RESEARCH ARTICLE

Studies on Histology, Histochemistry and Micrometry of Hippocampus of Goat (*Capra hircus*)

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Abstract

The brain of six adult goats 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 for various layers of cornu ammonis and dentate gyrus. The mean values of thickness of different layers of cornu ammonis were measured by micrometry. Microscopic study revealed that the hippocampus was consisting 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 study revealed that almost all the fibres of hippocampus were myelinated.

Key Words: Goat, Hippocampus, Histochemistry, Histology, Micrometry.

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Introduction

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 marks. 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 (Kumarvel *et al.,* 2014). Goats 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. Hence, this study was aimed to know the histology, histochemistry and micrometry of hippocampus of goat.

Materials and Methods

The fresh whole brain (without duramater), immediately after slaughter of six adult goats, were procured aseptically from the local abattoirs of Anand town, Gujarat. The samples were preserved and fixed in 10% neutral buffer formalin. Each brain was carefully dissected to expose the hippocampus in the laboratory of Department of Veterinary Anatomy and Histology, College of Veterinary Science & AH, AAU, Anand. The samples of hippocampus were used for the histology, histochemical and micrometrical studies. The samples were processed by paraffin embedded tissue sectioning method.

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Tissue sections of 4-6 µm thickness were obtained by a rotary microtome. The sections were then stained by following different 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; Aldehyde-Thionin-PAS method for Nissl's substance and mucopolysaccharides; Vogt's method for nerve cells, and Sevier-Munger method for neural tissues (Luna, 1968). Morphological characteristics of brain (cerebral cortex) were evaluated by gross observations and analyzing cellular details, tissue architecture and overall staining characteristics as per Rubayat *et al*. (2022).

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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 (Snedecor and Cochran, 1994).

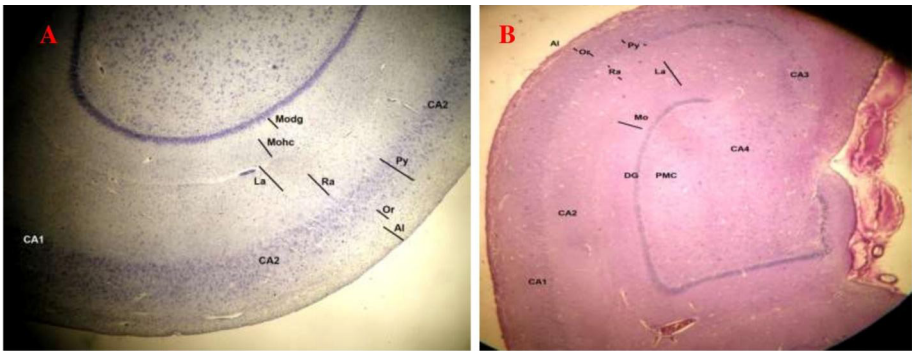
Results and Discussion

The goat's hippocampi consisted of six layers, *viz*., alveus, stratum oriens, stratum pyramidale, stratum radiatum, stratum lacunosum and stratum moleculare (Fig. 1A). The stratum pyramidale formed the principal cellular component of the hippocampus proper (Fig. 1B). The hippocampus commissure showed presence of blood veins, RBCs, basket cells and nerve fibers in H&E staining (Fig. 1B). 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.

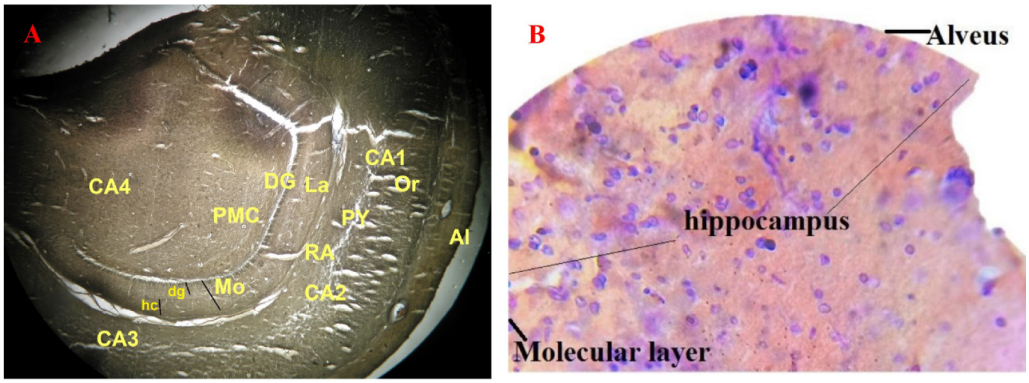
The CA1 contained small pyramidal cells. Field CA2 had a narrow, dense band of large pyramidal cells and field CA3 had a broad, loose band of large pyramidal neurons. CA4 formed the loosely structured end field and was enclosed by the narrow, dark band of cells of the dentate gyrus (Fig. 1B). The dentate 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 moleculare layer of dentate gyrus was in contact with the moleculare layer of the hippocampus (Fig. 1B).

