CYTOARCHITECTONIC STUDY OF THE TRIGEMINAL GANGLION IN HUMANS

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Abstract

The trigeminal ganglion (TG), a cluster of pseudounipolar neurons, is located in the trigeminal impression of the temporal pyramid. It is covered by a sheath of the dura mater and arachnoid and is near the rear end of the cavernous sinus. The peripheral processes of the pseudounipolar cells are involved in the formation of the first and second branch and the sensory part of the third branch of the fifth cranial nerve, and the central ones form the sensory root of the nerve, which penetrates at the level of the middle cerebellar peduncle, aside from the pons, and terminate in the sensory nuclei of the trigeminal complex. We found that the primary sensory neurons involved in sensory innervation of the orofacial complex are a diverse group. Although they possess the general structure of pseudounipolar neurons, there are significant differences among them, seen in varying intensities of staining. Based on our investigations we classified the neurons into 7 groups, i.e. large, subdivided into light and dark, medium, also light and dark, and small light and dark, and, moreover, neurons with an irregular shape of their perikarya. Further research by applying various immunohistochemical methods will clarify whether differences in the morphological patterns of the neurons are associated with differences in the neurochemical composition of various neuronal types.

Keywords: trigeminal ganglion (TG), pseudounipolar neurons, cytoarchitecture, human.

Introduction

The first macroscopic description of TG belongs to Merkel [1], followed and extended by Krause [2] and Schaffer [3,4]. The authors report that the TG composition is characterized by ganglion cells (pseudounipolar neurons), satellite cells and their appendages. Histological preparations visualize that the perikarya of the pseudounipolar neurons are tightly enveloped by small satellite cells with intimately related neurolemmas. The interior of the ganglion is pierced by a lot of connective tissue filaments, consisting mainly of collagen type III fibers. Externally the ganglia are dorsally enveloped by a multi-layer perineural sheath and an outer fibrous layer-epineurium, as described by Schaffer [4]. Along with the general principles of the structure of various sensory ganglia, certain specific characteristics of TG, pertaining solely to it, have been observed. First, it has been described that in animals before birth, small islands of nerve cells appear near the neurons, having some signs of astroglial cells normally occurring in the CNS [5,6]. Observations and experiments confirm that they are linked together through communicating contacts of the type of gap junctions. Secondly, after staining with methods specific for nervous tissue, such as silver impregnation techniques, another type of cells with ultrastructural characteristics of neurons have been observed between the astroglial type of cells and also between myelin nerve fibers in the ganglion root. They have, however, characteristic morphological differences in comparison to the other pseudounipolar sensory neurons. What distinguishes them is primarily the lack of the satellite cuff around the perikaryon of the cell, their form, and last but not least, the arrangement of their cytoplasmic organelles. Therefore, it is presumed that they represent central nervous system neurons which have migrated to the periphery. Lastly, the TG lies on the border between the peripheral and the central nervous system and possesses the characteristics of both.

The diversity in the structure of the trigeminal sensory system in the human and animals has been the

Manuscript received: 26.04.2013
Accepted: 07.05.2013
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subject of extensive research over a long period of time, and the accumulated morphofunctional data are a source of disagreement on various morphological and functional issues. Despite a significant amount of experimental data on the normal TG cytoarchitectonics, there are still some gaps in literature.

Materials and methods

Human trigeminal ganglia have been used as material for the present morphological study. The experiments were done in strict observance of the ethical work principles, applied at the Medical University of Sofia and the legislative requirements for the protection of rights. Nissl staining method was applied to study the normal morphology of the trigeminal neurons. The experiments were conducted on twenty pairs of TGs from 20 patients aged 21 to 82. The material was taken during routine autopsies at the Department of Forensic Medicine and Deontology, and the Department of General and Clinical Pathology of the Medical University of Sofia. After removing the whole brain, the dura mater sheath on the anterior wall of the pyramid was carefully removed and by cutting the three main branches of the trigeminal nerve, the TG was excised. The ganglia were then placed in a solution of 4% neutral formalin and after 5 days fixation the procedure continued with dehydration of the tissue pieces in an ascending series of alcohols, followed by clearing in cedar oil. The samples were embedded in paraffin and then serial cross-sections of 20 μm each were cut. After they were mounted on slides some of the serial cross-sections were stained by the Nissl method with cresyl violet, toluidine blue or thionine according to the Landau modifications [7-10], while others were stained for demonstration of myelin by a technique, modified by Woelcke [11]. Consistent monitoring of the cytoarchitecture and myeloarchitecture of the structure was ensured by observing stained consecutive sections.

