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Rajashailesha NM

Assistant Professor, Department of Veterinary Anatomy, Veterinary College, Hassan, Karnataka, India

Prasad RV

Professor and Head, Department of Veterinary Anatomy, Veterinary College, Bangalore, Karnataka, India

Sunil Kumar NS

Assistant Professor, Department of Veterinary Anatomy, Veterinary College, Mannuthy, Thrissur, Kerala, India

Girish MH

Assistant Professor, Department of Veterinary Anatomy, Veterinary College, Bangalore, Bangalore, Karnataka, India

Jamuna KV

Professor, Department of Veterinary Anatomy, Veterinary College, Bangalore, Bangalore, Karnataka, India

Ganga Naik S

Professor and Head, Department of Veterinary Anatomy, Veterinary College, Hassan, Karnataka, India

Vinuthan MK

Assistant Professor, Department of Veterinary Physiology and Biochemistry, Veterinary College, Hassan, Karnataka, India

Chandrashekhara NC

Assistant Professor, Department of Veterinary Microbiology, Veterinary College, Hassan, Karnataka, India

Corresponding Author:

Rajashailesha NM

Assistant Professor, Department of Veterinary Anatomy, Veterinary College, Hassan, Karnataka, India

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

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

Abstract

The lateral cuneate nucleus (LCN) (or nucleus of von Monakow) is a relay nucleus conveying proprioceptive stimuli of the muscles from the upper half of the body to the cerebellum. The cytoarchitecture of lateral 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. The nucleus first appeared as a group of two or three neurons in the dorso-lateral tegmentum of the medulla immediately ventral to the dorsal surface of the brain stem. The nucleus was composed of small and medium sized neurons. The majority of medium sized neurons were oval, round and stellate in shape. The nucleus of these neurons was central or eccentric and the position of the nucleolus was variably central and darkly stained. The shape of the small neurons was triangular and fusiform with a large central nucleus. The average true diameter of the cell body and the nucleus respectively for medium sized neurons in the lateral cuneate nucleus were $34.12 \pm 0.56 \mu\text{m}$ and $14.26 \pm 0.19 \mu\text{m}$. Medium sized neurons were dispersed throughout the LCN. Medium sized neurons had central nuclei and darkly stained fine to coarse Nissl substance. Small sized neurons tended to concentrate mainly at two poles of the nucleus comparable to the cat. Average true diameter of the cell body and nucleus of the small sized neurons were $21.02 \pm 0.38 \mu\text{m}$ and $10.90 \pm 0.19 \mu\text{m}$, respectively. The Nissl substance in small neurons appeared coarse and darkly stained. These findings yield some light on the structure of the lateral cuneate nucleus of one of the largest animals (the buffalo).

Keywords: Lateral cuneate nucleus, neuron, nissl substance, buffalo, brain

Introduction

Many neuronal types were described in the nuclei of the dorsal column (DCN) in various species using different methods such as Golgi impregnation, horseradish peroxidase, Nissl stain, immunocytochemistry and electron microscope (Blomqvist and Westman, 1976; Berkley *et al.*, 1986; Tan and Gopalakrishnakone, 1986; Crockett *et al.*, 1993) [4, 2, 19, 5]. Several neuronal types were described in the DCN in different species such as the rat (Basbaum and Hand, 1973; Gulley, 1973) [1, 7], cat (Taber, 1961; Kuypers and Tuerk, 1964; Keller and Hand, 1970) [18, 12, 10], monkey (Biedenbach, 1972) [3] and camel (Zaqout *et al.*, 2012) [20]. In the cat, the areas of the DCN which receive a large inflow of cortical fibres contained many triangular, multipolar, and fusiform cells with long, sparsely ramifying dendrites (Kuypers and Tuerk, 1964) [12].

India is considered as the home tract of some of the best buffalo breeds. Indian buffaloes are water buffaloes and are important source of milk supply today and yield nearly three times as much milk as cows. Hopefully, this work will find special features for neurons in the LCN of the buffalo, which represent the second order neurons of pathways in conducting deep sensations from the upper half of body to the cerebellum via the juxtaresti form body (through the Cuneo cerebellar tract). It is considered to be equivalent to the thoracic nucleus (or dorsal nucleus of Clarke) which transmits proprioceptive stimuli of the muscles from the lower half of the body to the cerebellum via the inferior cerebellar peduncle (through the posterior spin cerebellar tract or poster lateral spin cerebellar fibers (Smith, 1957 and King and Lowell, 1999) [13, 11]. Lesions of the dorsal funiculus or this nucleus abolish or diminish above sensation.

