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Morphometric Analysis of the Rat Spinal Trigeminal Nucleus

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This study presents a morphometric analysis of neuronal size within the oral (SpVo), interpolar (SpVi), and caudal (SpVc) subnuclei of the spinal trigeminal nucleus (SpV) in rats. Using classical staining and morphometric techniques, we found distinct distributions of small-, medium-, and large-sized neuronal cell bodies across the subnuclei. In general, the SpV consists of over 80% of neurons within the 5–15 micrometer range of their perikarya, with 15% classified as medium-sized (15-20 micrometer range), and 5% as large (above 25 micrometers in diameter). Moreover, the three subnuclei exhibited differing proportions of neuronal soma sizes, suggesting functional specialization within the SpV. This morphometric analysis underscores the heterogeneity of neuronal populations within the spinal trigeminal nucleus and provides insights into its role in somatosensory processing. Understanding neuronal soma size distributions in the SpV enhances our knowledge of its functional organization and may inform future studies investigating sensory integration and pain modulation within the trigeminal sensory system.

Key words: spinal trigeminal nucleus, neuronal size, morphometric analysis, rat

Introduction

A crucial node in the somatosensory pathway, the spinal trigeminal nucleus (SpV) is involved in the transmission and processing of sensory data from the face and mouth cavity [14]. The nucleus is positioned to process thermal and nociceptive impulses within the medulla oblongata. The SpV is separated anatomically into three distinct subnuclei: subnucleus caudalis (SpVc), subnucleus interpolaris (SpVi), and subnucleus oralis (SpVo) [10]. These subnuclei have different cytoarchitectural characteristics

with some of the features being neuronal size, shape, and distribution pattern [14]. The neurons' size, shape, and complexity vary widely based on the underlying function. Hence, the objective of this work is to clarify the neuronal size properties of the SpV subnuclei, offering important insights into their contributions to somatosensory perception.

Materials and Methods

The study was conducted on mature Wistar rats, using a total of 12 male rats with body weights ranging from 180 to 300 g. Adherence to ethical guidelines for experimental animal research in Bulgaria was ensured, following protocols approved by the Ethics Committee of the Institute of Neurobiology, Bulgarian Academy of Sciences (registration FWA 00003059 with the US Department of Health and Human Services), and the Research Ethics Committee at the Medical University of Sofia, as per Directive 2010/63/EU on the protection of animals used for scientific purposes. Initially, the experimental animals were briefly anesthetized with ether, followed by intraperitoneal administration of thiopental (Sigma-Aldrich) at a dose of 40 mg/kg to maintain anesthesia. Cannulation of the ascending aorta via the left ventricle was conducted for perfusion. The circulatory system was washed with 0.05 M phosphate-buffered saline (PBS), pH 7.36, and fixed using 4% paraformaldehyde (Merck) in 0.1 M phosphate buffer for approximately 20 minutes. Following brain removal, the region of interest spanning from the midbrain to the upper spinal cord was dissected. Tissue blocks were postfixed overnight at 4°C in the same fixative, washed thoroughly with tap water, and subsequently processed for embedding in paraffin. Conventionally, 7 μm thick tissue sections were cut and mounted on chrome-gelatinized slides, stained with toluidine blue (500 mg of dye to 100 ml of distilled water) for 5-10 min. Following dehydration, sections were embedded in Entellan (Merck). Observations and photography of the specimens were conducted using an Olympus VS120-L100 Virtual Slide System light microscope. The scanned images were saved in TIF format, and subsequent morphometric analysis of the digital images was undertaken. Over one hundred and seventy images were taken from all three parts of the SpV and we measured the size of over 1000 neurons with the Fiji image processing package [12].

