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Morphologically atypical cervical dorsal root ganglion neurons in adult rabbit.

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Abstract:

Dorsal root ganglion (DRG) contains primary sensory neurons which are said to be heterogeneous with respect to their morphological, functional and neurochemical characteristics. Literature regarding their light microscopic features for their categorization in different subsets remains inconclusive. The present study was attempted to see as to what extent neuronal subsets of DRG can be appreciated by routine histological techniques and if there still exist some neurons with atypical features. In the present study 5 rabbits of either sex were perfusion fixed either by 10% formalin or Karnovsky's fixative. Cervical DRGs from both sides were procured and processed for paraffin embedding. 10 µm-thick sections stained with Haematoxylin and Eosin were observed under light microscope. It was noticed that DRG neurons are arranged in groups interspersed among the fascicles of nerve fibres. They appear round or oval in shape, ranging in sizes from 15 to 75 µm in cross section, and each being surrounded by 3 to 15 satellite glial cells (SGCs). Sensory neuron is characterized by large centrally placed euchromatic nucleus and prominent nucleolus (1 to 3 nucleoli per neuron). Features of most of the neurons matched with those of typical neuronal subsets described by different workers (1, 2, 3, and 4). However, a very small population of neurons presented atypical features e. g; a) large cell body with coarse Nissl granules forming a prominent perinuclear ring; b) large neuron with a pyramidal or triangular somatic outline (90/50 µm); c) neuron having eccentrically placed nucleus; d) a couple of neurons sharing common SGC-sheath. It was concluded that even by routine histological techniques in addition to typical neuronal subsets some neurons having atypical features can also be recognized which require appropriate categorization in accordance with their ultrastructural, neurochemical and functional characteristics.

Key words: Dorsal root ganglion (DRG), satellite glial cells (SGCs), sensory neuron, cervical, Nissl substance, atypical, heterogeneous, rabbit.

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Introduction

Mammalian sensory ganglia in general and DRGs in particular, have been the focus of intense research because of their importance in the transmission of sensory signals and their contribution to acute and chronic pain syndromes.

DRG consists of a heterogeneous group of primary sensory neurons. Many subpopulations have been identified on the basis of their light microscopic morphology [2], ultra structure [1,5], neurochemical and immunocytochemical [6,7,8,9] and electrical and functional properties [10,11], their relative sensitivity to certain harmful agents [3], and their involvement in some pathological conditions [4].

Each DRG neuron is surrounded by a sheath formed by SGCs. These cells are in close contact with the neurons and play multiple roles both in health and disease but their many functions still remain to be understood. There is evidence that satellite cells undergo both morphological and biochemical changes after nerve damage [12,13], but there is paucity of information on their possible contribution to pain mechanisms. The present study aims at looking for the possible existence of morphologically atypical DRG neurons that can be identified by routine light microscopy.

Material and Method

5 adult healthy rabbits of either sex, aged between 12 to 15 months, and weighing on average 1.5 Kg each were included in this study. Under deep general anaesthesia, they were fixed by intra-cardiac perfusion method using either 10% buffered formalin or Karnovsky's fixative. Cervical part of spinal cord and associated dorsal root ganglia of both sides were dissected out. DRG from each spinal segment was processed separately for paraffin embedding. Serial 10 µm-thick sections were cut by rotary microtome. Haematoxylin and Eosin stained sections were observed under light microscope. Salient findings were recorded primarily under high power and occasionally under oil immersion.

Results

The DRG contains two main types of cells namely neurons and glia. Neurons are larger and more prominent and lesser in number while and glia cells are smaller in size, more numerous and surround each neuron. Clusters of neurons are interspersed among nerve fascicles (Fig. 1). In cross sections, the neuronal clusters appear to assume different size and shapes. Almost all nerve cells are circular to oval in outline and are of variable size ranging from 15 to 75 µm in diameter. Each cluster of neuron possesses cells of different size and there appears to be no specific pattern in their arrangement. Neuronal cell is characterized by large centrally placed euchromatic, vesicular nucleus and prominent nucleolus. The number of nucleoli per neuron ranges from 1-3. The perikaryon is filled with Nissl substance which assumes different appearance in terms of its overall amount, distribution pattern, size of granules, and intergranular space. Features of most of the DRG neurons match with those described by different workers [1,2,3,4,5] for various subsets of neurons.

