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Sciatic Nerve Transection-Induced Morphological Changes in the Dorsal Root Ganglia of Rabbits: A Light Microscopic Study

Mohd. Salahuddin Ansari and Aijaz Ahmed Khan

Department of Anatomy, J.N. Medical College, Aligarh Muslim University, Aligarh, India.

Abstract

The dorsal root ganglion (DRG) is the gateway for almost all modalities of sensory inputs to the brain but the etiology of various pain syndromes induced by peripheral nerve injury remains unresolved. The present study was carried on 24 healthy adult rabbit of either sex divided into four groups having 6 rabbits each. Under general anesthesia right sciatic nerve of all experimental animals were transected. After different post-lesional intervals (2d, 1w, 1m, 3m) rabbits were sacrificed and perfusion fixed by 10% formalin. Lumbosacral DRG from both sides were processed for paraffin embedding. Light microscopic observation on Haematoxyllin and Eosin stained sections from control (Left side) showed clusters of DRG neurons of different sizes interspersed among the fascicles of nerve fibers. They had round somata filled with Nissl substance and centrally placed euchromatic nuclei. Each neuron was surrounded by 3 – 15 satellite glial cells (SGCs). In the experimental group (Right side) the DRG neurons quite often showed eccentrically placed nucleus in association with degranulation of Nissl substance. Most of the affected neurons were of large and medium size. The adjoining areas of degenerating neurons were also attended with degeneration of nerve fascicle, and also showed an apparent increase in the number of satellite glial cells. Interestingly, these changes were also noticed in the contralateral (left side) lumbosacral DRG as well though they were of much lower intensity. It is concluded that unilateral spinal nerve transection induces bilateral degeneration of neurons and nerve fibres and glial cell proliferation. These changes are marked ipsilaterally and their severity is not strictly time-dependent.

Key words: Dorsal root ganglia, sensory neurons, satellite glial cells, eccentric nucleus, 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 possible implication in the 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, ultrastructure [1], and other characteristics. Each DRG neuron is surrounded by a sheath formed by SGCs. These cells are in close contact with the neurons and are said to play multiple roles both in health and disease [2], but many other possible roles played in different conditions both health and disease still remains to be understood. There is evidence that satellite cells undergo both morphological and biochemical changes after nerve damage, but there is paucity of information on their possible contribution to pain mechanism. There have been several studies on DRG in different context but many queries still remain unresolved e.g. time dependent changes on the morphology of DRG neurons, pattern of neuronal loss in different part of DRG, volumetric changes in DRG, changes in neuron density, relative vulnerability of neurons with respect to their soma size and proliferation of other cellular constituents and fibers within the ganglia. The present study was attempted to explore some of these facts.

Material and Methods

After clearance from the Institutional Animal Ethics Committee, 24 adult rabbits of either sex, aged between 12 to 15 months and weighing an average of 1.5 kg, were included in this study. Rabbits were divided in 4 groups, having 6 rabbits each. Sciatic nerve from right side was

treated as experimental and that of the left side from the same rabbit was treated as control. Animals from group l, ll, lll and lV were left postoperatively for 2 days, 1 week, 1 month and 3 months respectively. After the said duration, under general anesthesia they were fixed by perfusion method using either 10% formalin. Lumbosacral part of spinal cord and associated DRG of both sides were dissected and DRG from each spinal segment was processed separately for paraffin embedding. Rotary microtome cut 10 µm thick sections were stained with Haematoxyllin and Eosin and relevant observations were recorded in sample photomicrographs.

Results

The neurons of DRG from control (Fig. 1A, 1B) had spherical or ovoid somata of varying sizes, aggregated in clusters between fascicles of nerve fibers. Neuronal clusters were marked at the periphery of the ganglion. The nerve fibers were arranged mostly in parallel and occasionally in a crisscross manner. Each neuronal soma had a capsule of SGCs. Neurons varied not only in their sizes but also staining characteristics. The neuronal cell nucleus commonly possessed two to three nucleoli and morphological features of most of the neurons matched with one or other group described by earlier workers [1] for the DRG of rat. In the DRGs from the experimental side, some neurons had eccentrically placed nuclei. Other neurons showed loss of Nissl substance or loss of its granularity as evidenced by uniformly pink stained perikarya. Neurons with eccentric nuclei were predominantly present in experimental DRG of 1week and 1month and rarely present in DRGs of 2 d and 3 month. Increase in number of glia around many neurons was apparent. This increase in the glia was in the form of ring shaped structures. Most of these were found encircling large and medium sized neurons. Occasionally, interneuronal glial cell clusters could also be noticed in the DRG from both ipsi- and contralateral to the operated side.

