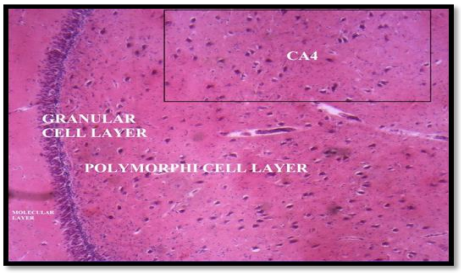

Figure 2: Photomicrograph of Section of Dentate Gyrusshowing different layers and CA4 (cornuammonis of Hippocampus Field 4), H & E staining, 40x.

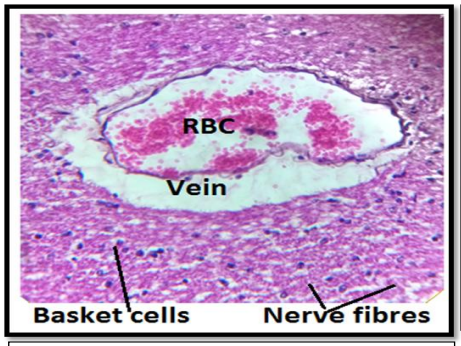

Figure-3: Photomicrograph of Section of Hippocampus Commissure, H & E staining, 40x.

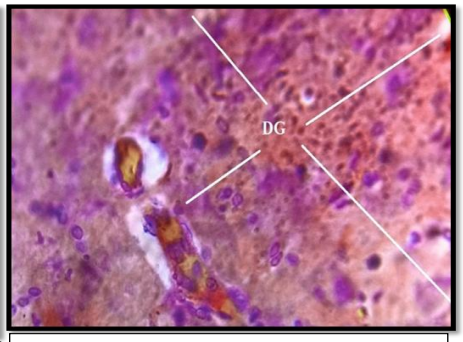

Figure-4: Photomicrograph showing Section of dentate gyrus PAS positive reaction in the myelinated nerve fibers, PAS Staining, 40X.

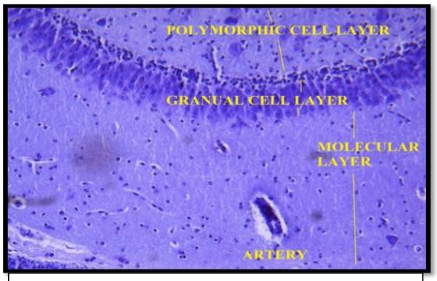

Figure-5: Photomicrograph showing Section of Dentate Gyrus: Molecular Layers, Granular Layers, Polymorphic Cell Layers, Artery VOGT'S staining, 40X.

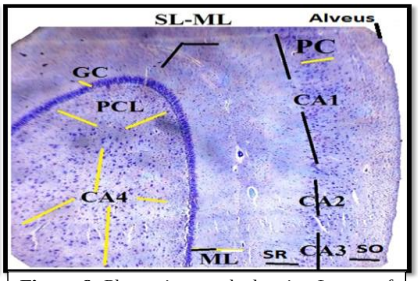

Figure-6: Photomicrograph showing Layers of Hippocampus and Dentate Gyrus (DG).SO= Stratum Oriens,PC = Pyramidal cells, SR = S. Radiatum, SL-ML = S. Lacunosum and Moleculare layer, ML = Moleculare layer of DG, GC = Granular cells; PCL = Polymorphic layerVOGT'S Staining, 4X.

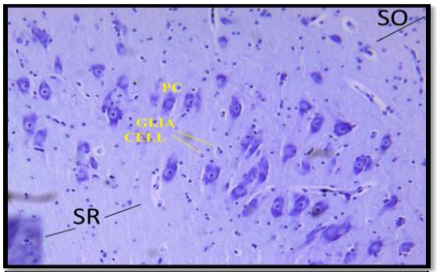

Figure-7: Photomicrograph section of Hippocampus showing layers: SO= Stratum Oriens, PC=Pyramidale cell, SR= Stratum Radiatum. VOGT's Staining, 40X.

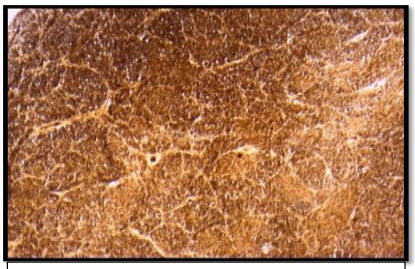

Figure-8: Photomicrograph showing Section of Hippocampus:Myelinated nerve fibers impregnated with Silver. Sevier - Munger Staining, 40X.

The cortical band of the hippocampus proper (Cornu of Ammonis) was divided into four fields according to its width, cell size and cell density. The dentate gyrus was composed of three layers and was embedded into the hippocampus. The layers of dentate gyrus include moleculare layer, granular cells layer and polymorphic cells layer. The molecular layer of dentate gyrus was in contact with the moleculare layer of the hippocampus.

The histological and histochemical study of the hippocampus were almost similar to that of Hart (1998); Stephan and Manolescu (1980) in the primates; Singh *et al.,* (2013); Kumaravel *et al.,* (2015) in buffalo; Cappaert *et al.,* (2015) in human beings;

However, the present findings of histo-morphologically six layered hippocampus was in contrast to the reports of Rao (1991) who worked on the ovine hippocampus and reported that the ovine hippocampus had four distinct layers i.e., the stratum alveus, the polymorphic layer, the pyramidal cell layer and the molecular layer.

