

NEW INSIGHTS INTO THE CYTOARCHITECTURE OF THE RAT SPINAL TRIGEMINAL NUCLEUS

Andrey Ivanov¹, Dimitrinka Atanasova^{2,3✉}, Nikolai Lazarov^{1,2}

Received on May 19, 2024

Presented by W. Ovtscharoff, Member of BAS, on May 28, 2024

Abstract

The spinal trigeminal nucleus (SpV) is a crucial relay station in the central nervous system for processing sensory information from the face, head, and oral cavity. While historically considered a continuous structure, pioneering work by Olszewski revealed its subdivision into three distinct subnuclei: oral, interpolar, and caudal. Understanding the cytoarchitectonics of these subnuclei is fundamental for elucidating their functional roles in sensory perception and pain modulation. In this study, we conducted a detailed examination of the structural organization within each subnucleus of the SpV using histological techniques. Our findings reveal distinct cytoarchitectonic features characteristic of each subnucleus by providing insights into the spatial distribution and density of neuronal populations across the rostrocaudal extent of the SpV. These results contribute to a deeper understanding of the neural circuitry underlying sensory processing in the trigeminal system and may have implications for the diagnosis and treatment of disorders involving altered trigeminal function.

Key words: spinal trigeminal nucleus, subnuclei, cytoarchitecture, trigeminal sensory system, rat

Introduction. The spinal trigeminal nucleus (SpV) is a vital structure within the central nervous system responsible for relaying sensory information from the face and oral cavity to higher brain centres. Situated within the caudal brainstem, specifically in the medulla oblongata, the SpV plays a crucial role in processing various sensory modalities, including pain, temperature, and touch,

<https://doi.org/10.7546/CRABS.2024.07.11>

essential for maintaining homeostasis and coordinating sensory responses [1]. Traditionally, the SpV was perceived as a continuous structure without clear subdivisions. However, groundbreaking work by OLSZEWSKI [2] in the mid-20th century revolutionized our understanding of its anatomical organization. Olszewski's meticulous anatomical studies on material from man and *Macaca mulatta* revealed that the SpV consists of three distinct subnuclei: the oral (SpVo), interpolar (SpVi), and caudal (SpVc) subnuclei [2]. Additionally, the caudal trigeminal subnucleus is subdivided into three distinct regions, mirroring the architecture of the apex of the spinal dorsal horn, the lateralmost area known as the subnucleus zonalis, followed by the subnucleus gelatinosus, and finally, the medial subnucleus magnocellularis. These subnuclei exhibit unique cytoarchitecture and receive inputs from different regions of the face and oral cavity, contributing to the complex sensory processing carried out by the SpV [3]. The cranial nerves V, VII, IX, and X carry the peripheral nociceptors that transmit the pain signal to these fibres [4]. The sensory fibres bundle and pierce the spinal trigeminal nucleus when they reach the brainstem. Functionally, the SpV serves as a critical relay station for sensory information travelling from the trigeminal nerve to higher brain centres, including the thalamus and cortex. Its involvement in processing nociceptive stimuli makes it particularly relevant in pain perception and modulation.

In light of the significance of the SpV and its subnuclei in sensory processing, this study aims to comprehensively examine the cytoarchitecture of the SpV. By meticulously analyzing the cellular organization within each subnucleus, we seek to enhance our understanding of the functional specialization and neural circuitry underlying sensory processing in the trigeminal system. A comparative analysis of these subnuclei, whose precise localization was determined according to coordinates from PAXINOS and WATSON's stereotaxic atlas of the rat brain, enhances our understanding of their complex microarchitecture at specific levels [5].

Material and methods. The experiments were conducted on mature Wistar rats. A total of 12 male rats weighing between 180 and 300 g were used in our investigation. All procedures adhered to the guidelines for experimental animal research in Bulgaria, following the protocols of the Ethics Committee of the Institute of Neurobiology at the Bulgarian Academy of Sciences (registration FWA 00003059 with the US Department of Health and Human Services), and those of the Research Ethics Committee at the Medical University of Sofia by Directive 2010/63/EU on the protection of animals used for scientific purposes.

