



Ectopic sympathetic ganglia cells of the ventral root of the spinal cord: an anatomical study

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Abstract: The sympathetic trunk ganglia contain the cell bodies of neurons. However, some patients who undergo sympathectomy can develop compensatory hyperhidrosis. To evaluate for ectopic pathways, the present anatomical study was performed. Ten adult cadavers underwent dissection of the spinal canal and removal of randomly selected ventral roots, which were submitted for histological analysis. Random ventral root samples were taken from cervical, thoracic, and lumbosacral regions in each specimen. Each histological section was then analyzed and the presence or absence of sympathetic cells documented for level and position within the ventral root. Of all samples, a sympathetic nerve cell was found in 80% of ventral roots. At least one sympathetic cell was found in these 80%. Most sympathetic cells were found in the proximal one-third of the ventral root. Such cells were found at all spinal levels and no specific level within a vertebral region was found to house a greater concentration of these cells. No statistical significance was found when comparing sides or sex. Our study confirmed that sympathetic cells exist in the majority of human ventral roots. Such data might better explain various clinical presentations and postoperative complications/findings.

Key words: Spine, Spinal cord, Anatomy, Sensation, Nerve roots

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Introduction

The sympathetic nervous system (SNS) is part of the visceral nervous system, also known as the autonomic nervous system (ANS). Anatomically, on each side of the spinal cord, a paravertebral sympathetic trunk exists and is attached to the ventral rami of the spinal nerves (Fig. 1). The sympathetic nerves leave the thoracolumbar (T1–L2) parts of the spinal cord and are distributed to the periphery and the viscera [1].

The SNS will increase blood pressure, heart rate, and dilate the pupils and bronchi and is the part of the ANS that responds to acute stimuli [2]. The SNS does this by secreting epinephrine from the adrenal medulla, which will also slow down the motility of the gastrointestinal tract [3]. The SNS plays an important role in temperature regulation of the skin, blood pressure control when rising from a seated position, and in the control of reproductive functions. Sympathetic output can be controlled on an organ system basis, which is crucial for maintaining homeostasis [4].

As we have identified occasional sympathetic neuronal cells bodies in ventral rootlets, the present study was performed to better elucidate this microanatomy in a large series of cadavers. As severe compensatory hyperhidrosis can complicate postoperative sympathectomy for hyperhidrosis, such anatomical findings might improve our understanding of such untoward outcomes.

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Materials and Methods

Ten adult fresh frozen cadavers (20 sides) underwent dissection of the spinal canal. Specifically, following laminectomy and opening of the dura mater, the ventral roots from their extension from the ventral horn of the spinal cord until their fusion with the dorsal roots at the intervertebral canal were harvested and submitted for histological analysis (Fig. 1). Five specimens were male and five were female with an average age at death of 85 years (range, 50–98 years). Random ventral root samples were taken from cervical, thoracic, and lumbosacral regions in each specimen. Additionally, for each harvested specimen, the adjacent sympathetic ganglion was also harvested so that its sympathetic cell bodies could be used for comparison. All specimens were placed in formalin until histology/immunohistochemistry (hematoxylin and eosin, Luxol Fast Blue, Masson's Trichrome), neurofilament (neuronal detection), S100, tyrosine hydroxylase (TH; sympathetic detection) was performed. For hematoxylin and eosin staining, sections were brought to distilled water and stained with hematoxylin for 4 minutes and then rinsed with running tap water. Next, differentiation was performed with 0.3% acid alcohol and this rinsed in running tap water followed by Scott's tap water substitute (10 g sodium hydrogen carbonate, 100 g magnesium sulphate and 5-l distilled water) then tap water again. Slides were then stained with eosin for 2 minutes. Finally, slides were dehydrated, cleared, and mounted. For

luxol fast blue staining, specimens were deparaffinized and hydrated in 95% ethyl alcohol and left in solution in an oven overnight. Next, excess stain was rinsed off with 95% ethyl alcohol and then distilled water. Differentiation was performed with lithium carbonate solution for 30 seconds then rinsed with distilled water. Continued differentiation in 70% alcohol was performed then with distilled water. Counterstaining with cresyl violet solution was performed and distilled water used to rinse. Differentiation with 95% ethyl alcohol for 5 minutes then alcohol for 5 minutes and xylene for 5 minutes.