However, few neurons (Figs. 2,3,4,5) in the present study revealed remarkable atypical features which do not fully match with the findings of aforesaid workers. For example – large size neuron with coarse granule Nissl substance arranged in the form of a prominent perinuclear ring and thus leaving the peripheral part of soma almost free of Nissl substance (Fig. 2). Occasionally, a large neuron was noticed to possess a triangular or pyramidal somatic outline with most of the other features remain similar to a typical sensory neuron (Fig. 3). Contrary to the most common pattern of having central nucleus some neurons are seen to possess eccentric nuclei (Fig. 4). Normally, each neuron is surrounded by SGCs which actually form a sheath and thus each individual neuron along with its SGCs can be identified as isolated units. But sometimes two medium sized or small neurons are seen to be placed so closely that part of SGC-sheath between adjacent neurons can not be resolved even at higher magnification and it appears that both neurons are housed inside the same capsule (Fig. 5). As seen in the cross section, the number of SGCs involved in making the perineuronal sheath varies with the size of neuronal cell body ranging from just 3 around small neuron to 15 around large neurons. The cytoplasm of SGCs can also be seen but only occasionally (Fig. 2).

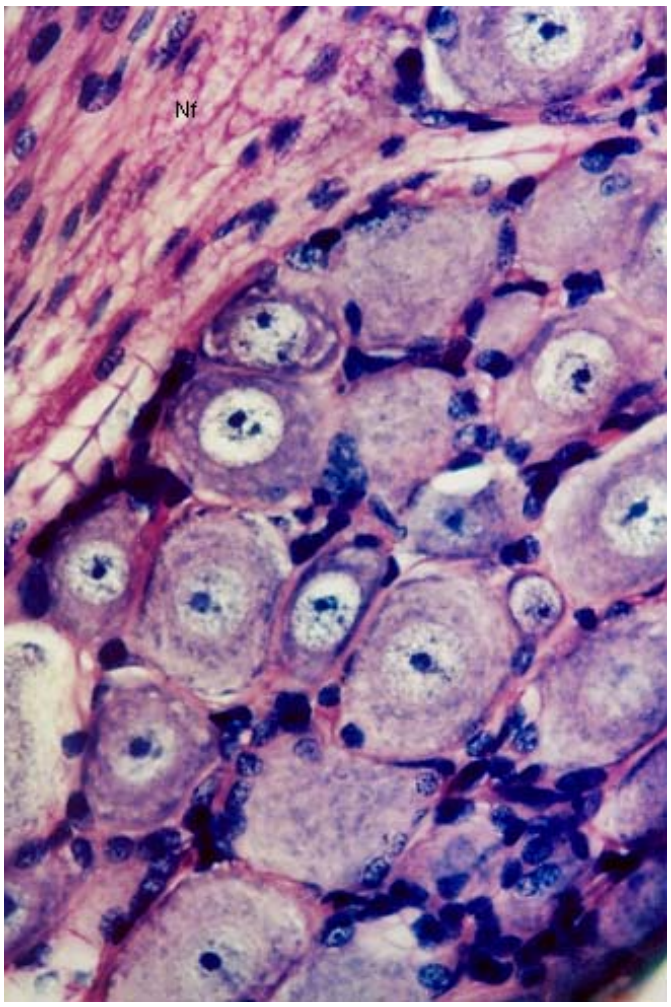

Fig. 1: *Rabbit DRG neurons. Almost all neurons are round or oval with centrally placed vesicular nucleus, prominent nucleolus, perikaryon contains Nissl substance. Each neuron is surrounded by many SGCs. Upper left quadrant show bundle of nerve fibres (**Nf**).*