Initially, the experimental animals were anesthetized with ether, followed by an intraperitoneal injection of thiopental (Sigma-Aldrich) at a dosage of 40 mg/kg to maintain anesthesia. Cannulation of the ascending aorta via the left ventricle was performed for perfusion. The circulatory system was flushed with 0.05 M phosphate-buffered saline (PBS), pH 7.36, followed by fixation using 4% paraformaldehyde (Merck) in 0.1 M phosphate buffer (PB) for approximately 20 min. 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, then thoroughly washed with tap water and subsequently processed for embedding in paraffin. Paraffin blocks were sectioned at 7-µm thickness, tissue sections were then mounted on chrome-gelatinized slides, and after rehydration, they were stained with Hematoxylin and Eosin (HE), toluidine blue or neutral red to describe the cytoarchitecture of the nucleus and its structural components. Following dehydration, sections were embedded in Entellan (Merck). The specimens were observed and photographed using an Olympus VS120-L100 Virtual Slide System research light microscope. The scanned digital images were saved in TIF format, and subsequent morphometric analysis of them was performed.

Results. The SpV represents the largest trigeminal nucleus and extends into the lateral tegmentum of the medulla oblongata and caudal part of the pons. The nucleus is composed of neurons that have a distinct cell body with sporadic Nissl bodies surrounded by a network of myelinated axons. Myelinated fibres can also be observed around the nucleus. Along its course from rostral to caudal, the SpV is subdivided into three anatomically distinct subnuclei: oral, interpolar, and caudal (Fig. 1–3).

Caudal trigeminal nucleus. The most prominent sensory trigeminal subnucleus spans from the lowermost part of the medulla oblongata, reaching from the caudal pole of the inferior olive and obex to the second cervical spinal cord segment (Fig. 1–3E). It seamlessly connects with the SpVi towards the rostral end, blending with the spinal cord's posterior horn caudally. The subnucleus zonalis presents as a thin layer housing a sparse population of neurons (Fig. 1–3F), notably characterized by the presence of large irregularly elongated multipolar cells, some reaching diameters of up to 21 µm (Fig. 3F). Amidst these, medium-sized neurons with discernible Nissl bodies are interspersed, alongside smaller neurons. Transitioning medially into the gelatinosus subnucleus, neuron density notably increases. Here, the gelatinosus subnucleus comprises predominantly small oval or elongated neurons, with typical diameters of up to 15 µm, displaying relatively large nuclei and scant cytoplasm forming a thin pale perinuclear ring. The magnocellular subnucleus, albeit relatively larger compared to the gelatinosus subnucleus (Fig. 1F), primarily consists of irregularly oval medium-sized cells, approximately 12 µm in diameter, with distinct basophilia (Fig. 1–2F). Despite its name, only a fraction of neurons are notably large, with diameters seldom exceeding 21 µm. Along its course, the caudal spinal trigeminal subnucleus is bordered laterally by the caudal part of the spinal tract of the trigeminal nerve. Dorsally, it is flanked by the cuneate fasciculus, while medially and ventromedially lies the dorsal subnucleus of the central reticular nucleus of the medulla oblongata, together with the ascending spinal tracts, marking its ventral boundary. At its transition into the spinal cord, the caudal spinal trigeminal subnucleus forms a distinct lump-like apex, extending medially from lamina V of the spinal cord.

