Original Research

Role of peripherin in defining specific populations of cell bodies in the dorsal root ganglia

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Abstract

Background: Dorsal root ganglion (DRG) cell bodies mediate the transmission of sensation from the periphery. They are unipolar in nature and enveloped by the satellite glial cells (SGCs). SCs have been reported to influence neuronal excitability via gap junctions. DRG cell bodies are classified upon cell size into small, medium, and large categories. This classification seems to be appropriate because the size of neuronal cell bodies determine their function as, for example, small-sized cell bodies preferentially transmit action potentials related to pain and temperature.

Objective: To correlate different sizes of cell bodies in DRG with Nissl staining and expression of peripherin.

Material and Methods: Male adult Sprague Dawley rats (n = 12) were randomly divided into two equal groups: group I for morphometric analysis of cell bodies after Nissl stain and group II (n = 6) for immunohistochemical study after staining with peripherin antibody.

Results: DRG cell bodies of right lumbar 4 level spinal nerves were classified into small to medium (10–40 μm) and large (>40 μm) depending upon the maximum diameter of the cell bodies using Nissl stain. Adjacent sections were stained with peripherin. Peripherin expression was noted in small- to medium-sized cell bodies (10–40 μm). Correlation between these two groups showed that cell bodies in the DRG can be classified into (1) small to medium (10–40 μm) and (2) large (more than 40 μm) categories.

Conclusion: Expression of peripherin by small to medium sized neuronscan provide additional guidelines for classifying DRG neurons. This could help in eletrophysiological assessment of neurons, depending upon various parameters.

Key Words: Ganglia, Cell bodies, Nissl stain, Peripherin, Satellite glial cells

Introduction

Sensory ganglia, particularly the dorsal root ganglia (DRG), have been the subject of intense research because of their importance in the communication of sensory signals such as pain. It is susceptible to ischemia owing to compression or chemical stimulation induced by inflammation that can cause chronic pain in lower extremity.[1] The cell bodies of sensory cell bodies are restricted within the dorsal root ganglia, and their axons travel large distances to innervate peripheral tissues. The cell bodies of DRG cell bodies lack the protection of the blood–brain barrier and are susceptible to various neurotoxins compared with central nervous system. For example, the dorsal root ganglion is the main site of platinum accumulation during treatment with platinum-based antitumor drugs.[2]

Previously, cell bodies of DRG were divided into two histological types called large light (LL) and small dark (SD), on the basis of their staining properties.[3] Recently, electron microscopic study has confirmed the presence of two types of DRG cell bodies, usually termed as A and B rather than large light and small dark.[4] However, functional importance of this classification is still in doubt. Many electrophysiological parameters such as velocity of conduction, modality, and adaptation rate serves to differentiate large number of functional types of sensory cell bodies, but it is still not clear how these are related to basic histological types.

In this study, maximum size of cell bodies in the DRG were measured and then correlated with presence of peripherin.

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Peripherin, a triton-insoluble protein, is 57-kDa type III neuronal intermediate filament (nIF), particularly expressed in peripheral nervous system. It is used to study peripheral nerve development and regeneration, as it is highly expressed during elongation of axon.[5] Exact function of the peripherin is still unknown although it has been suggested to be a determinant of shape and architecture of peripheral nerve axons.[6] Various sizes of cell bodies perform various functions, and these grouping roughly into small, medium, and large correspond to their functions, for example, small- to medium-sized cell bodies are concerned with nociception.[7] Neurofilaments have been recognized in the pathogenesis of many diseases for, e.g., amyotropic lateral sclerosis (AML), which is associated with peripherin.[8]

Materials and Methods

Animal Preparation

Adult male Sprague Dawley (SD) rats weighing 200–250 g were obtained from experimental animal facility of All India Institute of Medical Sciences after prior approval of the experimental procedure by Institutional Animal Ethics Committee (IEAC). Rats were maintained as three to four rats per cage with food and water *ad libitum* and a regular 12-h dark–light schedule. Rats were categorized into two groups: group I (n = 6) for morphometric analysis with Nissl stain and group II (n = 6) for immunohistochemical analysis using peripherin antibody. Standard protocol for perfusion fixation with 4% paraformaldehyde in 0.1 M phosphate-buffered solution (PBS) was done for isolating the DRG. After isolation, DRGs were processed for paraffin sectioning (5 mm) and stained with 1% Nissl stain (Cresyl violet) for localization of Nissl substance. These sections were taken and mounted on slides with DPX. In this study, six representative sections from the entire right DRG of each rat were selected. Every sixth section was selected for the study from the cranial end of the serial section of each DRG. For each section, counting of maximum diameter of cell bodies was started from left end, and each successive image was captured to the right side of the previous section. A total of 300 cell bodies in each rat were examined for determining the maximum diameter of cell bodies in the DRG. Images were saved and analyzed using Progres Image analysis system (Jenoptik, Germany). Later, sections were viewed using light microscope (Nikon E-600 compound microscope).