The hippocampus, hippocampus commissure and the dentate gyrus showed PAS positive reaction (Fig. 2A). 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. 2B). The stratum pyramidale formed the principal cellular component of the hippocampus proper.

The histological and histochemical findings of the hippocampus were almost similar to those of Stephan and Manolescu (1980) in the primates; Singh *et al.* (2013),



Figs 1A & B: A: Hippocampus: Photomicrograph showing layers of hippocampus and dentate gyrus. Al = Alveus, Or = Oriens, Py = Pyramidal, Ra = Radiatum, La = Lacunosum, Mohc = Moleculare layer of hippocampus, Modg = Moleculare layer of dentate gyrus, CA1 = Cornu Ammonis 1, CA2 = Cornu Ammonis 2, VOGT's Staining, 4x.; **Fig. 1B:** Dentate Gyrus: Photomicrograph of section of hippocampus and dentate gyrus showing different layers and Cornu Ammonis field. Al = Alveus, Or = Oriens, Py = Pyramidal, Ra = Radiatum, La = Lacunosum, Mo = Moleculare, DG = Dentate Gyrus, PMC = Polymorphic cells, CA1 = Cornu Ammonis 1, CA2 = Cornu Ammonis 2, CA3 = Cornu Ammonis 3, CA4 = Cornu Ammonis 4, H&E Staining, 4X.



Figs 2A & B: A: Hippocampus: Photomicrograph showing layers of hippocampus and dentate gyrus. Al = Alveus, Or = Oriens, PY = Pyramidal, RA = Radiatum, La = Lacunosum, Mo=Molecular layer, Mohc = Moleculare layer of hippocampus, Modg = Moleculare layer of dentate gyrus, CA1 = Cornu Ammonis 1, CA2 = Cornu Ammonis 2, CA3 = Cornu Ammonis 3, CA4 = Cornu Ammonis 4, PAS (Periodic Acid Schiff) Staining, 4x.; **B:** Dentate Gyrus: Photomicrograph of section of hippocampus and dentate gyrus. Sevier-Munger, Staining, 4X.

Kumarvel *et al.* (2014) and Gori *et al.* (2018) in buffaloes; and Cappaert *et al.* (2015) in human beings. However, the present findings of histo-morphologically six layered hippocampus of buffalo was in contrast to the report 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 findings on micrometry of the hippocampus performed by various layers of cornu ammonis and dentate gyrus are presented in Table 1.

The mean values of thickness of different layers of cornu ammonis, *i.e.* alveus, stratum oriens, stratum pyramidale, stratum radiatum, stratum lacunosum and stratum moleculare were 41.37 ±3.11 µm, 387.62±25.12 µm, 464.32±37.32 µm, 247.84±44.33 µm, 237.32±14.17 µm and 249.46±17.46 µm, respectively. The thickness of alveus observed in the present study was lower than that of the rat (75-100 µm, Hussein and George, 2009), however, the thickness of stratum radiatum (175-225 µm), stratum pyramidale (50 µm), stratum lacunosum and stratum moleculare (50-75 µm) in rats (Hussein and George, 2009), were quite lower than our findings in goat.

In the present study, the mean values of thickness of different layers of dentate gyrus (DG), *i.e.* stratum moleculare, stratum granulosum, and stratum polymorphic layer were 352.72±21.43 µm, 68.73±8.67 µm, and 247.33±45.37 µm, respectively. The thickness of stratum moleculare and stratum granulosum of DG in the present study was higher than thickness of stratum moleculare and stratum granulosum of rat (50-75 µm and 125-150 µm) measured by David and Pierre (2009) and Hussein and George (2009), respectively.