Results

Lightmicroscopic study of the trigeminal ganglion

During an observation of human TG with a small magnification of the light microscope (LM) three separate agglomerations, delicately separated from each other by fibers, passing between them, were seen (Figure 1). Each of the cell agglomerations was composed of a cluster of pseudounipolar neurons, diffusely scattered and forming the three branches of the trigeminal nerve (Figure 1, 3). It was clearly evidenced that each of these agglomerations could be subdivided into two parts, dorsomedial and ventrolateral (Figure 3). The cells, located in the dorsomedial subdivision, were more tightly packed than the cells located in the ventrolateral subdivision, and visibly had a smaller size. The ventrolateral trigeminal neurons had larger perikarya and were more distinctly separated. In fact, these areas contained the cell bodies of the neurons of origin of the three main branches of the trigeminal nerve, i.e. ophthalmic, maxillary and mandibular nerves (Figure 1, 3). At the beginning of the first and second branches single perikarya enveloped with a less dense cuff of satellite cells were often observed (Figure 6). In spite of the uniform cellular pattern at a low magnification (Figure 1), the larger percentage (over 80%) of trigeminal neurons were small- and medium-sized pseudounipolar cells, while a more detailed observation at a larger magnification revealed cells with bodies of various shape: round, oval, fusiform (polygonal) and elongated (Figure 1, 6). The sizes of their cell bodies also widely varied ranging from 15 to 110 μm in diameter (Figure 4). Apart from their external morphology, the trigeminal ganglion cells differed in the structural features of their nuclei and cytoplasm, as well as the nucleus cytoplasm ratio. The morphological picture revealed that dark stained nucleoli were observed in some of the nuclei (Figure 4, 5). The thorough study of serial horizontal cross-sections of the TG from rostral to caudal allowed us to differentiate the following types of trigeminal neurons according to the size of their perikaryon: large-, medium- and small-sized neurons (Figure 4, 5). Depending on the intensity of staining of their cell bodies, each of these neuronal types was observed in two varieties, light and dark neurons. According to the shape of the perikarya, the TG cells could be divided into typical (oval) and atypical (elongated and/or fusiform) neurons. The location of the different TG neurons in the human was established in relation to their morphological patterns. In particular, the large light neurons (with a diameter >40 μm) were predominantly located at the periphery of the ganglion, near the site of origin of the three main branches of the trigeminal nerve, though they also occurred inside the TG as agglomerations (Figure 1, 3, 6). They were typical pseudounipolar neurons with a pale cytoplasm and large hypochromic oval nuclei with dark nucleoli (Figure 4, 6).

In the human TG, the neurons of average size were the largest cell population. They were diffusely scattered in the three agglomerations of the TG (Figure 3, 4). Their cell bodies were 10-40 μm in size and with an oval shape. The nucleus was centrally located and had an oval shape (Figure 4). According to the density of cytoplasmic staining these neurons were divided into two subtypes: medium-sized light and medium-sized dark neurons (Figure 6). The small neurons were ubiquitous and were also classified as light and dark, according to the staining of their cytoplasm (Figure 4). Their size was about 10 μm or smaller. They had a slightly oval in shape nucleus, usually located eccentrically, and a nucleolus was rarely noticed.

Along with the described typical oval neurons there were also neurons with a fusiform or polygonal shape. These were mostly medium in size, but also large-

and small-sized were seen. These cells were usually located centrally in the neurophil, but sometimes they were found at the initial segments of the three trunks of the nerve (Figure 2). Near the individual well-defined agglomerations some elongated TG cells were observed. Their perikarya were mostly medium- sized, though some large- and small-sized neurons were also visualized. In the ganglion, stained by the Heidenhain method according to the Woelcke modification [11], we found fibers penetrating the TG or originating from it (Figure 3, 4, 5). These bundles of fine fibers ran in a fan-like manner in a ventrodorsal direction between the neuronal perikarya and thus they discriminated the agglomerations where the neurons of the three branches of the trigeminal nerve were mainly clustered.

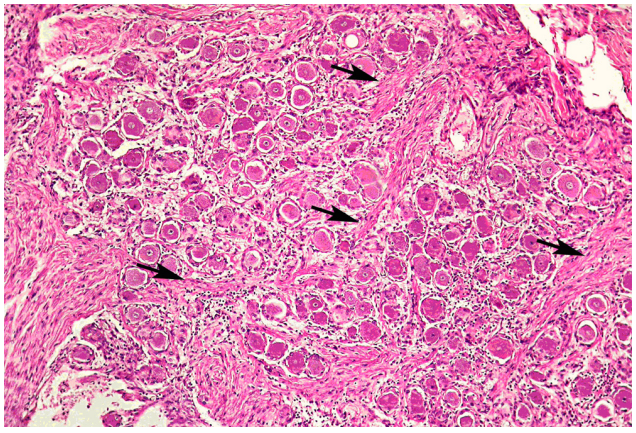

Figure 1. Low magnification reveals tightly grouped perikarya of TG pseudounipolar neurons with different sizes. They are surrounded by connective tissue fibers (arrows), which separate the agglomerations, located at the sites of origin of the trigeminal nerve main branches. It is seen that the trigeminal neurons differ in their staining intensity. HE x 100.

