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Cytoarchitecture of the Spinal Trigeminal Nucleus in Rats

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The spinal trigeminal nucleus (SpV) is one of the three nuclei in the trigeminal sensory nuclear complex which extends over the whole length of the brainstem. The SpV travels adjacent to the spinal trigeminal tract and is responsible for relaying various sensory modalities including temperature, deep or crude touch, and pain from the ipsilateral portion of the face. It is continuous with the substantia gelatinosa, while the tract is continuous with Lissauer‘s tract. The purpose of the study is to scrutinize the structure and cytoarchitecture of this nucleus in the rat. The SpV is the largest trigeminal nucleus and is found in the lateral tegmentum of the medulla and caudal pons. Spinal trigeminal neurons are composed of soma with sporadic Nissl bodies surrounded by a network of myelinated axons. Our results show that its three structural divisions, i.e. the oral, interpolar, and caudal subnuclei, share a common neuronal organization though they are associated with the transmission of different kind of sensory information from the orofacial region.

Key words: spinal trigeminal nucleus, hematoxylin staining, neutral red staining, toluidine blue

Introduction

The trigeminal nerve (the fifth cranial nerve, CN V) is the nerve responsible for facial sensation and motor functions such as biting and chewing. This nerve is the largest of the cranial nerves. Its name („trigeminal“ = tri-, or three and – geminus, or twin: three twins) derives from the fact that each of the two nerves (one on each side of the brain bridge) has three main branches: the ophthalmic nerve (NOpth – nerve ophthalmicus, V1), maxillary nerve (NMax – nervus maxillaris, V2) and mandibular nerve (NMan – nervus mandibularis, V3) [7].

In embryonic development, the motor division of the trigeminal nerve originates from the basal plate of embryonic ridges, and sensory division originates from the

cranial neural crest. Sensory information from the face and body is processed by parallel pathways in the central nervous system [7].

The trigeminal sensory nuclei comprising the trigeminal sensory nuclear complex are divided into three parts. From caudal to rostral direction (ascending from the medulla to the midbrain), they are the spinal trigeminal nucleus, principal sensory and mesencephalic trigeminal nuclei. Parts of the trigeminal nucleus receive different types of sensory information; the spinal trigeminal nucleus receives fibers associated with pain and temperature signals, the main sensory nucleus receives fibers with contact information, and the mesencephalic nucleus receives fibers from the proprioceptors and mechanoreceptors of the jaws and teeth. The fibers associated with pain from the peripheral nociceptors are transferred to the cranial nerves V, VII, IX and X. Upon entering the brain stem, the sensory fibers are grouped and sent to the spinal trigeminal nucleus [5].

The spinal trigeminal nucleus contains the sensory map of pain and temperature of the face and mouth. From this nucleus, the secondary fibers intersect the midline and ascend in the trigeminothalamic tract to the contralateral thalamus. The fibers for pain and temperature are sent to multiple thalamic nuclei [2].

Inside the spinal trigeminal nucleus, information is presented in the form of layer analogy. The lower parts of the nucleus (in the upper cervical part of the spine and lower medulla) represent the peripheral areas of the face (scalp, ears and chin). The higher levels (in the upper medulla) represent the central areas (nose, cheeks and lips). The highest levels (in the pons) are the mouth, teeth and pharyngeal cavity areas [4].

The purpose of the study is to scrutinize the structure of the spinal trigeminal nucleus in rats.

Materials and Methods

The experiments in this study were performed on adult (12-week-old; n= 6) normotensive rat material, the Wistar breed. The experimental procedures were consistent with the European Communities Council Directive 2010/63 /EU, were conducted in accordance with national rules on animal experiments, and were approved by the Ethics Committee of the Institute of Neurobiology, Bulgarian Academy of Sciences. The experimental animals were anaesthetized with ether followed by intraperitoneal injection of thiopental (40 mg/kg body weight). After anesthesia, they were perfused through the ascending aorta with the cannula inserted through the left ventricle of the heart. Initially, the circulatory system was flushed for about 5 minutes with 0.05 M phosphate-buffered sodium chloride solution (PBS) at pH 7.36. After washing, the system was started with a retainer consisting of 4% paraformaldehyde (Merck) in 0.1 M phosphate buffer (PB) for about 20 minutes. After perfusion, we removed under a magnifying glass the area from the midbrain to the cervical part of the spinal cord. The formed tissue blocks were left overnight in the same retainer at 4 ° C. After fixing, the material was washed from the rest of the retainer until the next day with tap water. The incorporation of the material into paraffin necessitates its dehydration through an ascending series of alcohols, starting with placing it in 50% ethanol for 2 h, after which the tissue is transferred to 70% ethanol until the next day, followed by 80% ethanol for 2 h, 96 % ethanol – 2×20 min, 100% ethanol – 2×15 min. The next step involves clarifying the material in cedar oil in penicillin vials, which are left open to allow the ethyl alcohol to evaporate. The material remains in cedar oil until it becomes amber or until it sinks to the bottom of the bottle, which may take several days. After washing twice with xylene (2 times for 10 minutes), the material was paraffin embedded.

Routine staining with Neutral red

This method of staining is the most widespread in histological practice, because, thanks to the appropriate combination of colorants, belonging to two opposite groups - basic and acidic dyes, it gives a general idea of the state of the structure under study [3].

Neutral Red is a weak cationic azine dye that is used extensively as a nuclear stain in a variety of biological stain applications. Standard protocol has been used for dewaxing and rehydration after which Neutral Red staining solution has been added and samples incubated for 5 minutes at room temperature followed by 2-minute wash with distilled water. Sections have been dehydrated, cleared in xylene and mounted.