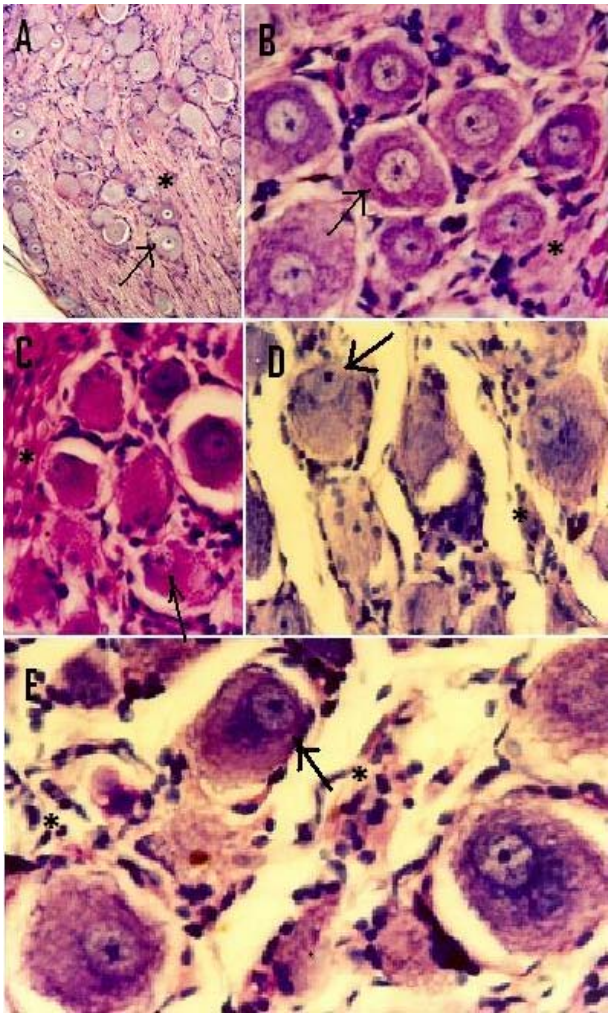

Figure 1*. Photomicrograph showing DRG from control (A & B), the distribution and characteristic features of neurons (arrow) and nerve fascicle (*) are evident. Those from the experimental side (C, D, & E) at 2d, 1w and 1m respectively show neurons with eccentric nuclei (↑) and degenerating nerve fascicles (*). H & E stain. X 200, 400 and 1000.*

Discussion and conclusion

The post-lesional gross morphological changes noticed in the proximal stump of the sciatic nerve, reported earlier were in the form of nerve fibre sprouting and formation of endneuroma [3]. In the present study of the DRGs light microscopic morphological changes were in the form of Nissl substance degranulation and nuclear eccentricity of neurons and degeneration of nerve fibres which was found to be consistent with many previous studies such as those reporting cell loss in the DRGs of rat after intercostal [4] and sciatic nerve [5] transection. Occasionally, atypical sensory neurons may possess eccentric nucleus without Nissl substance dissolution [6]. Cell loss among DRG neurons appears to be regulated by signals from the peripheral tissue [7] through nerve growth factor (NGF) which increases its receptor, p75 mRNA *in vitro* [8] and it partly prevents the loss of p75, induced by axotomy *in vivo* [9]. In the present study, it was noticed that in conjunction with the neuronal cell loss, there appeared to be proliferation of glial cells which were mainly associated with large and medium size neurons. The glial rings occurred much more frequently than the rings of nonadrenergic terminals observed after similar sciatic lesions [10]. From their distribution and frequency of occurrence, it appeared that glial rings occurred only around axotomized cells [11]. A number of morphological, biochemical and functional changes have been described in the contralateral DRG after peripheral nerve injury [12]. A transneuronal mechanism in the dorsal horn via crossed collaterals of damaged afferent axons seemed to bear a possible explanation.

Selective lesions of L5 ventral root mimicked the response to sciatic nerve transection and, direct trauma to the primary afferent appeared not mandatory for this response [7]. Further, lesions of dorsal root alone had little effect on the appearance of the rings. Two possible explanations, one based on positive [13] and other based on negative signal [14] have been suggested to account for this phenomenon. This response could be mimicked by neuromuscular blockade, indicating that the response was mediated by motor nerve activity [14]. Because large diameter neurons contain trkC [15], it is possible that muscle derived NT3 repressed the secretion of diffusible factors by sensory neurons that upregulate glial expression in adult animals. Thus, large sensory neurons deprived of NT3, either by muscle denervation (down regulation) or by peripheral axotomy, could trigger the glial response. NGF receptors are located on both neurons and glial cells of the DRG [7]. Findings of various studies [16, 17] suggested that up regulation of glial p75 after nerve trauma, might provide an ideal microenvironment for sprouting in response to neurotrophins secreted from glial cells [18]. Furthermore mRNA-NGF was significantly increased in DRGs after sciatic lesions [19] despite a reduced supply from the periphery caused by decreased synthesis of neuronal NGF receptors [20]. Role of other neurotrophins expressed in proliferating satellite cells of damaged nerves [13] remains to be investigated [21], specially with respect to the contralateral neuropathic pain [22].

It is concluded that unilateral spinal nerve transection induces bilateral degeneration of neurons and nerve fibres and glial cell proliferation and these changes are marked ipsilaterally, but their severity are not strictly time-dependent.

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Correspondence:

Aijaz Ahmed Khan
Department of Anatomy,
JN Medical College
Aligrh Muslim University
Aligarh, India
E-mail: aijazahmedkhan7@live.com