Neurofilament H (200 kDa) antibody (Chemicon, Temecula, CA, USA; rabbit polyclonal, 1:1,000 dilution, incubation time 60 minutes, room temperature) was used. S100 (Ab-2) antibody (common marker for neural tissue) (Lab Vision, San Diego, CA, USA) with rabbit polyclonal and a dilution of 1:800 and an incubation time of 60 minutes at room temperature was used. TH antibody (Millipore, Temecula, CA, USA) with rabbit polyclonal at 1:100 and an incubation time of 60 minutes at room temperature was used. Sections were made through each harvested ventral root including longitudinal axial sections. Using a light microscope, each histological section was then analyzed and the presence or absence of sympathetic cells documented for level and position within the ventral root. For ease of analysis, each ventral root was divided into thirds. Statistical analyses (Statistica for Windows) were performed between sides and sex with statistical significance set at $P < 0.05$.

Additionally, a literature search was conducted using PubMed and Google Scholar for relevant articles relating to evidence for the presence of sympathetic ganglion cells in the ventral roots. Search terms included: "ectopic sympathetic ganglia in ventral/anterior roots," "sympathetic ganglia, ventral roots," "ectopic sympathetic ganglia," "ventral roots, hyperhidrosis," and "sympathetic trunk overstimulation." Articles in English were included from 1881-2017. Literature was excluded if there was no discussion pertaining to sympathetic root ganglia anatomy.

Results

Of all samples, a sympathetic cell (Figs. 2–7) was found in approximately 80% of ventral roots and identified via positive immunohistochemical staining. Of cervical ventral root samples, at least one sympathetic cell was identified in 81% of 10 sides, of thoracic ventral root samples, 78% of 10 sides contained at least one sympathetic cell and for lumbosacral

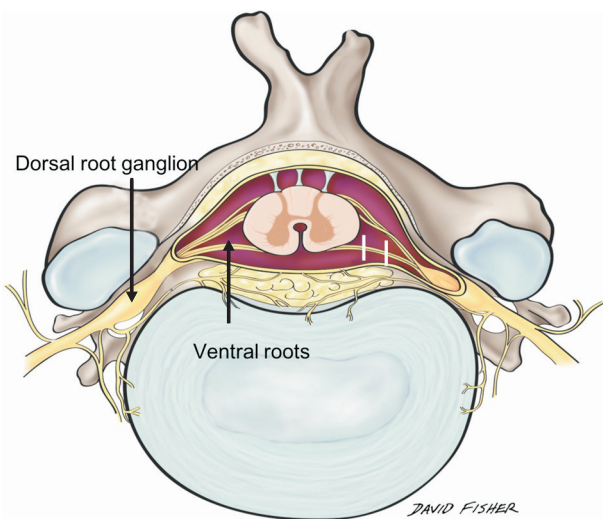


Fig. 1. Schematic drawing of the cervical region illustrating ventral roots and the adjacent dorsal root ganglion. On the left side, the current study's method of dividing the ventral root into a proximal, middle, and distal third is shown.

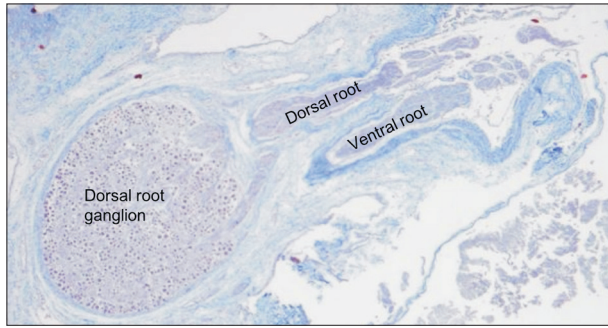


Fig. 2. Axial section through the dorsal and ventral roots (Masson's trichrome, $\times 20$).