Results

Our morphometric analysis using the Fiji program identified three distinct subgroups of neurons within the SpV based on the sizes of their cell bodies, as shown in **Figure 1.** Neurons classified as small had somatic diameters ranging from 5 to 15 μm, with a mean diameter of 9.89 μm ± 2.16 (n = 798). These neurons represent approximately 79.7% of the total population of neurons within the SpV (Fig. 1). On average, medium-sized neurons possess perikarya with a mean diameter of 16.66 μm ± 1.51 (n = 151), falling within the range of 15 to 20 μm. These neurons make up approximately 15% of the total population of neurons in the nucleus. Neurons categorized as large had a somatic diameter of greater than 25 μm, with a mean diameter of 26.68 μm ± 4.58 (n = 50). In

addition, one very large (giant) neuron was observed with dimensions exceeding 40 μm in somatic diameter (n = 1). Large-sized neurons collectively represent approximately 5% of the total population of neurons within the rat SpV.

Further analysis revealed different distributions of neuronal soma sizes within individual SpV subnuclei (**Figs. 2, 3, and 4**). Specifically, in the SpVc, small cells constituted the majority of neurons (almost 85%), medium-sized neurons represented 14.93%, followed by large-sized neurons, which accounted for less than 1% of all neurons in the nucleus (**Fig. 2**). The SpVi was dominated by small neurons (86%), followed by medium-sized neurons (7.46%), large neurons (6.47%), and sporadic giant neurons (less than one percent) (**Fig. 3**). Specifically, the largest cell body diameter per neuron was measured in the SpVi at 43.93 μm. It is in this subnucleus that the large in size neurons scattered among smaller ones make an impression. In the SpVo, small neurons are almost three times more numerous than medium-sized cells, making up 70% and about 23%, respectively, while large neurons account for almost 7% of all cells in it (**Fig. 4**).

The average diameter of small neurons in the rat SpV ranged from 8.84 to 10.52 μm, that of medium neurons was 16.39 to 16.80 μm, and that of large neurons ranged from 25.45 to 27.82 μm in all subnuclei.

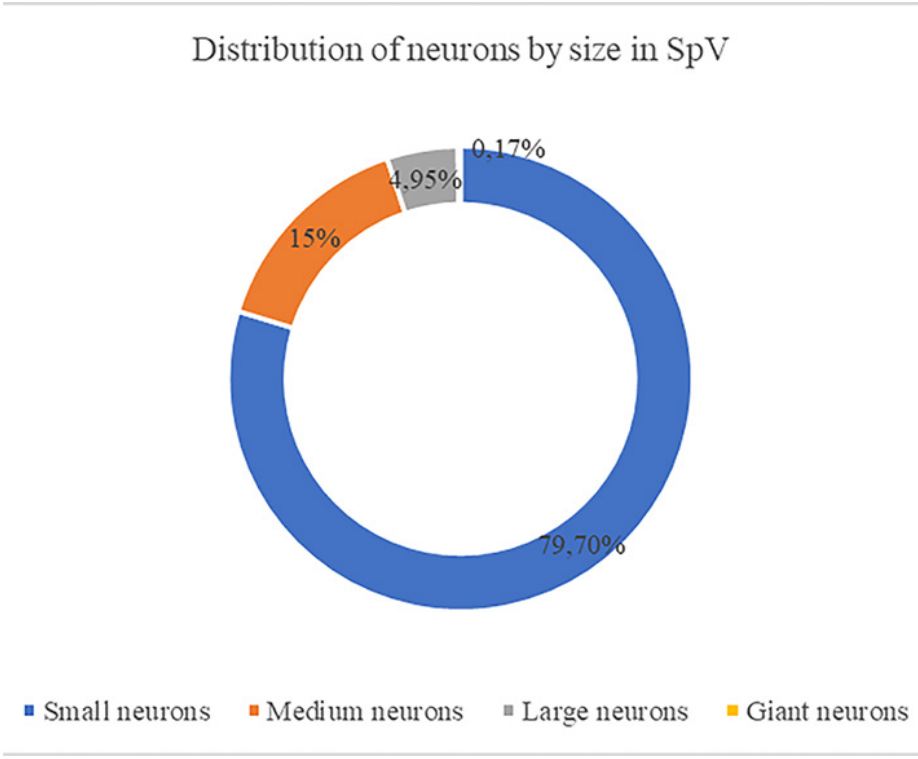

Fig. 1. Percentage distribution of different-sized neurons in the spinal trigeminal nucleus (SpV). Small-sized neurons make up almost 80% of the total neuronal population in the SpV, followed by medium-sized with 15%, and neurons large in size that constitute up 5% of the population. Giant neurons are less than 1%.