Immunohistochemical Study Using Peripherin

Immunohistochemistry was done on free-floating sections contained in 0.1 M PBS. Cryosections (10 mm) were obtained on gelatine-coated slides using cryostat (LEICA CM 1950) at –20°C. The sections were incubated with hydrogen peroxide, rinsed in PBS, and incubated in 10% normal goat serum. Sections were then incubated with antiperipherin (1:1,000; Millipore) antibody diluted in PBS containing Triton X-100 and NGS at room temperature for 2 h at 4°C. The sections were then processed using Vectastain® ABC kit (Vector, Burlingame, CA) and rinsed with the PBS-TX between each incubation period. Visualization of immune complex with 3,3 diaminobenzidine complex (0.025%; DAB, Sigma Laboratories) was done.

Results

Histological Study

Unipolar cell bodies of different sizes were observed ranging from large round cell bodies to much smaller ones [Figure 1]. Neurons in DRG are arranged in clusters or groups. The center of the ganglia was occupied by nerve fiber bundles. Satellite glial cells (SGCs) were smaller in size but more numerous and surrounded by the neuronal cell bodies [Figure 1A and 1B]. The number of SGCs involved in making sheath varied with the size of neuronal cell bodies. It ranged approximately between two and six SGCs around the large cell bodies. DRG neurons have large pale staining, vesicular centrally placed nuclei. Nucleus in the DRG contained intensely staining nucleoli. Nucleoli in some of the cell bodies were absent; this is because section may pass above or below the nucleoli. Few cell bodies revealed interesting feature of having centrally placed nucleus with double nucleoli [Figure 1B]. Larger cell bodies were seen with lighter-colored Nissl bodies (large-light type or type A) when compared with the smaller cell bodies with more darkly stained Nissl bodies (small-dark cell bodies or type B).

Classification of Cell Bodies According to Size

Total numbers of cell bodies measured were 1,800 (300 cell bodies per rat) [Figure 2]. Cell bodies were categorized according to the maximum diameter of the neuronal cell bodies. Observation in individual rats showed that maximum number of cell bodies was in the 20.00–29.99-μm diameter category in all the six rats, which mainly constitutes the small diameter cell bodies. Composite analysis for all six rats again showed that maximum cell bodies were distributed in 20.00–29.99 μm (60.22%). Fewer cell bodies were present in 50.00–59.99 μm ranges, which were generally much larger in size. The mean diameter of all the neurons in the DRG was 28.20 ± 0.18 mm (range, 10.1–59.9 mm).

Peripherin Staining for Dorsal Root Ganglia

Immunohistochemical Localization

Lower magnification represents two populations of cell bodies. One showed intense cytoplasmic (dark) staining, and the other was light stained [Figure 3A]. Intense staining is seen in cell bodies having diameter less than 40 mm, while the light-stained cell bodies were >40 μm in diameter. In addition, nerve fibers in the DRG were stained [Figure 3B]. Higher magnification showed formation of circular zone of peripherin staining around the nucleus (similar to perinuclear ring) [Figure 3D].

Classification of Cell Bodies According to Size

Peripherin stains variable sizes of neuronal cell bodies in DRG. Cell bodies were categorized according to maximum

