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Morphological heterogeneity in the cervical dorsal root ganglion neurons of mice

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Abstract

The dorsal root ganglion (DRG) neurons are said to be heterogeneous with respect to their morphology, function and neurochemistry. Light microscopic features for their categorization into different subsets remain inconclusive. The present study was attempted to outline certain important features of heterogeneity in cervical DRG neurons of mice. Five adult mice of either sex were perfusion fixed with 10% buffered formalin. Cervical DRGs from both sides were procured and processed for paraffin embedding. Observations were recorded from 10 µm-thick sections stained with Haematoxylin and Eosin. DRG neurons in general were arranged in clusters interspersed among the nerve fascicles. Most of them were round or oval in shape, ranging in sizes from 8 to 25 µm in cross section, had large centrally placed euchromatic nucleus and prominent central nucleolus. Each neuron was surrounded by 2 to 5 satellite glial cells. Some interesting observations included a) frequent (~ 30%) occurrence of binucleolate neurons; b) coarse Nissl granules forming a prominent peripheral ring and c) eccentric nucleus with central or eccentric nucleoli. It was concluded that mice cervical DRG neurons possessing the afore-said interesting features required appropriate categorization in concurrence with their ultrastructural, neurochemical and functional characteristics.

Key words: Dorsal root ganglion, satellite glial cells, cervical, Nissl substance, atypical, heterogeneous, mice.

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Introduction

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 both acute and chronic pain syndromes. DRG consists of a heterogeneous group of primary sensory neurons. Many sub-populations have been identified not only on the basis of their light microscopic morphology [1], ultra-structure [2,5], relative sensitivity to certain harmful agents [3], their involvement in certain pathological conditions [4] but also on neurochemical and immunocytochemical [6,7,8,9], electrical and functional properties [10,11]. Each DRG neuron is surrounded by a sheath formed by satellite glial cells (SGC). These cells, because of their proximity with the neurons are believed to play important roles both in health and disease but their many possible functions still remain to be uncovered. Though their role in chronic pain mechanism is not fully clear, satellite cells have been shown to undergo both morphological and biochemical changes after nerve damage [12,13]. Involvement of some atypical neurons in some of the pathological conditions especially associated with pain syndromes cannot be ruled out. The present study was aimed to identify and assess the population of neurons with atypical features using routine light microscopic techniques.

Material and Method

5 adult healthy mice of either sex, aged between 1.5 to 2 months, and weighing on average 30 gm each were included in this study. After sacrificing them by overdose of 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 with rotary microtome. Haematoxylin and Eosin stained sections were observed under light microscope (Olympus BX40, Japan). Interesting findings were recorded primarily under high power (X40 objective).

Results

The cervical DRG of mice contained two main types of cells namely neurons and glia. Neurons were larger, prominent but lesser in number while the glia cells were smaller in size, more numerous and many of them surround each neuron. Clusters of neurons were interspersed among nerve fascicles. In cross sections, the neuronal clusters assumed different size and shapes. Almost all neurons were circular to oval in outline and were of variable size ranging from 8 to 25 µm in diameter. Each cluster of neuron possessed cells of different size and there was no specific pattern in their arrangement with respect to their size. Neuronal cells were characterized by large centrally placed euchromatic, vesicular nucleus and prominent nucleolus. The number of nucleoli per neuron varied from 1-3. The perikaryon was filled with Nissl substance which assumed different appearance in terms of its overall amount, distribution pattern, size of granules, and intergranular space. Features of most of the DRG neurons matched with those described by different workers [1,2,3,4,5] for various subsets of neurons.

