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NM Rajashailesha
Assistant Professor, Dept of Veterinary Anatomy, Veterinary College, Hassan, Karnataka, India

RV Prasad
Rtd Professor and Head, Dept of Veterinary Anatomy, Veterinary College, Bangalore, Karnataka, India

NS Sunil Kumar
Assistant Professor, Dept of Veterinary Anatomy, Veterinary College, Mannuthy, Kerala, India

MH Girish
Assistant Professor, Dept of Veterinary Anatomy, Veterinary College, Bangalore, Karnataka, India

S Ganga Naik
Professor and Head, Dept of Veterinary Anatomy, Veterinary College, Hassan, Karnataka, India

MK Vinuthan
Assistant Professor, Dept of Veterinary Physiology and Biochemistry, Veterinary College, Hassan, Karnataka, India

KV Jamuna
Professor and Head, Dept of Veterinary Anatomy, Veterinary College, Bangalore, Karnataka, India

Corresponding Author:
NM Rajashailesha
Assistant Professor, Dept of Veterinary Anatomy, Veterinary College, Hassan, Karnataka, India

Cytoarchitecture of medial cuneate nucleus in the buffalo (*Bubalus bubalis*)

NM Rajashailesha, RV Prasad, NS Sunil Kumar, MH Girish, S Ganga Naik, MK Vinuthan and KV Jamuna

Abstract
The medial cuneate nucleus represents the rostral termination of the fasciculus cuneatus, the dorsal column spinal pathway that carries information regarding proprioception, fine tactual discrimination (stereognosis) and vibratory sensations from the upper extremities and upper trunk. The cytoarchitecture of medial cuneate nucleus of the buffalo has been described by materials collected from eight buffalos. Serial and semi serial sections of brain stem were stained with nissl stain. Nucleus was consisting of round, triangular, oval, fusiform and stellate cells. Majority of the medium sized neurons were round and multipolar cells. The position of the nucleus of these cells was central or eccentric and the position of the nucleolus was variably central and darkly stained and the Nissl substance was stained moderate to deep. The numbers of medium sized neurons were more in the caudorostral directions. Hopefully, this study will improve our understanding of the role of the medial cuneate nucleus as major centre for sensory information processing and contribute to our understanding of comparative neuroanatomy and neurophysiology.

Keywords: cytoarchitecture, medial cuneate nucleus, buffalo and brain

Introduction
The dorsal column nuclei (DCN) are among the most completely studied structures in the mammalian central nervous system and functions as relay stations from the anatomical, physiological and behavioural point of view (Norton, 1969) [16]. The DCN (gracile and cuneate nuclei) are sensory nuclei which are insinuated in the ascending somesthetic pathway from the periphery to the somatosensory thalamus (Tan and Gopalakrishnakone, 1986) [24].
Receptors mediating proprioception, kinesthesia, stereognosis and vibration sense through nerve fibres which enter directly into the dorsal columns of the spinal cord and ascend ipsilaterally, without synapsing to the gracile (Gr) and cuneate (Cu) nuclei. The fibres from the hindlimb ascend in the more medial portion of the dorsal column in the fasciculus gracilis, while those fibers from the upper extremity (forelimb) ascend in the more lateral fasciculus cuneatus (Mendoza and Foundas, 2008) [13]. This arrangement allows assay of interactions between distinct components of the somatosensory system during the course of development (Rhoades and Wall *et al*., 1993) [18]. The phylogenetic analysis reveals that there is a steady increase in size of the dorsal fasciculi and their bulbar nuclei from lower animals to man (Chang and Ruch, 1947) [4]. These nuclei make their first definite appearance in reptiles and reach their maximum stage of development in mammals (Ariens-Kappers *et al*., 1936, Goller, 1963, Rajashailesha *et al*., 2017 and Rajashailesha *et al*., 2019) [1, 9, 14, 15]. It is believed that the development of the dorsal fasciculi and their nuclei in the mammalian and primate series is correlated with increasing sensory discrimination in the skin and the increased development of proprioceptive sense in the limb (Chang and Ruch, 1947) [4].
The water buffalo (*Bubalus bubalis*), also called domestic water buffalo or Asian water buffalo is a large bovid originating in the Indian subcontinent, Southeast Asia, and China. The domestic Asian water buffalo is found on all five continents, with a global population of some 202 million. Macgregor (1941) [12] recognised river and swamp buffalo among Asian water buffalo. The river buffalo is native to the Indian subcontinent and has spread west as far as the Balkans, Greece, Egypt and Italy within recorded historical times, whereas swamp buffalo are found throughout south-east Asia, from Assam and Bangladesh in the west to the Yangtze valley of China (Cockrill 1984) [6]. The river buffalo having a black body and generally curved horns, whereas the swamp buffalo is usually dark grey with white chevrons (one or two white stripes on the throat), socks and tip of tail, and relatively straight, occasionally long pale-