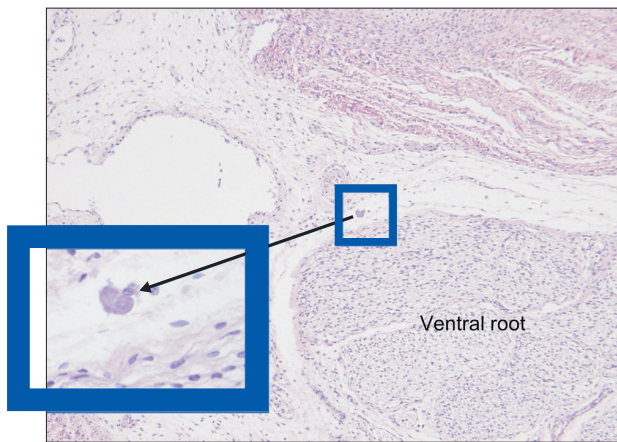


Fig. 3. Example of a sympathetic cell (small box) and its relation to the ventral root of the lumbar region (H&E, $\times 12.5$). The larger box illustrates the magnified version of this cell, which is just on the periphery of the ventral root in this specimen.

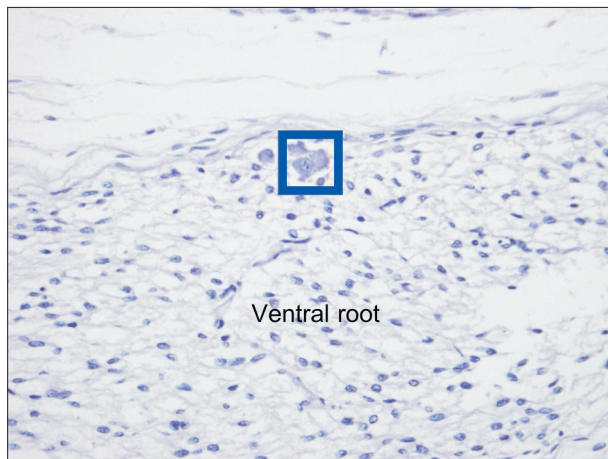


Fig. 4. Sympathetic cell embedded in the ventral root of the cervical region (Luxol Fast Blue, $\times 25$).

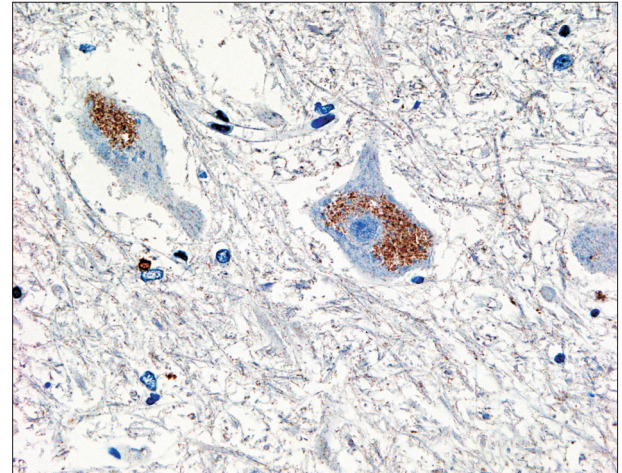


Fig. 5. Sympathetic neuronal cell body in the thoracic region shown with NeuN ($\times 40$).

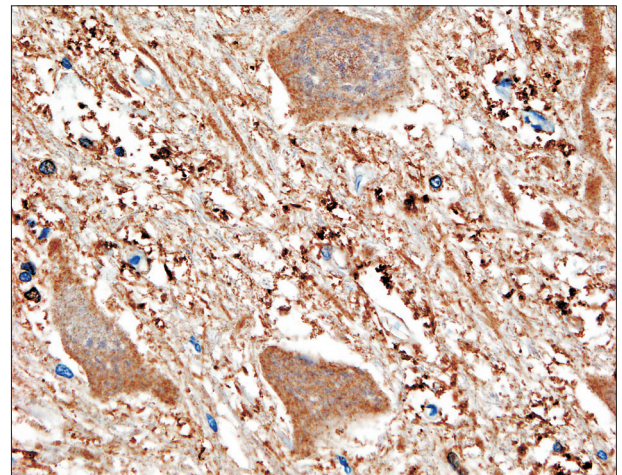


Fig. 6. Sympathetic neuronal cell body of the lumbar region shown with S100 ($\times 40$).