Though extensive study is done on the extent and cytoarchitecture of this nucleus in man (Olszewski and Baxter, 1954) [15], cat (Taber, 1961) [18], ox (Goller, 1963) [6], sheep (Rao, 1964) [16], horse (Salam, 1971) [17], however lack of information on cytoarchitecture of lateral cuneate nucleus in the buffalo formed the basis for this study.

Materials and Methods

Brains of eight buffaloes were obtained from the Slaughter House, Bangalore. The heads, as a whole were collected immediately after slaughter and were perfused with 10 percent buffered formalin through the common carotid artery till a clear fluid came out. The perfused heads were kept for two weeks in 10 percent buffered formalin. The cranium was opened carefully and the brain along with the brainstem were removed and preserved in 10 percent 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 20 μ m thickness stained with toluidine 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 were stained with neutral red and cresyl fast violet (Keller, 1960)^[9].

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)^[14].

The total numbers of neurons in these nuclei were determined by using the formula $A \times B$, 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 were 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 50 μ m), medium (26 to 50 μ m) and small (less than 25 μ m in diameter).

Results and Discussion

Type and structure of neurons

In the buffalo Lateral cuneate nucleus first appeared as a group of 2 or 3 cells in the dorsolateral tegmentum of the medulla, immediately ventral to the dorsal surface of the brain stem. Similar observation was made in the horse by Salam (1971)^[17].

The LCN of the buffalo was composed of small sized triangular and fusiform cells, medium sized oval, round and stellate cells (Figs. 1 & 2). The nucleus of the medium sized cells was central and eccentric and the position of the nucleolus was variably central and darkly stained (Fig. 4). The small cells presented a large central nucleus with Nissl substance coarse and darkly stained (Fig. 3). However, in the horse, Salam (1971)^[17] reported round and oval shaped small and medium size neurons. The fusiform and stellate shapes were observed only in large neurons which was not a feature in the buffalo. But cells had variably central nuclei and darkly

stained fine to coarse Nissl bodies as observed in the buffalo (Fig. 3 & 4). The cells of the LCN in man (Olszewski and Baxter, 1954)^[15] were much larger and stained intensely. Majority of the cells were large and oval with peripherally arranged Nissl granules. In the sheep (Rao, 1964)^[16] LCN was composed of multipolar and medium sized stellate cells with central nuclei comparable to that of the buffalo. In the camel six types of neurons were identified based on soma size and shape, density of dendritic trees, morphology and distribution of spines, and appendages (Zaqout *et al.*, 2012)^[20].

In the buffalo, medium sized cells were dispersed throughout the LCN and small cells tended to concentrate mainly at two poles of the nucleus comparable to the cat (Taber, 1961)^[18]. Whereas in the sheep, Rao (1964)^[16] noticed predominance of large and medium sized cells at the caudal pole of the nucleus and in the rostral pole was composed only of large cells. However based on morphology large neurons were not described in the present study.

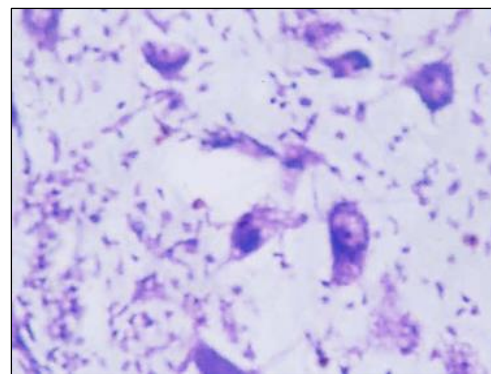


Fig 1: Photomicrograph showing types and distribution of neurons in the lateral cuneate nucleus (Cresyl fast violet-X100)

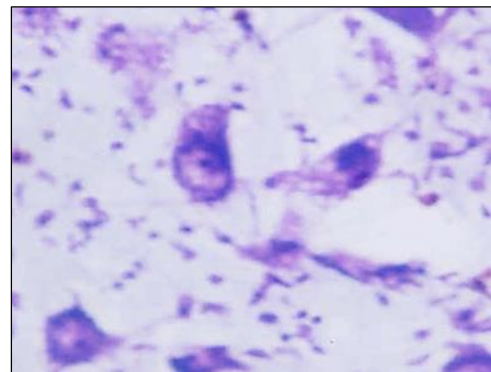


Fig 2: Photomicrograph showing some characteristic neurons in the lateral cuneate nucleus (Cresyl fast violet-X200)