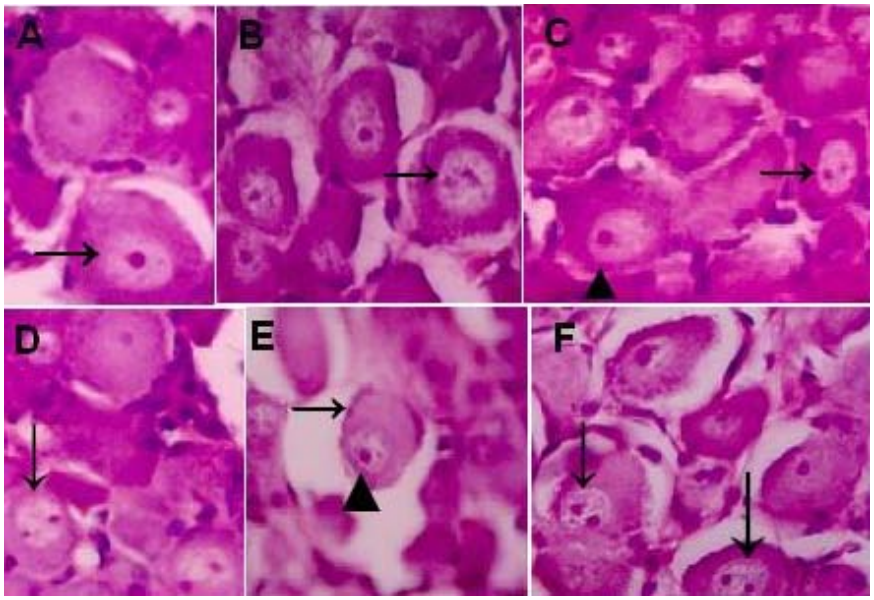

Figure 1 *Sample photomicrographs showing of cervical dorsal root ganglion neurons of mice having both typical and atypical features. Centrally placed nucleus with single, central prominent nucleolus (horizontal arrow) and diffuse Nissl substance (A). Centrally placed nucleus containing double nucleoli (arrows in B,C,D,F) and eccentrically place nucleus in C and E (dark triangle) and prominent peripheral ring of Nissl substance in E (arrow). H & E stain, x400.*

However, few neurons (Figs.1 A to F) revealed interesting features which did not fully match with the findings of aforesaid workers. For example–neuron having centrally placed nucleus with double nucleoli (Fig 1-B,C,D,F). The nucleoli were either placed together in the centre or else located separately at diagonally opposite side, and eccentrically placed nucleus (Fig. 1-C,E) and prominent peripheral rim of Nissl substance in (Fig 1- E). Thus, contrary to the most common pattern of having central nucleus some neurons possessed eccentric nuclei. Commonly, each neuron was surrounded by SGC which is said to form a sheath and thus each individual neuron along with its SGCs could be identified as isolated units. As it was seen in the cross section, the number of SGCs involved in making such perineuronal sheath varied with the size of neuronal cell body and thus it ranged from just 2 around small neuron to 5 around large neurons. However, the cytoplasm of SGC could not be satisfactorily resolved at this magnification.

Discussion and Conclusion

Sensory neurons of 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 mice remained scanty and therefore the findings of the present study were compared with low power electron-microscopic features of DRG neurons of rat [1]. It was noticed that the light microscopic features of almost all DRG neurons were comparable to one or the other groups (A_1, A_2, A_3, B_1, B_2, and C) of rat lumbar DRG [1]. However, a substantial number of neurons possessed double nucleoli which stood in clear contrast with the cervical

DRG neurons of rabbit [16] but closely matched with the trigeminal ganglion neurons of different mammals [17] and thus possibly suggested a species variation. Though significance of binucleolate neurons is not clear, role of nucleolus in the biogenesis of ribosomes is well documented [18] and therefore the binuclolate neurons possibly represented the neuronal subsets engaged in high level of synthetic activity. Again finding of neurons with coarse Nissl substance forming a single peripheral ring was not encountered in the cervical DRG neurons of rabbit [16] but has been reported in the trigeminal ganglion neurons of mammals [17]. Thus certain atypical features of cervical DRG neurons of mice matched with the rigeminal ganglions neurons of rabbit. Eccentric location of nucleus is considered to be an important feature of autonomic ganglion neurons and therefore, in the present study, the occasional occurrence of eccentric nucleus in an otherwise normal DRG (Fig. 1-C, E) assumed significance. The neuronal perikaryal response to axonal injury includes reduction in axonal caliber, development of chromatolysis and nuclear eccentricity [19]. However, in our study, the nuclear eccentricity noticed was neither due to apparent axonal injury nor it was associated with obvious chromatolytic changes. Therefore, it remains to be resolved as to whether these neurons represented a minor sub-population of normal DRG neurons or else those neurons which are undergoing routine apoptosis as a part of ageing process. From afore-said observations it may be emphasized that caution must be exercised while looking for experimental degenerative changes in DRG and considering nuclear eccentricity as one of its criteria [13,19,-20].

Mammalian DRG neurons are anatomically isolated from one another and are not synaptically interconnected. Neurons in very close contact which appeared to share common SGCs as reported earlier [16] was not seen in the present study. In such cases it is believed that cross-depolarization contributes to mutual cross-excitation. This intraganglionic dialog appeared to be mediated by an activity-dependent diffusible substance (s) [20] and provided a suitable morphological substrate for intraganglionic communication and which would have practical consequences for sensory conduction in health and disease [21].

The SGCs number associated with each neuron in the present study seemed to positively correlate with the size of neuronal cell somata which was found to be in agreement with certain other studies [22]. SGCs in sensory ganglia are believed to share many characteristics with their central counterparts and 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 [23].

Conclusion

From the present study it was concluded that a small subpopulation of cervical DRG neurons on their morphological grounds, may be considered as *atypical*. Remarkably large number of neurons bearing double nucleoli remained a matter of curiosity and appeared only partly to be a species variation and therefore, needed suitable categorization with respect to their other characteristics.

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