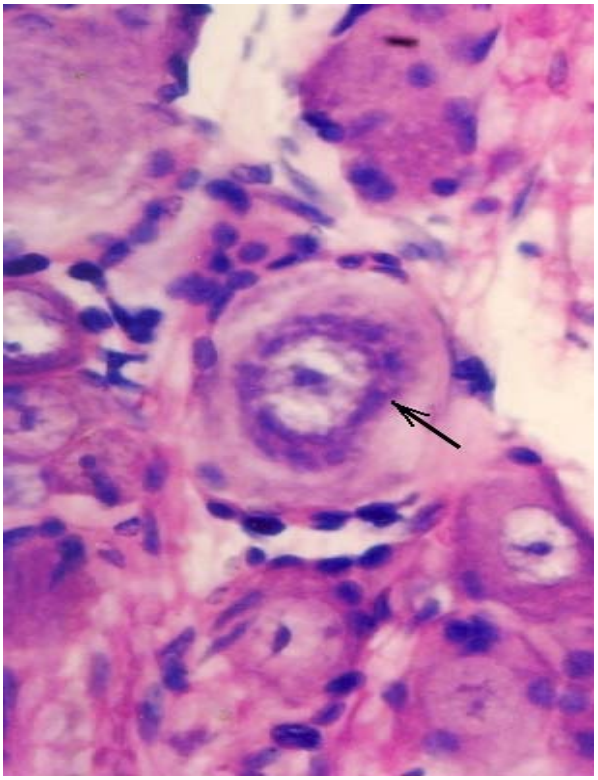

Fig. 2: *DRG neurons from of rabbit. Centrally placed large neuron has prominent perinuclear ring of Nissl substance (↑).Surrounding neurons are of medium size.*

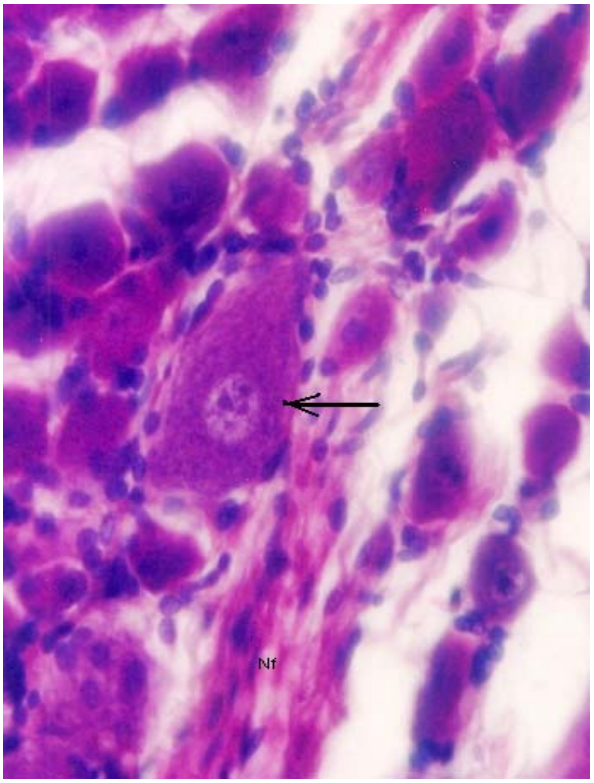

Fig. 3: *Large DRG neuron with triangular soma (↑) shows centrally placed vesicular nucleus with three nucleoli. Lower field shows a fascicle of nerve fibres (**Nf**).*