pale-coloured horns. The two types also differ in chromosome number, i.e. river 2n = 50 and swamp 2n = 48 (Ulbrich & Fischer 1967; Fischer & Ulbrich 1968) [25, 8]

Water buffaloes are the ideal animals for work in the deep mud of paddy fields because of their large hooves and flexible foot joints. They are often referred to as "the living tractor of the East". They are the most efficient and economical means of cultivation of small fields. In most rice-producing countries, they are used for threshing and for transporting the sheaves during the rice harvest. They provide power for oilseed mills, sugarcane presses and devices for raising water. They are widely used as pack animals in India and Pakistan, for heavy haulage, also. In their invasions of Europe, the Turks used water buffaloes for hauling heavy battering rams. Their dung is used as a fertilizer and as a fuel when dried (Cockrill, 1977) [5].

The domestic Asian water buffalo is an important animal resource, supplying draught power, contribute 72 million tonnes of milk and three million tonnes of meat annually to world food, much of it in areas that are prone to nutritional imbalances in at least 67 countries, and more people depend on this species for their livelihoods than on any other domestic animal (FAO 2000) [7]. In India, river buffaloes are kept mainly for milk production and for transport, whereas swamp buffaloes are kept mainly for work and a small amount of milk (Singh and Barwal 2010) [20]. The present study was undertaken to elucidate the cytoarchitecture, neuronal types and total neuron population of medial cuneate nucleus of the buffalo.

Materials and Methods

Six heads, as a whole were collected immediately after slaughter from Slaughter House, Bangalore, Karnataka and were perfused with 10 per cent buffered formalin through the common carotid artery till a clear fluid came out. The perfused heads were kept for two weeks in 10 per cent buffered formalin. The cranium was opened carefully and the brain along with the brainstem were removed and preserved in 10 per cent buffered formalin for a further period of two weeks.

The brain stem from the level of first cervical to trapezoid body (medulla oblongata) were cut and processed for paraffin technique. The cytoarchitectural description of the nuclei was based on the transverse serial sections of 20µm thickness stained with toludine blue to study the size and shape of the cell body, Nissl pattern, size and position of the nucleus and nucleolus from the transverse sections that were stained with neutral red and cresyl fast violet (Keller, 1960) [10].

The true neuron population in the right and left side of the median raphe was determined by counting the neurons in all the serial transverse sections obtained from one animal. Further, for the estimation of total neuron population of a nucleus in other five animals, systematically sampled every 10th section from each of the five animals were used in this study. Only those neurons that had a distinct nucleolus were counted. Neuron counts for the right and left nuclei were recorded separately and were compared statistically using unpaired‘t’ test (Snedecor and Cochran, 1996) [21].

The total numbers of neurons in these nuclei were determined by using the formula AxB, where A is the number of neurons counted in each sampled section and B is the number of sections up to the next counted section, and by adding the products of AB for all the sections counted. The counting of neurons from the systematically sampled sections was done from caudal to the rostral end of the nucleus.

An ocular micrometer was used to measure the size of the neurons. The neurons were measured at a magnification of X600. The length and width of a cell was measured and the average was taken to arrive at its diameter. Similarly the size of the nucleus was also determined. These diameters were considered as the true diameters. The true diameter of the cell body formed the basis for classification of neurons in the nuclei under study. The neurons were classified as large (greater than 50mm), medium (26 to 50mm) and small (less than 25mm) based on their diameter.