Table 1: The micrometrical parameters of the hippocampus of the goat (N=6)

Measurements		Name of Stratum (Layer)		Mean ± SE (µm)
1.	Thickness of different layers of cornu ammonis (µm)	Alveus		41.37±3.11
		Stratum oriens		387.62±25.12
		Stratum pyramidale		464.32±37.32
		Stratum radiatum		247.84±44.33
		Stratum lacunosum		237.32±14.17
		Stratum moleculare		249.46±17.64
2.	Thickness of different layers of dentate gyrus (µm)	Stratum moleculare		352.72±21.43
		Stratum granulosum		68.73±8.67
		Polymorphic cells layer		247.33±45.37
3.	Thickness of different fields of stratum pyramidale (µm)	CA1		345.43±24.36
		CA2		189.75±37.62
		CA3		242.18±44.39
		CA4		209.13±27.12
4.	Number of neuron present in the different fields of stratum pyramidale (No./ mm^2)	CA1		36.53±1.76
		CA2		25.58±2.37
		CA3		14.66±3.31
		CA4		18.72±4.32
5.	Length and width of neuron present In the different fields of stratum pyramidale (µm)	Length	CA1	15.76±1.63
			CA2	17.73±1.65
			CA3	24.45±1.31
			CA4	16.61±1.71
		Width	CA1	10.61±1.26
			CA2	14.72±3.94
			CA3	12.46±2.79
			CA4	20.44±1.31
6.	Area of neuron present in the different fields of stratum pyramidale (μm^2)	CA1		131.26±4.2
		CA2		204.87±3.3
		CA3		239.14±1.4
		CA4		266.83±3.2

In the current study, the mean values of thickness of the stratum pyramidale in the different field of CA1, CA2, CA3 and CA4 were 345.43±24.36 µm, 189.75±37.62 µm, 242.18±44.39 µm and 209.13±27.12 µm, respectively. Further, the mean values of number of neuron in the different fields of stratum pyramidale per mm^2 *i.e*. CA1, CA2, CA3 and CA4 were 36.53±1.76, 25.58±2.37, 14.66±3.31 and 18.72±4.32, respectively (Table 1).

In the present study, the mean values of length of neurons observed in the stratum pyramidale of different field of CA1, CA2, CA3 and CA4 were 15.76±1.63 µm, 17.73±1.65 µm, 24.45±1.31 µm and 16.61±1.71 µm, respectively. Furthermore, the mean values of width of neurons found in the stratum pyramidale of different field of CA1, CA2, CA3 and CA4 were 10.61±1.26 µm, 14.72±3.394 µm, and 12.46±2.79 µm and 20.44±1.31 µm, respectively (Table 1). These findings are in agreement with those of Gori *et al*. (2018), who recorded the mean values of length of neurons in the stratum pyramidale of different fields of CA1, CA2, CA3 and CA4 in buffaloes as 14.43±0.70 µm, 18.33±1.66 µm, 22.2±1.11 µm and 18.87±1.40 µm, respectively, and the mean values of width of neurons as 11.10±0.70 µm, 12.21±0.70 µm, 13.32±0.85 µm and 18.87±2.22 µm, respectively. Further, 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), while the length and width of the CA3 and CA4 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 the neuron present in CA1 had the least width. The mean values of area of neuron located in the different fields of stratum pyramidale, *i.e.,* CA1, CA2, CA3 and CA4 were 131.26±4.2 μm^2, 204.87±3.3 μm^2, 239.14±1.4μm^2 and 266.83±3.3 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 arctic fox measured by Sierakowska *et al*. (2015), who reported higher area of neuron in CA1 (153.8 μm^2) than CA2 (125.1 μm^2), CA3 (108.1 μm^2) and CA4 (136.9 μm^2).

Conclusion

The study of histology and histochemistry 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 behaviour of an animal. The present study will help in better understanding of the process and physiology of behaviours especially lactation and also the emotional behaviour of animals through a better understanding of the anatomy of hippocampus by using micrometrical studies.

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