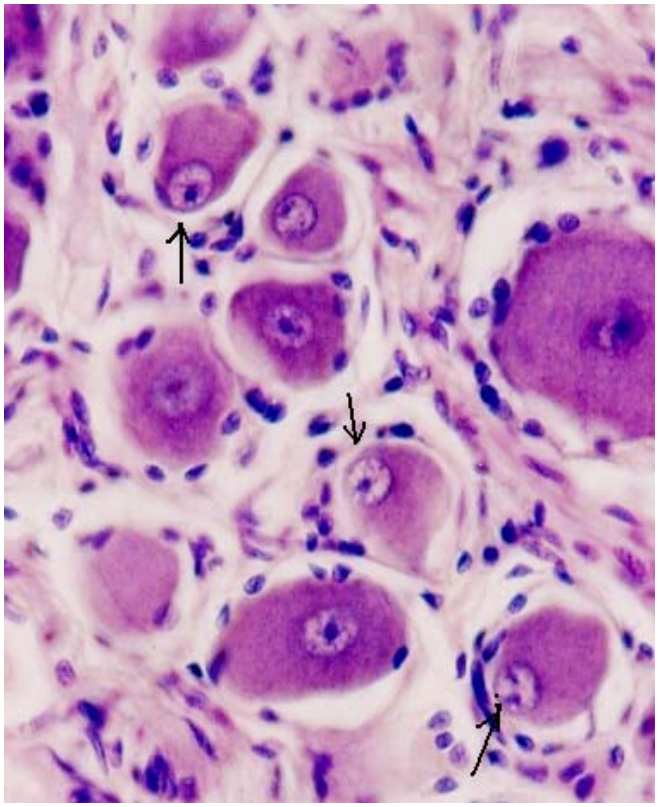

Fig. 4: *DRG neurons with usual centrally placed nuclei as well as unusual eccentrically placed nuclei (↑) interspersed among fascicles of nerve Fibres.*

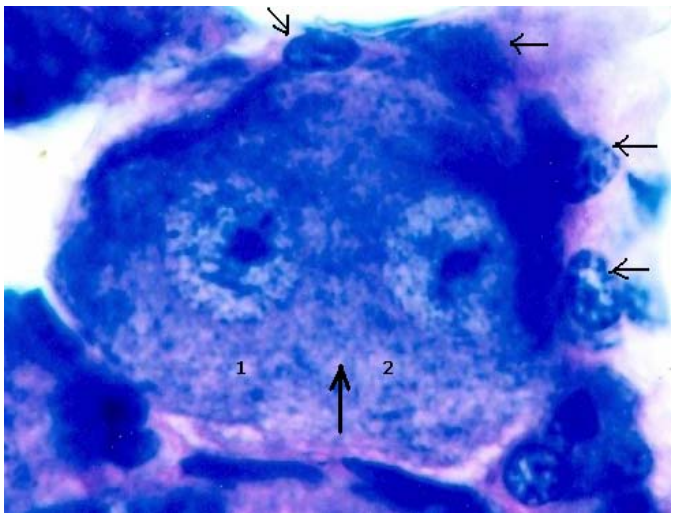

Fig. 5: *Two DRG neurons (1 and 2) enclosed in common sheath formed by perineuronal glial satellite cells (thin and small ↑ ↑ ↑). Sheath tissue is not visible at the line of junction between two cells (large and **thick** ↑) even under oil immersion.*

Discussion and Conclusion

Sensory neurons in DRG are anatomically, functionally, and neurochemically diverse [1,2,3,4,5]. In the present

light microscopic study only morphological criteria were taken for identification and classification of DRG neurons. Literature on neurons of cervical DRG of rabbit is scanty and therefore the findings of the present study being compared with low power electron-microscopic features of DRG neurons of rat [1]. The light microscopic features of almost all DRG neurons fell within one or the other groups (A_1, A_2, A_3, B_1, B_2, and C) of rat lumbar DRG [1]. However, few neurons revealed features which did not match with those described previously. In general, there is good correlation between form and function and DRG neuronal cell body size and axonal conduction velocity are generally positively correlated. Though, the generalization that large cells give rise to myelinated axons and small cell to unmyelinated axons has been claimed to be oversimplification as it may not be true for the intermediate size of cells [2]. Differential susceptibility of DRG neurons and their atrophy in various experimental studies [3,4,14,15] may be the morphological basis for the development of decreased sensory nerve conduction velocity noticed in many peripheral neuropathies. Features of large neuron (Fig. 2) wherein the configuration of Nissl substance stands out so distinctly different that it is difficult to unequivocally equate it with any single subtype of DRG neuron. In fact its soma size matched with type A_1, while location of Nissl substance with type A_3 and size of Nissl granules with type B_1 [1]. How this morphological feature is really going to affect its functional properties in health and disease, similar to those mentioned above remains to be resolved.