Results and Discussion

Type and structure of neurons

The medial cuneate nucleus in the buffalo composed of round, triangular, oval fusiform and stellate cells (Fig. 1 & 2). Majority of the medium sized neurons were round and multipolar, with a very few round and oval cells. In the caudal pole of the MCN consists of small round, oval or triangular shaped neurons whereas the rostral pole of MCN presented both small and medium sized neurons. Such variations in shape of neurons with respect to poles were not reported earlier. However, in general MCN neurons were medium to small in size with round, oval, fusiform and stellate shaped cells were described in man (Olszewski and Baxter, 1954) [17], cat (Cajal and Ramon, 1909; Kuypers and Tuerk, 1964) [3, 11] and horse (Salam, 1971) [19] , whereas in European Bison medial cuneate nucleus was formed of four types of neurons. Type-I triangular cells, Type-II multipolar cells, Type-III fusiform cells and Type-IV round cells (Szteyn and Robak, 1989) [22]. Six types of neurons were identified by Zaqout *et al*., (2012) [26] in camel based on soma size and shape, density of dendritic trees, morphology and distribution of spines, and appendages.

In the buffalo the nucleus of the neurons was central or eccentric and the position of the nucleolus was variably central and darkly stained. Nissl substance was moderate to deeply stained (Fig. 3 & 4). Similar observations were recorded in horse by Salam (1971) [19]. In man (Olszewski and Baxter, 1954) [17], the cells possessed moderately stained cytoplasm and peripherally arranged Nissl granules, whereas in the cat (Taber, 1961 and Kuypers and Tuerk, 1964) [23, 11] cells of MCN with a central nucleus and fine, evenly dispersed Nissl granules were described.

Size of neurons

The average true diameter of the cell body and nucleus of medium sized neurons of the MCN in the buffalo was 33.86±0.67 µm and 13.55±0.23µm respectively. The average true diameter of the cell body and small sized neurons was 20.4±0.28 µm. and 10.65±0.18µm, whereas in the horse, as reported by Salam (1971) [19] the oval cells were small and ranged in size from 12 to 22µm, with an average of 16µm. The fusiform cells were small to medium in size and ranged from 23 to 42 µm, with an average of 31µm. The stellate cells were medium to large in size and ranged from 26 to 45µm with an average of 35 µm. Similarities in the size of the cell body and nucleus were found in horse (Salam, 1971) [19] and in buffalo. The approximate ratio between diameters of nucleus and cell body for medium and small sized neurons were 1:2.54 and 1:1.96, respectively (Table-1).

Neuron population

The neuron population in the right medial cuneate nucleus

ranged from 9,120 to 9,470 with a mean of 9,336 ± 51 and in the left from 9,230 to 9,520 with a mean of 9,394 ± 42. However, there was no significant difference in the neuron population of the left and right nuclei of the same specimen. The total neuron population of the MCN in the buffalo (right and left side combined) was 18,730 ± 64 of which right side comprised of 9336±51neurons and left side has 9394± 51(Table-2). Where as in cat (Bermejo *et al*., 2003) [2] the total number of neurons in the cuneate nucleus possessed approximately 10,740. The estimated population of medium sized neurons for both left and right nuclei together was 8,850. The estimated population of small sized neurons was 9,980. Thus the proportion of medium to small sized neurons in the medial cuneate nucleus in the buffalo was 8,850: 9,980. The approximate ratio of medium to small cells was 1: 1.13 (Table-3). From these findings it shows that total number of neurons increase with larger sensory area of the animal, being lowest in cat (small animals) to the highest recorded in the buffalo (large animals) and this can be postulated due to better development of somatosensory modality in buffalo. Further electrophysiological studies are required to assess the correlation of the sensory inputs with the central connections.

Table 1: True diameter of the medium and small sized neurons and the ratio between the diameter of nucleus and cell body in the medial cuneate nucleus.