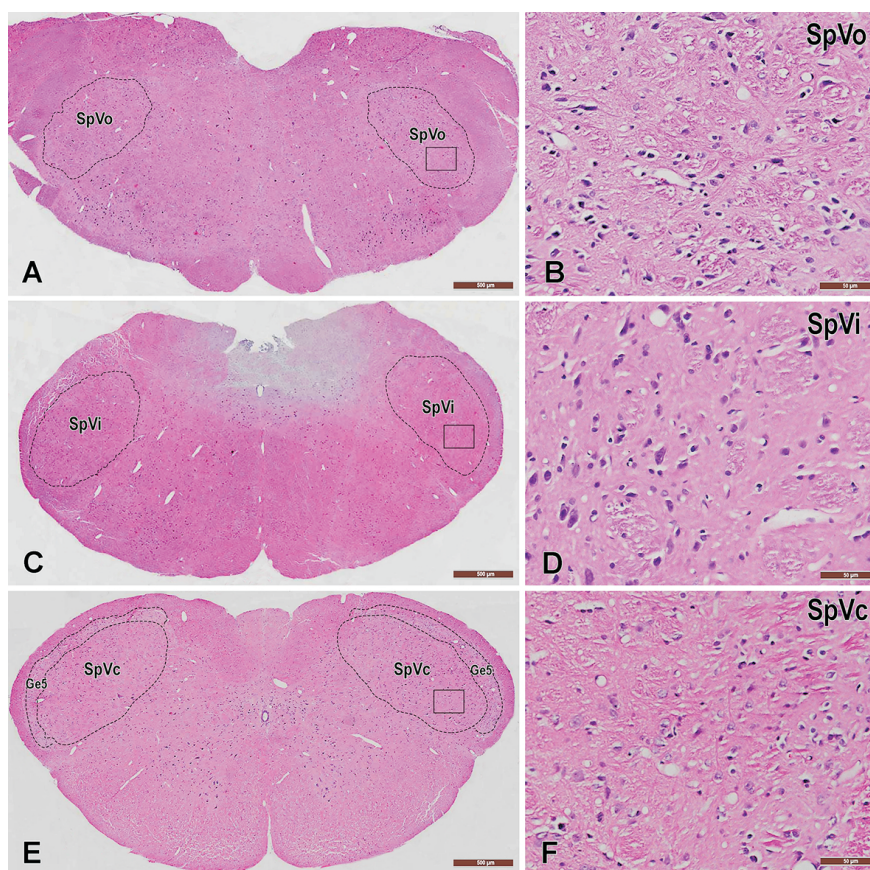


Fig. 1. A coronal H&E-stained section through the rat brainstem at the level of the spinal trigeminal nucleus (SpV). (A) Low-power photomicrograph showing the location of the oral spinal trigeminal nucleus (SpVo). (B) The higher magnification of the boxed area in (A) demonstrates small- and medium-sized, irregularly shaped neurons. (C) Low-power view showing the location of the interpolar spinal trigeminal nucleus (SpVi). (D) The higher magnification of the boxed area in (C) shows a heterogeneous population of neurons with variation in the size of the cell bodies. (E) Low-power photomicrograph showing the location of the caudal spinal trigeminal nucleus (SpVc). (F) Small neurons can be seen on a higher magnification of the boxed area in (E). Ge5, gelatinous subnucleus. Scale bar: 200 µm (A, C, E), 50 µm (B, D, F)

Interpolar trigeminal subnucleus. This nucleus is located in the medulla oblongata. Rostrally it is continuous with the SpVo and caudally with the SpVc. The caudal pole of the SpVo is located somewhat rostral to the caudal pole of the inferior olivary nucleus. The population of neurons is heterogeneous (Fig. 1–3D). The majority of cells are small to medium in size (ranging between 6 and 15 µm), irregularly oval or elongated, with moderate, diffuse basophilia (Fig. 2D). They are similar to cells in the oral subnucleus. Characteristics of this nucleus