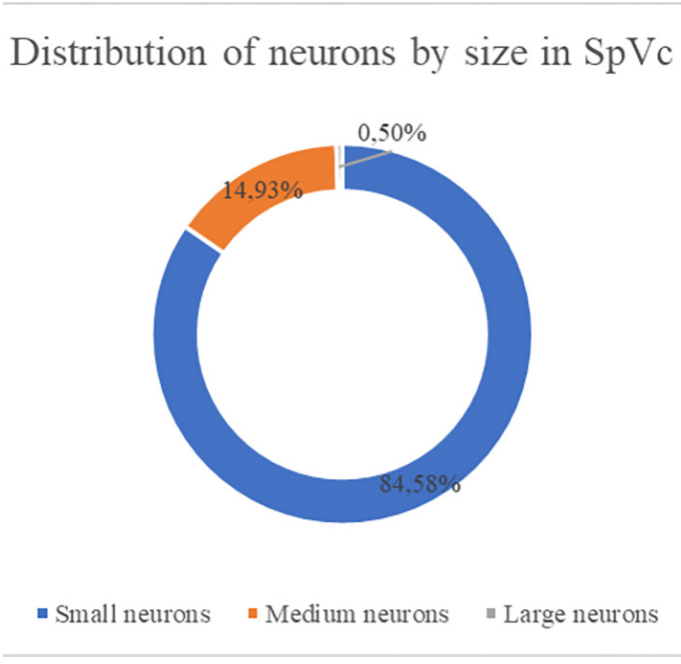

Fig. 2. Percentage distribution of different-sized neurons in the caudal spinal trigeminal subnucleus (SpVc). The predominant neuronal population consists of small cells, comprising nearly 85% of all neurons. Medium-sized neurons constituted approximately 14.93%, with large-sized neurons making up less than 1% of the total neuronal count.

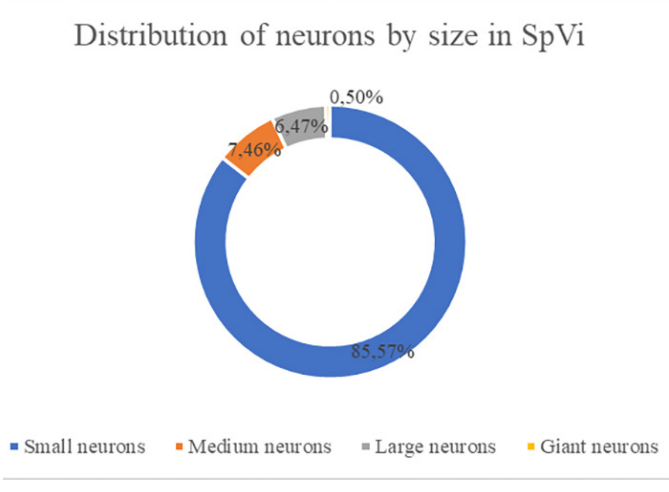

Fig. 3. Percentage distribution of different-sized neurons in the interpolar spinal trigeminal subnucleus (SpVi). Small neurons are the predominant type, accounting for 86% of the total neuronal population, followed by medium-sized neurons at 7.46%, large neurons at 6.47%, and occasional giant neurons, constituting less than one percent of the total neuronal population.