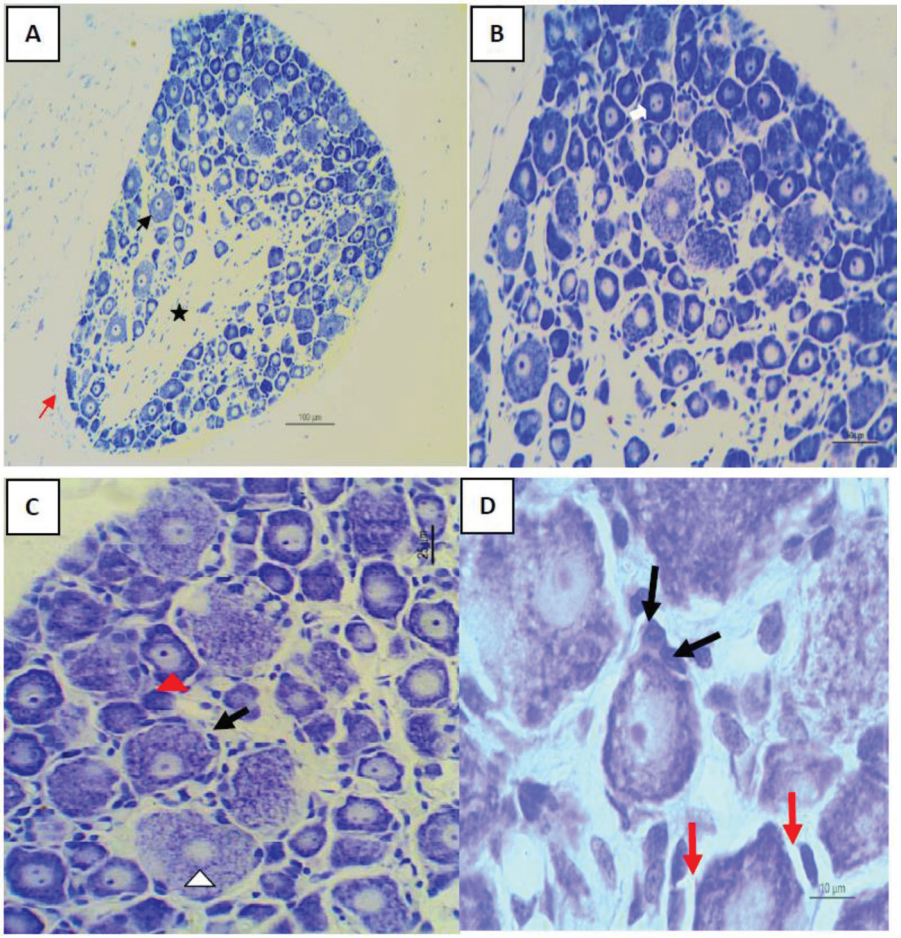

Figure 1: (A) Nissl staining of dorsal root ganglia. Varying sizes of cell bodies were observed. The tissue between the cell bodies is composed of nerve fibres with Schwann cells (*). Whole of the section is surrounded by connective tissue capsule (red arrow). (B) Few cell bodies display two nucleoli in nucleus (white arrow). (C) Satellite cells were observed around the periphery of cell bodies (black arrows). Larger cell bodies (white arrow head) have light-stained cytoplasm (large light) when compared with small cell bodies (red arrow head), which possesses darker cytoplasm (small dark). (D) The satellite glial cells are observed, and gap is seen between neuron and glial cells (red arrows).

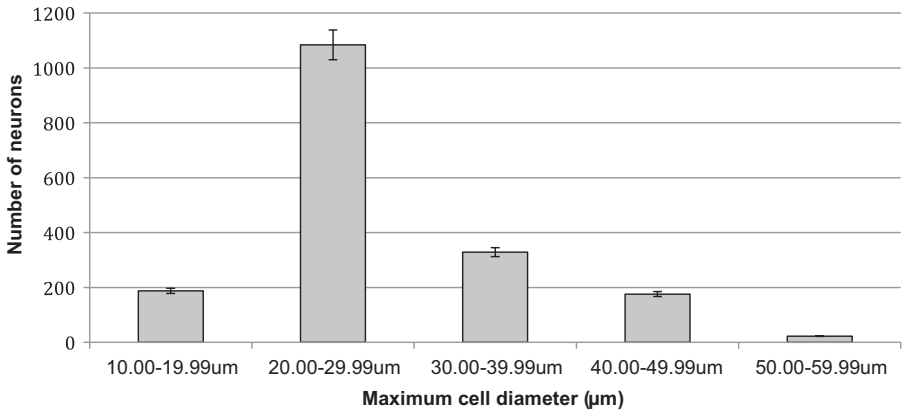

Figure 2: Frequency distribution showing cell diameter and number of cell bodies in DRG after Nissl staining. Data are shown as mean ± SEM.