The morphological feature of neuron (Fig. 3) wherein shape of soma is pyramidal is rather very uncommon. It is contrary to usual description and in fact to our knowledge this is first report of its kind. In fact sectioning of pseudounipolar neuron in any known plane is unlikely to give rise to pyramidal somatic shape. The presence of fine dynamic projections on the surface of neuron in relation to its contact with satellite cells [16] resulting into such gross alteration in the shape of its soma is also not plausible. Moreover, it does not appear to be an artifact either. How this rather unusual somatic shape which otherwise appears normal will affect its functional properties is not clear; may be that it will affects the distribution pattern of ion channel present on its surface which is believed to lend neurons their unique functional attributes [17].

Sensory and autonomic ganglion neurons are known to be characterized by the presence of centrally placed and eccentrically placed nuclei respectively. In the present study, occasional neurons from an otherwise normal DRG revealed presence of eccentric nuclei (Fig. 4). The neuronal perikaryal response to axonal injury includes reduction in axonal caliber, development of chromatolysis and nuclear eccentricity [18]. However, in our study, the nuclear eccentricity noticed is neither due to apparent axonal injury nor it is associated with obvious chromatolysis. Therefore, it remains to be resolved as to whether these neurons represent a minor subpopulation of normal DRG neurons or else those neurons which are undergoing routine apoptosis as a part of ageing process. Occurrence of nuclear eccentricity in normal DRG assumes significance because caution must be exercised while looking for experimental degenerative changes in DRG and observing nuclear eccentricity as one of the criteria [13,18]..

Primary afferent neurons in mammalian DRG are anatomically isolated from one another and are not synaptically interconnected. And as such they are classically thought to function as independent sensory communication elements. In the present study a couple of neurons appear to share a common sheath formed by SGCs (Fig. 5). Although, presence of ultrathin interneuronal sheath element cannot be ruled out with certainty by light microscopy, the significance of such intimate association among certain neurons is not very clear. It has recently been shown that most DRG neurons are transiently depolarized when axons of neighboring neurons of the same ganglion are stimulated repeatedly [19]. Cross-depolarization contributes to mutual cross-excitation. This intraganglionic dialog appears to be mediated, at least in part, by an activity-dependent diffusible substance (s) released from neuronal somata and/or adjacent axons, and directed to neighbouring cell somata and/or axon [19]. Thus it appears that such type of intimate association (Fig. 5) may provide a suitable morphological substrate for the aforesaid intraganglionic communication which is based on novel, non-conventional neural mechanism and which has practical consequences for sensory conduction in health and disease [19,20].

Although much less is known about SGCs in sensory ganglia, it appears that these cells share many characteristics with their central counterparts. It has been shown that SGCs also promote formation of dynamic projections from the surface of neuronal perikarya as compared to the extracellular matrix [16]. Like Schwann cells SGCs cytoplasm contains peroxisomes which may influence oxygen levels in the vicinity of' perikarya, and they may also contribute to the processing and breakdown of material which gains access to the extra cellular spaces near neurons [21]. In the present study the SGCs number seems to positively correlate with the size of neuronal cell somata and this is in agreement with the finding in other species showing the volume of SGC-sheath to be directly proportional to both the volume and surface area of the related neuronal cell body [22].

Conclusion

It is concluded that a small subpopulation of cervical DRG neurons on their morphological grounds, may be considered as *atypical*. These neurons possibly do not represent species or regional variation, and therefore,

need suitable categorization with respect to their ultra-structural, physiological and neurochemical characteristics.

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