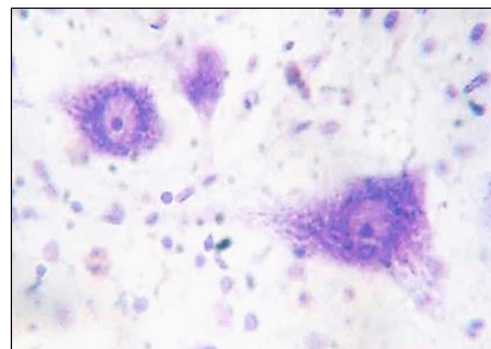


Fig 3: Photomicrograph showing small sized neurons of lateral cuneate nucleus (Cresyl fast violet-X400)

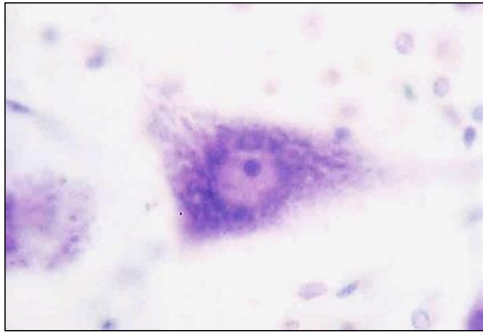


Fig 4: Photomicrograph showing medium sized neuron in the lateral cuneate nucleus (Cresyl fast violet-X600)

Neuron population

The total neuron population of LCN in the buffalo (right and left side combined) was 91,043±942 of which right side comprised of 45,326±449 and left side was 45,717±515 (Table-1). Whereas in the cat, Heino and Westman (1991) [8] reported that the total number of neurons in the LCN was 33,000. The larger proportion of neuron in the LCN probably reflects the proprioceptive stimuli of the muscles from the larger upper half of the body to the cerebellum.

The estimated population of medium sized neurons for both left and right nuclei together was 57518, while average population of small sized neuron was 33780. Thus the proportions of medium to small sized neurons in the LCN of

buffalo were 57518:33780. The approximate ratios of medium to small neurons were 1.7:1 (Table-2).

Size of neurons

In the present study the neurons of the LCN were classified into small and medium based on their diameter. The average true diameter of the cell body and nucleus of the medium sized neurons of the LCN in the buffalo was 34.12±0.56µm and 14.26±0.19µm respectively. Average true diameter of the cell body and nucleus of the small sized neurons was 21.02±0.38µm and 10.90±0.19µm (Table-3) indicating the diameter of the cell body was appear to be twice the diameter of the nucleus. Whereas in the horse, Salam (1971) [17] observed oval and round cells were small to medium sized, ranging from 23 to 33µm with an average of 29µm. The fusiform cells were medium to large in size and range from 35 to 47µm with an average of 42µm. the stellate cells were predominantly large and range in diameter from 32 to 56µm with an average of 47µm. Thus the neurons were smaller in the LCN of buffalo compared with that in the horse. From these observations it appears that number of neurons of LCN increases with the increase in the size and surface area of the animals, being lowest in cat (small animal) to highest recorded in horse and buffalo (large animals). This can be functionally correlated to the larger skin surface area to be covered in transmitting somatosensory modality.

Table 1: Comparison of neuron population in the left and right lateral cuneate nucleus in the buffalo

Side of Brain	Buffalo number						Mean ± SE	‘t’ (P> 0.05)
	B1	B2	B3	B4	B5	B6		
Right	43980	47000	44875	44670	45210	46220	45326±449	0.57
Left	44350	47950	45740	45190	44920	46150	45717±515	
Total	88330	94950	90615	89860	90130	92370	91043±942	

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

Table 2: Distribution and relative proportion of medium and small neurons in the nuclei

Nucleus	Medium neuron	Small neuron	Relative proportion
Lateral cuneate nucleus	57518	33780	1.7:1

Table 3: True diameters of the medium and small neurons and the ratio between the diameter of nucleus and cell body in the lateral 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	34.12 ± 0.56	14.26 ± 0.19	1: 2.43
	Small	21.02 ± 0.38	10.90 ± 0.19	1: 1.95

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