Staining with Toluidine blue

Toluidine blue staining is another method of demonstrating acidic tissue components (Davidoff). The dewaxed and water-driven sections were transferred to a 0.5% solution of toluidine blue for 5-10 min. The sections were rinsed in distilled water and then differentiated in 70% ethanol until the excess paint was washed, observing under a microscope. This is followed by dehydration, treatment with xylene and incorporation into Entellan.

Results and Discussion

This nucleus is the largest trigeminal nucleus and is found in the lateral tegmentum of the medulla and caudal pons. The SpV travels adjacent to the spinal trigeminal tract. The SpV is continuous with the substantia gelatinosa, while the tract is continuous with Lissauer‘s tract (**Fig. 1**) [6].

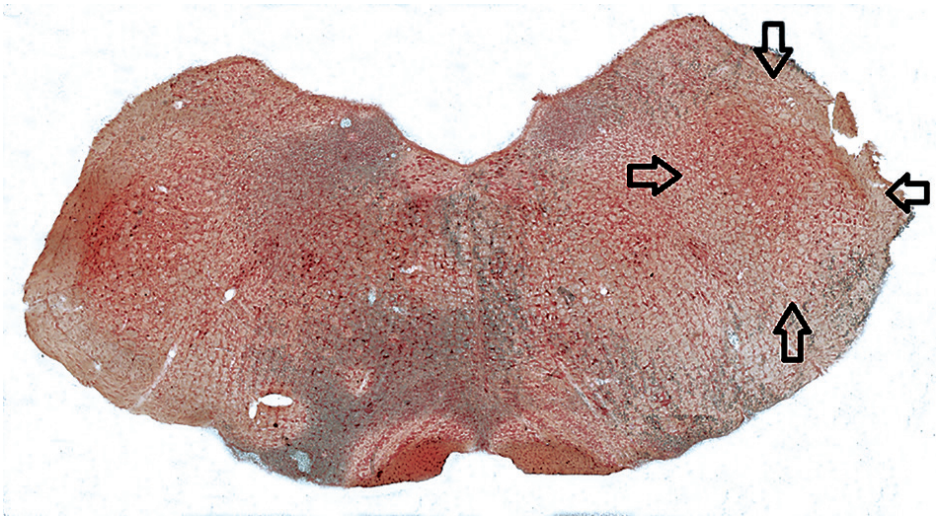

Fig. 1. Neutral red stained coronal section of the medulla oblongata showing cell types of the spinal trigeminal nucleus outlined from the spinal trigeminal tract laterally. The arrows indicate the boundaries of the spinal trigeminal nucleus. Magnification: 50×.

The spinal trigeminal neurons have a distinct cell body, soma with sporadic Nissl bodies surrounded by a network of myelinated axons (**Fig. 2**). Around the nucleus, myelinated fibers may be observed. These fibers are associated with pain signaling from the peripheral nociceptors which are transferred to the cranial nerves V, VII, IX and X [1]. Upon entering the brain stem, the sensory fibers are grouped and sent to the spinal trigeminal nucleus. This incoming fiber bundle can be identified in pons- and medulla-cross sections as the spinal tract of the trigeminal nucleus, which is parallel to the spinal trigeminal nucleus. The spinal tract of the fifth cranial nerve is analogous to dorsolateral fasciculus in the spinal cord.

Furthermore, along the caudal-rostral direction, three separate parts of the spinal nucleus may be distinguished – the oral part, the caudal part and the interpolar part. The differentiation of the three anatomical parts of the nucleus may be associated with their different functions [8]. The first part is associated with the transmission of discriminative (fine) tactile sensations from the orofacial area and is an extension of the main sensory nucleus of the fifth cranial nerve. The interpolar nucleus' function is also associated with the transmission of tactile information as well as tooth pain, while the caudal part transmits information on nociception and thermal sensations from the head [9, 10].

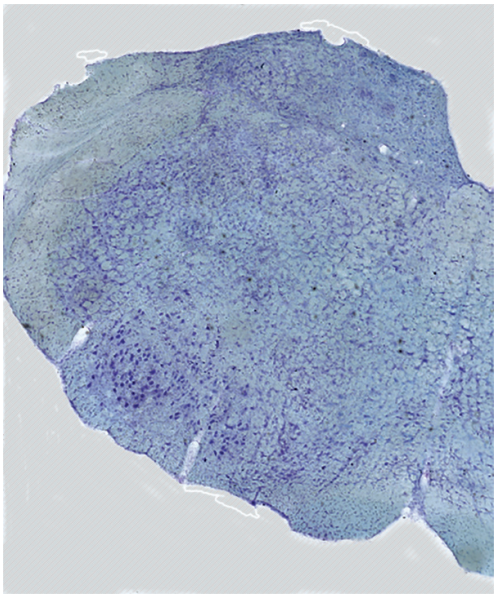

Fig. 2. Coronal section of the medulla oblongata with toluidine blue staining depicting neuronal organization of the spinal trigeminal nucleus. Magnification: 50×.

Taking into account the achievements in recent years in elucidating the morphology of the spinal nucleus, there is still scope for more investigation revealing the neurotransmitter affiliation of its cell population. The receptor profile of the neurons in the spinal nucleus, the role of endogenous neurotransmitters and neuromodulators has not been fully scrutinized. Therefore, more studies will be needed in order to better fathom the anatomy of the spinal trigeminal nucleus and its association with the functions it executes.

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