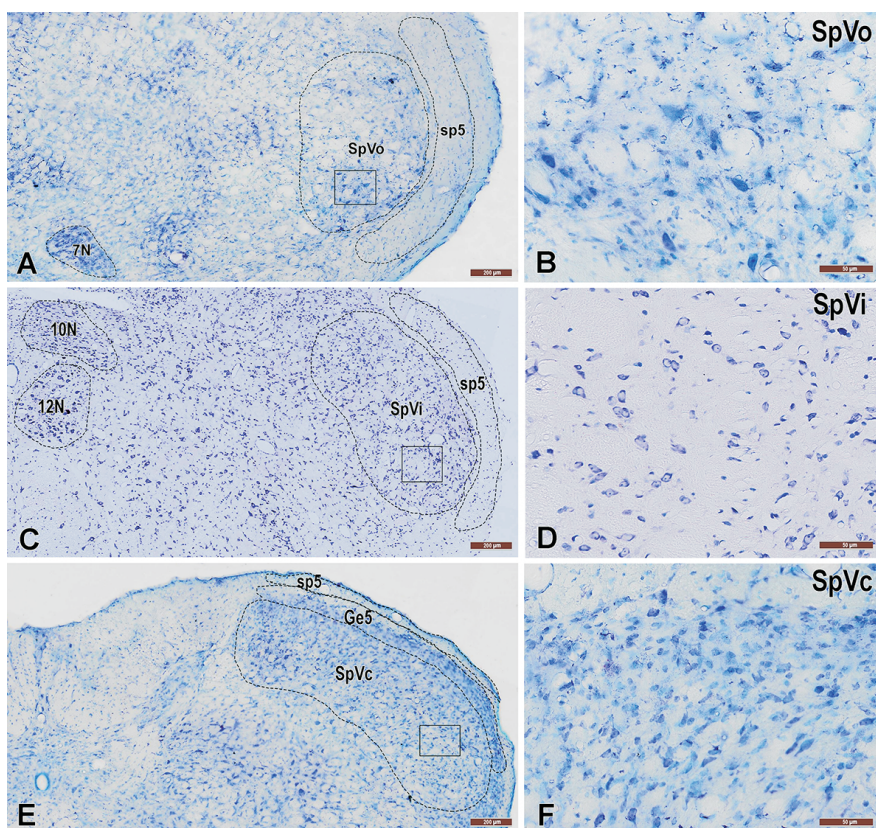


Fig. 2. A coronal section, stained with toluidine blue, across the rat brainstem at the level of the spinal trigeminal nucleus (SpV). (A) A section from the rat brainstem at the level of the oral spinal trigeminal nucleus (SpVo). The boxed region in (A) is shown at a higher magnification in (B). Note the distribution of the neurons around unstained bundles of nerve fibres. (C) Low-power photomicrograph showing the location of the interpolar spinal trigeminal nucleus (SpVi). (D) The higher magnification of the boxed area in (C) shows the polygonal neurons with moderately stained cytoplasm. (E) Low-power photomicrograph showing the location of the caudal spinal trigeminal nucleus (SpVc). (F) Small neurons of the gelatinous nucleus (top) and the magnocellular nucleus can be observed on a higher magnification of the boxed area in (E). Ge5, gelatinous subnucleus; sp5, spinal trigeminal tract; 10N, dorsal nucleus of the vagus nerve; 12N, hypoglossal nucleus. Scale bar: 200 μm (A, C, E), 50 μm (B, D, F)

are large neurons (20–35 μm) elliptical perikarya, with intense, diffuse basophilia (Fig. 1D, 2D). Such large neurons are usually singly scattered among small cells. On transverse sections, the SpVi has an irregular oval shape with a long axis directed dorsomedially. Rostrally it is a thin lamina that gradually increases in size in a caudal direction. Along its entire length rostrocaudally, the SpVi borders the spinal trigeminal tract laterally. In the caudal direction, the solitary tract nucleus moves medially and is replaced by the cuneate nucleus. Medially, the