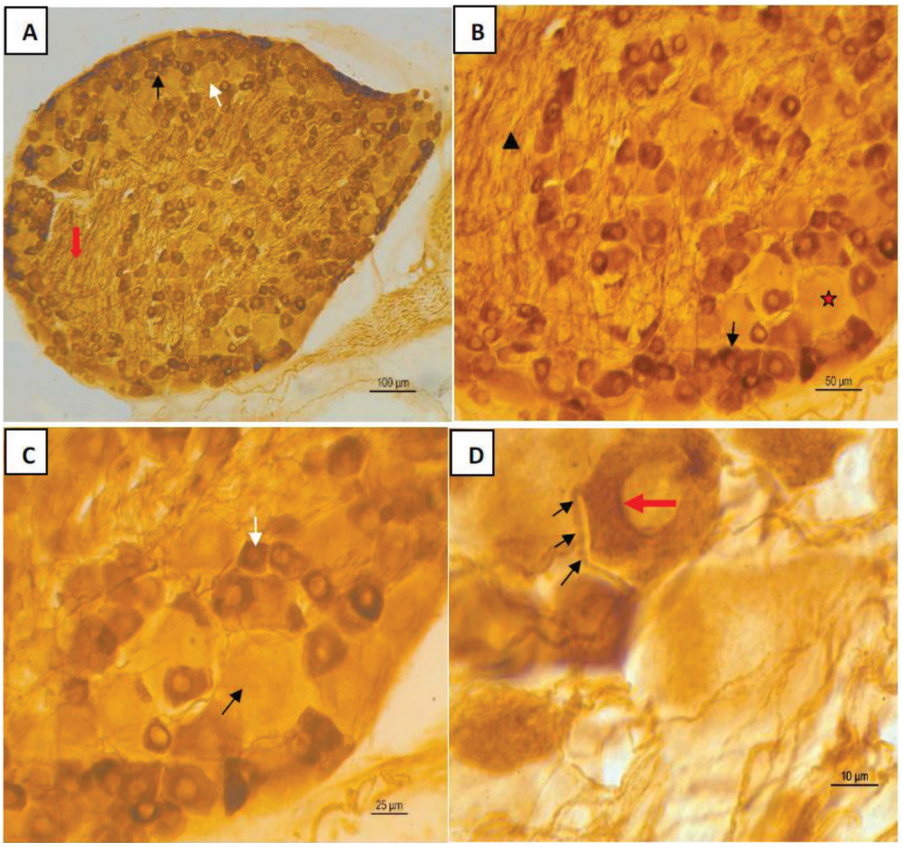

Figure 3: Peripherin immunostaining of DRG sections. (A) Low magnification view shows entire section of DRG with multiple darker (black arrow) and lighter cell bodies (white arrow) surrounded by connective tissue (red arrow) within the DRG section. (B) Maximum small- to medium-sized cell bodies (black arrow) were stained along with nerve fibres (black arrow head). Larger neuronal profile is shown by (*). (C) Several dark-stained smaller cell bodies (white arrow) along with light-stained large cell bodies (black arrow) were seen. (D) Formation of ring-like structure around the nucleus of neuron (red arrow) and the axon emerging from the neuron (multiple black arrows) can be seen.

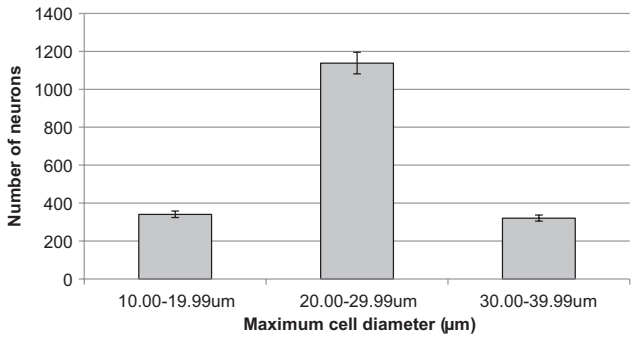

Figure 4: Frequency distribution showing cell diameter and number of small- to medium-sized cell bodies in DRG after peripherin immuno staining. Data are shown as mean ± SEM

diameter of neurons stained with peripherin. Observation showed that maximum number of neurons fall in the category of 20.00–29.99 µm diameter (63.22%). But, the neurons that are greater than 30 µm but less than 40 µm were also stained. So, the peripherin maximally stains the cell bodies up to 40 µm. Therefore, cell bodies greater than 40 µm were categorized as larger ones. The mean diameter of neuron was 25.00 ± 0.13 mm (range, 10.1–59.9 mm) [Figure 4].