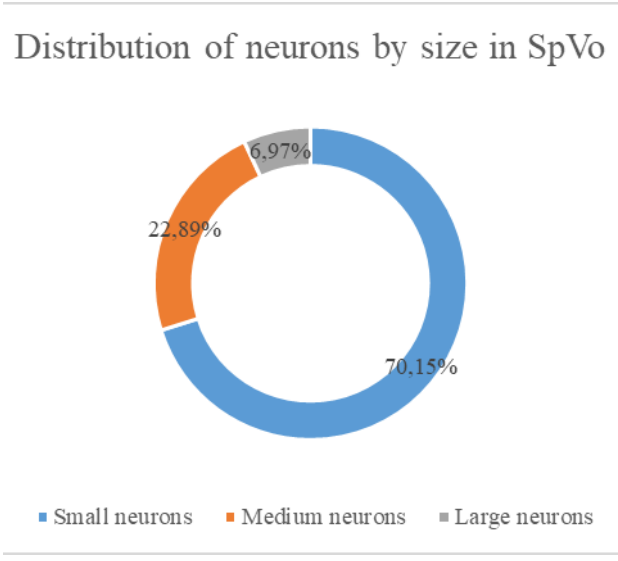

Fig. 4. Percentage distribution of different-sized neurons in the oral spinal trigeminal subnucleus (SpVo). Small neurons outnumber medium-sized cells by nearly threefold, comprising approximately 70% of the total, whereas medium-sized neurons represent about 23%. Large neurons constitute nearly 7% of all cells within this region.

Discussion

The SpV is a critical component of the trigeminal sensory system that is responsible for processing sensory information from the face and head [14]. Understanding the structural characteristics, specifically the size and morphology of neurons in these subnuclei is essential for unraveling the functional organization of the trigeminal sensory system. Supporting the descriptions already made with statistical analysis, our results show that this part of the nucleus contains unique neurons that can be divided into three groups according to the size of their cell bodies.

In the study of the SpV, understanding the size, shape, and distribution of its neuronal population is crucial for elucidating its functional organization and potential implications in sensory processing. We have examined the diverse morphologies of neurons within the rat SpV, encompassing bipolar, pyramidal, pear-shaped, boat-like neurons, among others, identifying at least 7 different neurons types (unpublished data). While the shape of neurons has been explored, the relationship between neuronal soma size and shape, and how these factors collectively contribute to sensory processing, remains an open question.

Certainly, building upon our prior investigations into the cytoarchitectonics of the SpV, this study endeavors to deepen our understanding of its organizational principles by examining the size characteristics of its constituent neurons. Our previous work has provided invaluable insights into the laminar organization, cellular composition,

and structural arrangements within the nucleus [7]. By leveraging this foundational knowledge, we tried to extend our inquiry to elucidate the size-related features of neurons within the SpV.

The size of neurons within the SpV can vary significantly, indicating potential functional diversity and specialization. Neuronal size is often correlated with various physiological properties such as firing rate, synaptic connectivity, and neurotransmitter phenotype [1]. Investigating neuronal soma size can provide insights into the metabolic demands, dendritic arborization, and integration of synaptic inputs within the spinal trigeminal circuitry. Several studies using various histological techniques and immunohistochemistry [4, 5, 6, 8, 13] have consistently reported differences in neuronal soma size among SpVo, SpVi, and SpVc. Previous studies of the morphology of neurons in the SpV categorized neurons in this nucleus based primarily on the size and shape of their cell bodies [6, 8, 9].

Studies on the human oral subnucleus have delineated two major categories of neurons [11, 13]. The first category consists of small rounded or fusiform cells (8-10 μm in diameter) clustered in small clusters or evenly spaced. The second category comprises large neurons (22 μm in diameter) with various morphology. Two of the subnuclei of the SpV have been extensively studied in different species. In the feline interpolar subnucleus, for example, one study has revealed the presence of five different cell types [9]. These types exhibit different morphologies and the diameters of these neurons vary widely from 6-12 μm to 15-25 μm. On the other hand, three major types of neurons are present in the rat oral subnucleus based on their shape and size [2, 3]. The sizes of the neuronal bodies range from 5-15 μm to 25-50 μm in diameter. Focusing on the size of neurons within the subnuclei of the SpV represents a logical progression in our understanding of its functional organization and sensory processing mechanisms.

Conclusion

In conclusion, the structural heterogeneity of neurons within the three subnuclei of the SpV has been well described in the literature, and there are mixed reports regarding the size of neurons within them. Moreover, the discrepancies in the external morphology of spinal trigeminal neurons observed in different studies highlight the complexity of this brain structure. Hence, by complementing previous investigations on neuronal shape and cytoarchitectonics, this study reveals the intricate relationships between neuronal morphology, size, and distribution, ultimately advancing our comprehension of somatosensory integration and relay within the trigeminal sensory pathway.

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