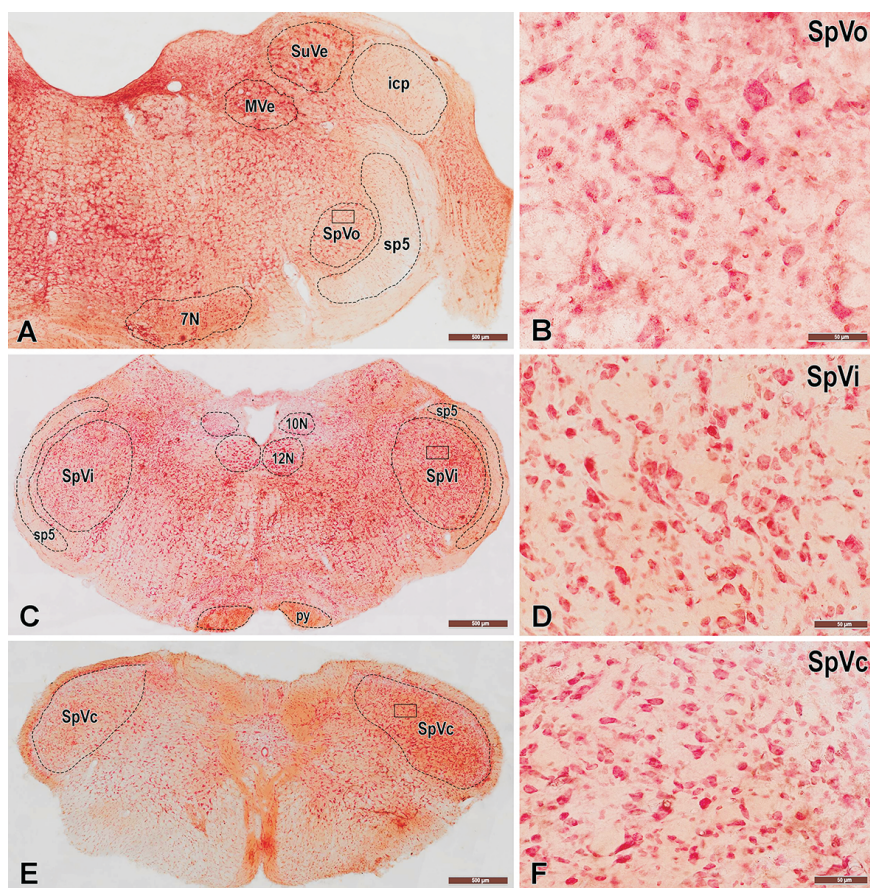


Fig. 3. A cross-section of the rat brainstem at the level of the spinal trigeminal nucleus (SpV) stained with neutral red. (A) The low-magnification section demonstrates the SpVo. (B) Higher magnification of the rectangle in (A) reveals medium-sized perikarya with intensely stained cytoplasm. Low-(C) and high (D)-power photomicrographs indicate the position of the SpVi and the scattered neurons with varying sizes in the subnucleus, respectively. (E) Low-power photomicrograph showing the location of SpVc. (F) Small neurons with high density can be observed on a higher magnification of the boxed area in (E). SuVe, superior vestibular nucleus; MVe, medial vestibular nucleus; icp, inferior cerebellar peduncle; 7N, facial nucleus; 10N, dorsal nucleus of the vagus nerve; 12N, hypoglossal nucleus; sp5, spinal trigeminal tract; py, pyramidal tract. Scale bar: 500 µm (A, C, E), 50 µm (B, D, F)

SpVi is bounded by the parvocellular reticular nucleus, a nucleus of the medulla oblongata, and near the transition to the SpVc by the central reticular nucleus of the medulla oblongata. There are relatively few neuronal perikarya along the entire medial border, as this area is traversed by prominent fibre bundles (Fig. 2A, B).

Oral trigeminal subnucleus. Located in the caudal pons and rostral

medulla oblongata, this subnucleus extends rostrally into the main sensory trigeminal nucleus and caudally into the SpVi. Neuronal perikarya exhibit a spectrum of sizes, ranging from small to medium. The rostral segment of the SpVo predominantly features medium-sized perikarya, contributing to its larger overall size, while small irregularly oval perikarya are also present. These small cells possess barely discernible dendritic poles (Fig. 1A), with relatively large nuclei enveloped by lightly stained cytoplasm. Conversely, medium-sized neurons may exhibit either oval or fusiform morphology, with the latter potentially reaching diameters of up to 20 μm (Fig. 3B). Characterized by cytoplasm with diffuse basophilia (Fig. 2B), these neurons contribute to the diverse cellular composition of the subnucleus. In the pontine region, the SpVo abuts laterally against the spinal trigeminal tract, often intersected by its fascicles, leading to uneven division into distinct groups across individual sections. Dorsally adjacent to the SpVo lie the vestibular sensory nuclei, while ventromedially, a narrow oligocellular and parvocellular strip, representing the ventrolateral extension of the parvocellular reticular nucleus, separates it from the facial nucleus.

Discussion. The SpV is the largest trigeminal nucleus and is located in the lateral tegmentum of the brainstem and caudal part of the pons, adjacent to the spinal trigeminal tract. Caudally, the nucleus transitions into the substantia gelatinosa of the spinal cord, while the tract continues with Lissauer's bundle [5].

The topographic-anatomical separation of neurons in the SpV offers researchers two complementary perspectives to consider a conventional classification based on purely anatomical features and a modern segmentation scheme based on molecular methods. While the classical notion that divides the nucleus into three distinct subnuclei, the oral, interpolar, and caudal, is a cornerstone of neuroanatomical research, the molecular approach to segmentation represents a more recent endeavor that addresses the complex molecular characteristics of neurons within this nucleus.

During the early stages of neural tube development in vertebrates, the morphological and functional complexity of the brain emerges as different regions and subregions begin to define and differentiate. One mechanism contributing to this complexity is the process of segmentation, which involves the division of the proneuromeres into transverse regions, known as neuromeres, along the rostrocaudal axis [6]. These neuromeres display distinct molecular and cellular identities and develop into specific brain regions containing unique neuronal populations through proliferation and neurogenesis [7–9]. Although migratory processes during development can disrupt some segmental boundaries, mapping experiments have shown adherence to distinct segmental regions [10–12]. The current segmental model for the vertebrate brain, known as the prosomere model, specifies seven prosomers in the forebrain and 11 rhombomeres in the hindbrain [1, 13, 14]. This segmentation process contributes to forming distinct brain regions with specific neuronal populations.