The mean value of thickness of different layers of cornu ammonis i.e. alveus, stratum oriens, stratum pyramidale, stratum radiatum, stratum lacunosum and stratum moleculare was 42.87 ± 5.22 µm, 392.98 ± 72.84 µm, 462.04 ± 48.62 µm, 242.93 ± 40.42 µm, 233.40 ± 38.64 µm and 257.22 ± 32.17 µm, respectively. The thickness of alveus observed in the present study was lower than that of the rat measured by Hussein and George, 2009 (75- 200 µm). The thickness of stratum oriens, stratum pyramidale, stratum radiatum, stratum lacunosum and stratum moleculare was higher than the thickness of stratum oriens (75- 100 µm), stratum pyramidale (50 µm), stratum radiatum (175-225 µm), stratum lacunosum and stratum moleculare (50- 75 µm) as reported by Hussein and George (2009).

In the present study, the mean value of thickness different layers of Dentate Gyrus (DG) i.e. stratum moleculare, stratum granulosum, and stratum polymorphic layer was 347.72 ± 21.41 µm, 73.83 ± 8.59 µm, and 245.31 ± 43.57 µm, respectively. The measurement of thickness of stratum moleculare and stratum granulosum of DG in the present study was higher (250 µm and 60 µm, respectively) than thickness of Stratum moleculare and Stratum Granulosum of rat measured by David and Pierre (2009) and Hussein and George (2009), (50-75µm and 125-150 µm, respectively). In the current study, the mean values of thickness of the stratum pyramidale in the different field of CA1, CA2, CA3 and CA4 was 338.21±21.13 µm, 191.16 ± 22.46 µm, 245.08 ± 14.54 µm and 2112.61 ± 170.71 µm, respectively. In this study, the mean value of number of neuron in the different fields of stratum pyramidale per mm^2 i.e. CA1, CA2, CA3 and CA4 was 33.50 ± 0.76, 22.50 ± 1.02, 16.66 ± 1.11 and 17.33 ± 0.33, respectively.

In the present study, the mean value of length of neurons observed in the stratum pyramidale of different field of CA1, CA2, CA3 and CA4 was 14.43 ± 0.70 µm, 18.31 ± 1.66µm, 22.2 ± 1.11 µm and 18.87 ± 1.40, respectively. Furthermore, the mean value of width of neurons found in the stratum pyramidale of different field of CA1, CA2, CA3 and CA4 was 11.1 ± 0.70µm, 12.21 ± 0.70 µm, and 13.32 ± 0.85µm and 18.87 ± 2.22 µm, respectively. The length and width of the CA1 and CA2 found in the present study were lower than length and width of the CA1 (19.2 ± 1.59 µm and 12.9 ± 0.51 µm) and CA2 (24.3 ± 0.74 µm and 15.2 ± 0.57 µm) of arctic fox measured by Sierakowska *et al.,* (2015). The length and width of the CA3 and CA4 in the present study were higher than length and width of the CA3 (18.6 ± 1.3 µm and 12.2 ± 1.2 µm) and CA4 (17.2 ± 0.76 µm and 10.4 ± 1.1 µm) of arctic fox measured

by Sierakowska *et al.,* (2015). The length of the neuron of CA3 in the present study was the highest and neuron of the CA1 was the shortest. The neuron of the CA4 had the highest length and width of neuron present in CA1 had the least width. In the present study, the mean value of area of neuron located in the different fields of stratum pyramidale i.e. CA1, CA2, CA3 and CA4 was 124.76 ± 6.61 μm^2, 176.99 ± 21.28 μm^2, 230.67 ± 14.87 μm^2 and 269.84 ± 21.44 μm^2, respectively. Furthermore, it was found that the area of neuron increased from CA1 to CA4, which was in contrast with the result of area of neuron of arctic fox as measured by Sierakowska *et al.,* (2015) who reported that the area of neuron in CA1 (153.8 μm^2) was higher than CA2 (125.1 μm^2), CA3 (108.1 μm^2) and CA4 (136.9 μm^2).The mean value of thickness of different layers of cornu ammonis was measure by micrometry. i.e. alveus, stratum oriens, stratum pyramidale, stratum radiatum, stratum lacunosum and stratum moleculare was 42.87 ± 5.22 µm, 392.98 ± 72.84 µm, 462.04 ± 48.62 µm, 242.93 ± 40.42 µm, 233.40 ± 38.64 µm and 257.22 ± 32.17 µm, respectively. The mean value of thickness of different layers of Dentate Gyrus (DG) i.e. stratum moleculare, stratum granulosum, and stratum polymorphic layer was 347.72 ± 21.41 µm, 73.83 ± 8.59 µm, and 245.31 ± 43.57 µm, respectively.

The study of histology and histochemistry which is very useful to understand the microscopic structure, composition, myelinated nerve fibers and various layers of cornu ammonis and dentate gyrus of hippocampus. Hippocampus is considered as an important component of the limbic system. The limbic system is responsible for emotional behavior of an animal. The present study will help in better understanding of the process and physiology of behaviors especially lactation and also the emotional behavior of animals through a better understanding of the anatomy of hippocampus by using micrometrical studies.

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