Discussion

In this study, varying sizes of neuronal cell bodies were measured using Nissl stain and correlated with peripherin immunostaining. The maximum diameter of the cell bodies was evaluated so as to obtain an estimate of the small, medium, and large cell bodies. Detailed stereological measurements were not done. Nissl stains the rough endoplasmic reticulum and polyribosomal complexes in these cell bodies, which can be seen as Nissl granules under the light microscope.[9] Many of the small dark cells contain substance P or calcitonin gene-related peptide, and many of them are nociceptive in function.[10] In our study, cell bodies appeared to be of various sizes ranging from larger to smaller ones. Larger cell bodies appear to be lighter when compared wih smaller, which were intensely stained. Moreover, larger cell bodies were relatively

fewer in comparison with smaller ones. According to Nissl stain, cell bodies in DRG were grouped as small to medium (10–40 μm) and large (>40 μm). All the cell bodies revealed pale staining, vesicular nucleus, and intensely stained nucleoli. The size of the nucleolus varied from 2 to 6 μm. The nucleolus is the cellular site of rDNA gene transcription and processing of peribosomal RNA transcripts.[11] The prominence of the nucleolus of dorsal root ganglion cell bodies presumably reflects a high requirement for ribosomal gene transcription, ribosome production, and protein synthesis to maintain their large cell bodies and extensive axonal projections. In few cell bodies, double nucleoli were present but the significance was not clear, although these could be related with more extensive ribosomal protein synthesis.[12] SGCs were more numerous in number but smaller in size and situated around the entire periphery of the cell bodies. SGCs are unique in that they usually form a sheath around the individual cell bodies, which is tight but still permeable to large molecules. So, the individual neuron along with its SGCs forms isolated morphological and functional units.[13] The neuron and the SGCs are separated by a gap of about 20 nm and constitute the extracellular space around the cell bodies. Its small volume allows SGCs to control the neuronal environment.[14]

This histological classification between large and small cell bodies roughly corresponds to functional aspect of the cell bodies. The small- to medium-sized cell bodies mainly transmit the sensation carried by C and Aδ fibers such as pain and temperature, and large-sized cell bodies mainly transmit the sensation related to proprioception, vibration, and joint sense. In this study, cell bodies in the DRG were also evaluated for immunostaining of peripherin. In the previous literature, it was already mentioned that peripherin mainly shows the immunoreactivity in small- to medium-sized cell bodies in DRG but the exact range of cell bodies were not mentioned.[15] In our study, after immunohistochemical localization of peripherin, maximum size of the neurons stained was up to 40 μm. So, according to peripherin localization, small- to medium-sized cell bodies were up to the size of 40 μm, and the cell bodies above that range will fall into the large category of cell bodies. Peripherin expression was seen chiefly in the cytoplasm. In the small cell bodies, there is a formation of dark ring-like structure around the nucleus in the "Golgi zone," suggesting the synthesis area for peripherin protein. Along with the smaller cell bodies, nerve fibers in relation to these small cell bodies were also stained. Previously, similar peripherin immunoreactivity was found in fine nerve fibers.[16] Similar results were obtained in another study performed in relation to the Nav1.8 immunoexpression. They had defined the cell bodies ranging from small (27–31 μm), medium (31–40 μm), to large (40–50 μm) for the DRG.[17] Our finding correlates with findings noted by these authors.

Neurofilaments (NFs) in the mature central nervous system are composed of four subunits, light (NF-L), middle (NF-M), and high (NF-H), being about 200 kDa), and α-internexin.[18] In most adult peripheral nerves, however, α-internexin is markedly downregulated during the embryonic development to hardly any detectable levels, and its disappearance from the PNS coincides with the appearance of another intermediate filament protein, peripherin.[19] Previous data in literature have suggested that all DRG cell bodies express NF-M and NF-H, while only NF-L defines a distinct class of rat sensory ganglion cells, and in particular, only the large-light cell bodies were seen NF-L positive.[20] Mutation in peripherin is now widely applied as cause for many neurological disorders such as recessive congenital Leber's amaurosis[21] and AML.

Conclusion

In conclusion, the result of this study provide a method of classifying the DRG neurons, which could be useful for conducting electrophysiological measurements using defined parameters.

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