ventral root samples, 82% of 10 sides were found to have at least a single sympathetic cell. Neurofilament and S100 (Figs. 6, 8) both stained the cytoplasm of the ganglion cells well and TH (Fig. 7) stained some neurons and occasional background axons. The TH demonstrated granular cytoplasmic immunoreactivity. NeuN (Fig. 5) stained both the nuclei and cytoplasm of these neuronal cell bodies and cytoplasm immunopositivity was more evident and pronounced. At least one sympathetic cell was found in these 80% with a range of 1–4 cells (mean, 1.5) per ventral root. In general, the ectopic sympathetic cells were found in the proximal one-third (i.e., closer to the spinal cord) of the ventral root (Fig. 2). However, occasionally, these cells were found more distally (i.e., near

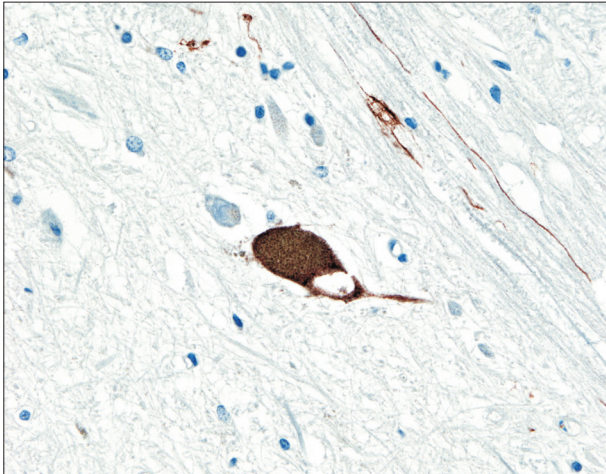


Fig. 7. Sympathetic neuronal cell body of the sacral region shown with tyrosine hydroxylase ($\times 40$).

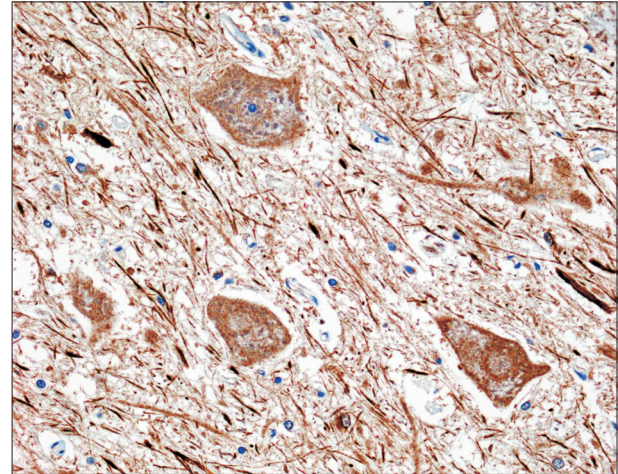


Fig. 8. Sympathetic neuronal cell body of the sacral region shown with neurofilament ($\times 40$).

the intervertebral foramen) in the ventral root but these were always in the middle third with none found in the distal third. Such cells were found at all spinal levels and although not significant, were most often seen in cervical and lumbosacral levels. No specific level within a vertebral region was found to house a greater concentration of these cells. Roughly one-half of all ectopic sympathetic were found within the periphery of the ventral root (Fig. 3) and one-half within the ventral root (Fig. 4). All ectopic sympathetic cells had a similar histology to the sympathetic cells sampled from the sympathetic ganglia of the sympathetic trunk of the adjacent spinal level. No statistical significance was found when comparing sides or sex.

Discussion

Our study confirmed the presence of sympathetic ganglion cells in the ventral roots of human cadaveric specimens. Although the exact function cannot be elucidated from a postmortem study, the circuitry of such cells can be theorized based on clinical and experimental animal data.