Number of animals	Size of neuron	Mean true diameter in µ (Mean± SE)		Ratio between diameter of Nucleus: Cell body
		Cell body	Nucleus	
6	Medium	33.86 ± 0.67	13.55 ± 0.23	1: 2.54
	Small	20.4 ± 0.28	10.65 ± 0.18	1: 1.96

Table 2: Comparison of neuron population in the left and right medial cuneate nucleus in the buffalo

Side of Brain	Buffalo number							't' (P> 0.05)
	B1	B2	B3	B4	B5	B6	Mean ± SE	
Right	9390	9290	9315	9120	9430	9470	9336±51	0.87
Left	9520	9410	9486	9350	9230	9370	9394 ±42	
Total neurons	18910	18700	18801	18470	18660	18840	18730±64	

Note: The mean neuron population in the right and left medial cuneate nucleus showed no significant difference (P> 0.05).

Table 3: Distribution and relative proportion of medium and small neurons in the nuclei

Nucleus	Medium neuron	Small neuron	Relative proportion
Medial cuneate nucleus	8850	9980	1:1.13

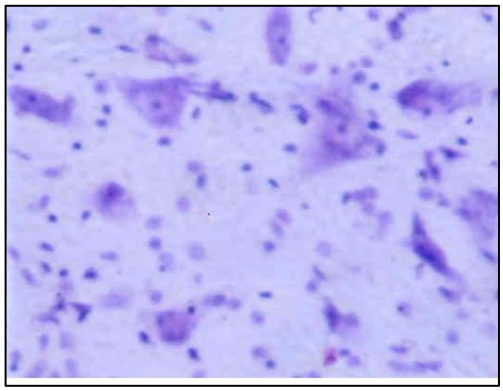

Fig 1: Photomicrograph showing types and distribution of neurons in the medial cuneate nucleus. (Cresyl fast violet-X200)

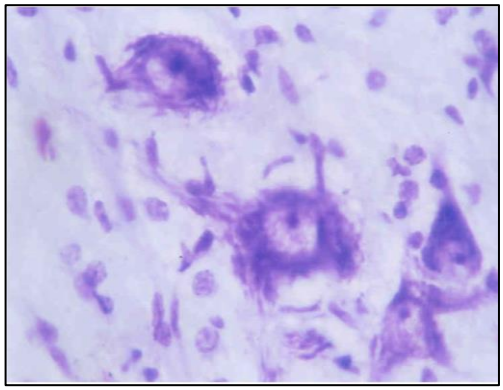

Fig 2: Photomicrograph showing some characteristic of small sized neuron in the medial cuneate nucleus. (Cresyl fast violet-X400)

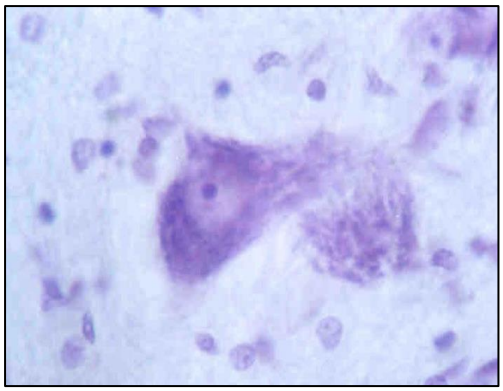

Fig 3: Photomicrograph showing some characteristic medium sized neuron in the medial cuneate nucleus. (Cresyl fast violet-X600)

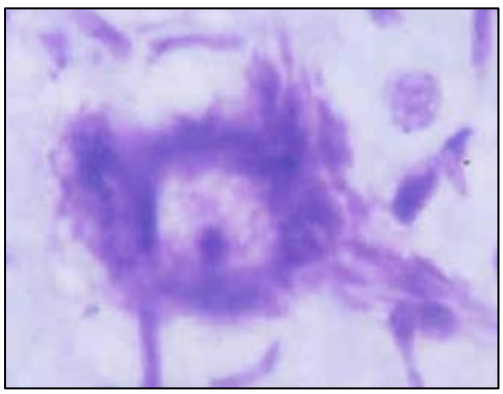

Fig 4: Photomicrograph showing some characteristic medium sized neuron in the medial cuneate nucleus. (Cresyl fast violet-X1000)

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