The trigeminal sensory column, located in the hindbrain, is an example of such a brain structure, consisting of the main trigeminal sensory nucleus in the rostral part of the hindbrain and the SpV extending caudally. On the other hand, as described, it is further subdivided into SpVo, SpVi, SpVc, each characterized by unique molecular markers and connectivity patterns [2]. The trigeminal column consists of second-order sensory neurons that receive information from trigeminal nerve fibres. The prosomer model suggests that this column is divided into segmental units derived from the corresponding rhombomeres [11, 15]. Experimental studies have localized the main sensory trigeminal nucleus to midbrain rhombomeres (r2-r3) in mice [16]. The SpV comprises multiple rhombomeres (r4 to r11 in mice) in the pontine, retropontine, and medullary regions of the hindbrain [11, 17]. Understanding the organization of these subunits sheds light on the more subtle organization of the trigeminal sensory column, which is potentially influenced by underlying rhombomeric structures.

Despite the emergence of molecular segmentation as a promising way to understand neuronal organization, our current study remains rooted in the traditional framework of the classical division of the spinal trigeminal nucleus. By adhering to this well-established anatomical categorization, we try to provide a comprehensive study and extensive analysis of the morphological and functional characteristics of spinal trigeminal subnuclei, shedding light on their distinctive roles in neuronal circuits.

Considering the results of the present study in elucidating the morphology and distribution of neurons in the rat SpV, a comparison can be made with the earliest descriptions of its cytoarchitectonics in primates. We have already investigated the cytoarchitectonics of the rat SpV as a complex structure in the brainstem [18], so this study sheds more light on the specific neuronal organization in each SpV subnucleus. Before Olszewski's work, the SpV was thought to be a continuous structure that was a direct extension of the posterior horns of the spinal cord, reaching the main sensory nucleus of the fifth cranial nerve in the pons. This manner of presenting uniformity in structure has given rise to the idea that the entire nucleus also possesses uniformity in function. Olszewski's study illustrates the cytoarchitecture of the SpV, focusing on three distinct subnuclei evident at the level of the pyramid crossing. In the caudal subnucleus, SpVc, three parts are identified, each housing different neuronal groups. The gelatinosus region consists of small, densely arranged oval cells, while the magnocellularis contains multipolar neurons categorized by size. The marginalis region features large multipolar neurons with lengthy dendrites, bordering the gelatinosus area. The interpolaris subnucleus, SpVi, displays uniformity with small and large cellular elements. The oralis subnucleus, SpVo, contains few but large neurons. Neuronal shapes and sizes vary across these regions, shedding light on the architecture of the SpV [1].

The SpV is a critical component of the trigeminal sensory system responsi-

ble for processing sensory information from the face and head. Understanding the structural characteristics, particularly the size, and morphology, of neurons in these subnuclei, is essential for revealing the functional organization of the trigeminal sensory system. Olszewski's description, although based on observations of material different from that used in the present study, shows similarities with the observed organization of the caudal part of the SpV in the rat.

Conclusion. In conclusion, the subnuclei of the SpV have unique organizational patterns in their cytoarchitecture, which is indicative of their functional specialization in the processing of sensory data, especially that of touch and pain from the face and mouth cavity. To fully comprehend its function in sensory processing and its connection to neurological conditions like migraine and trigeminal neuralgia, more investigation into the cellular makeup and connections of the nucleus is necessary.

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¹*Department of Anatomy and Histology, Faculty of Medicine, Medical University of Sofia, 1 St. G. Sofiiski St, 1431 Sofia, Bulgaria*
e-mails: aivanov@medfac.mu-sofia.bg, nlazarov@medfac.mu-sofia.bg

²*Institute of Neurobiology, Bulgarian Academy of Sciences, Akad. G. Bonchev St, Bl. 23, 1113 Sofia, Bulgaria*
e-mails: d.atanasova@inb.bas.bg, nlazarov@bio.bas.bg

³*Department of Anatomy, Faculty of Medicine, Trakia University, 11 Armejska St, 6000 Stara Zagora, Bulgaria*
e-mail: dimitrinka.atanasova-dimitrova@trakia-uni.bg