Experimental and histological evidence

Bell and Magendie laid the foundation (i.e., Bell Magendie law) for the understanding that dorsal and ventral spinal roots have specificity to sensory and motor functions, respectively [5]. Since then, it has been generally accepted that the dorsal root contains sensory axons and that the ventral root consists of axons from motor neurons [6]. However, histological studies conducted by Matthews and colleagues [7, 8] showed the

evidence of presence of some motor fibers in the dorsal roots. They were able to see that one month after a dorsal root section in cats, activity in the nerve fibers remained. Matthews and Barron suspected from their histological studies that certain fibers in the dorsal roots were motor in nature [5]. It is thought that the axons may take different routes and that some of the efferent fibers might come directly from cells within the spinal cord, while others could arise from neurons whose cell bodies are in spinal ganglia at differing spinal levels [5].

There is evidence from animal studies on catecholamine (CA) containing neurons that sympathetics, verified with positive TH staining, may leave through the ventral roots [9]. TH is a useful marker for CA synthesis and is the first enzyme in CA biosynthesis and catalyzes L-tyrosine to L-DOPA conversion. These neurons showed spontaneous activity, and there is evidence they were sprouting in the ventral roots or dorsal roots as evidenced by cutting the dorsal ramus and ventral ramus significantly reduced spontaneous activity of the sympathetic fibers [10].

Sensations perceived after stimulation of the ventral roots

Some patients have been reported non-somatic motor sensations when the ventral roots were stimulated [11-14]. These findings have been confirmed in several animal studies, specifically by the findings of receptive fields carried by fibers in the ventral root [15-23]. Coggeshall et al. [19] showed, by using electron microscopy, that in animals, like the cat, nearly

30% of the fibers are unmyelinated and arise from the dorsal root in the seventh lumbar and first sacral ventral roots. These findings suggest that a large number of fibers of the ventral root may be sensory. After this discovery, Clifton et al. [20] performed a physiologic study of S3 and S4 segments in cats. They found evidence of unmyelinated afferent fibers attached to receptive fields in the periphery, which means that much of the information enters the spinal cord via unmyelinated fibers through the ventral roots [6]. Therefore, our study identified sympathetic cell bodies in the ventral root is not surprising.

Clinical relevance

Clinically we can apply these concepts of the presence of sympathetic ganglia cells in the ventral roots by looking at the effects of sympathectomies and hyperhidrosis. Sweating is thought to be mediated primarily by the SNS especially in times of stress. However, there are times in which the SNS goes on overdrive leading to conditions such as essential hyperhidrosis. Essential hyperhidrosis ranges in prevalence from 1%–3% of the population [24, 25] and is defined by experiencing a disproportionate amount of sweating for an extended period of time. It may affect multiple parts of the body; however the most common areas are the hands, armpits, feet, head and inguinal area [26-28]. A treatment option for essential hyperhidrosis is to perform a sympathectomy. This surgery, for palmar hyperhidrosis, entails removing the sympathetic ganglia between T2–T4 or T5 and is often successful. However, in a study done by Baumgartner and Toh, it was shown that in some patients compensatory hyperhidrosis occurred. This might be explained by the presence of sympathetic ganglia in the ventral roots remaining even after the sympathectomy. In their study, these authors reported 309 patients with either palmar hyperhidrosis or axillary hyperhidrosis [29]. The procedure completely corrected the hyperhidrosis in nearly 100% of patients with palmar hyperhidrosis and 68% of those with axillary hyperhidrosis [29]. However, of the patients who underwent thoracoscopic sympathectomy, 1.3% of them developed severe compensatory hyperhidrosis [29].

In sum, our findings elucidate ectopic sympathetic neurons in the ventral root of the human spinal cord. Such findings are important for better understanding of the variations of the human nervous system [30-33].

Conclusions

Our study confirmed that sympathetic ganglion cells exist

in the majority of human ventral roots. While the function of these ectopic neurons needs further investigation, such a finding might contribute to poor outcomes following some sympathectomies where hyperhidrosis is recalcitrant or in patients who develop compensatory hyperhidrosis following such procedures.

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Conceptualization: ML, RST. Data acquisition: MMA, EH, JI. Data analysis or interpretation: CM, MMA, EH, JI. Drafting of the manuscript: CM, MMA, EH. Critical revision of the manuscript: JI, RST. Approval of the final version of the manuscript: all authors.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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