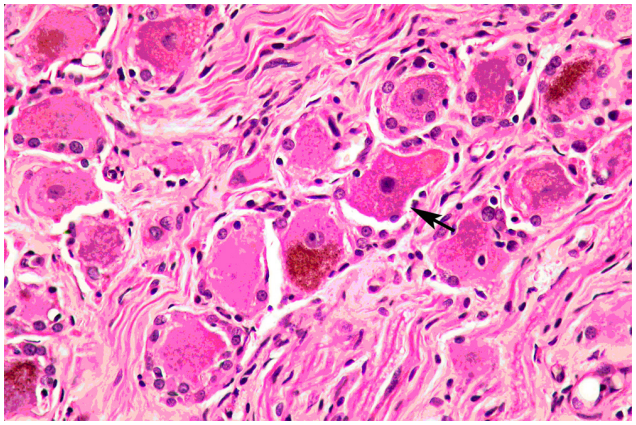

Figure 2. A neuron of a medium size and polygonal shape, located within a group of oval neurons and surrounding fibers (arrow). HE x 150.

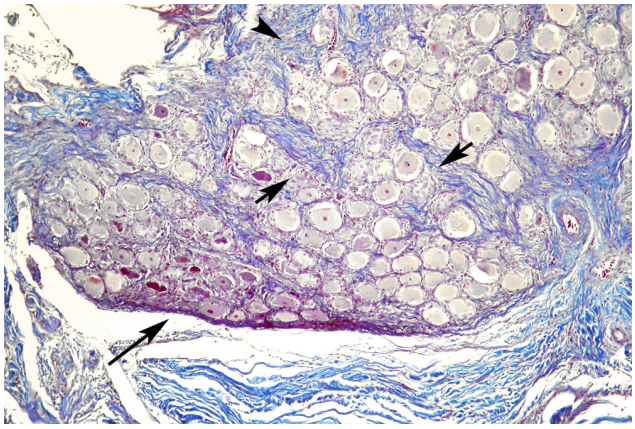

Figure 3. Low magnification shows the agglomeration, giving rise to the third branch of the trigeminal nerve. Please note the presence of numerous medium-sized neurons and a clear distinction between the two parts (short arrows), dorsomedial (arrowhead) and ventrolateral (long arrow), where the respective branch of the nerve originates. Azan x 100.

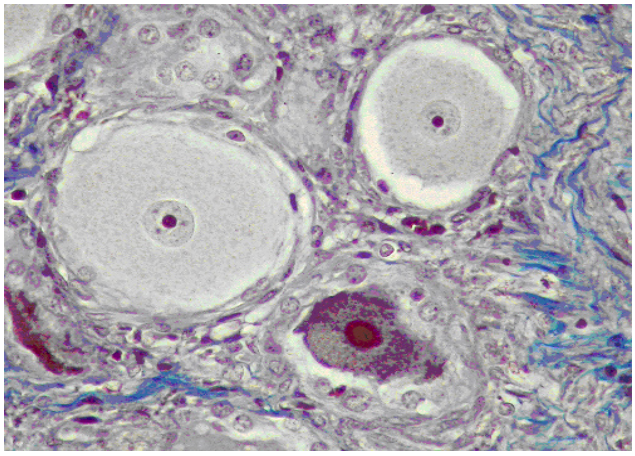

Figure 4. It is possible to observe the difference in neuronal size (large light, small dark and medium-sized) and the difference in their staining intensity. Azan x 200.

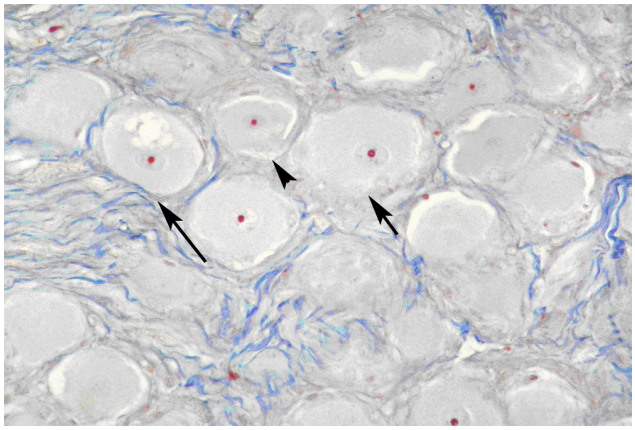

Figure 5. Large (short arrow), medium-sized (long arrow) and small (arrowhead) light neurons in the human TG. Azan x 150.

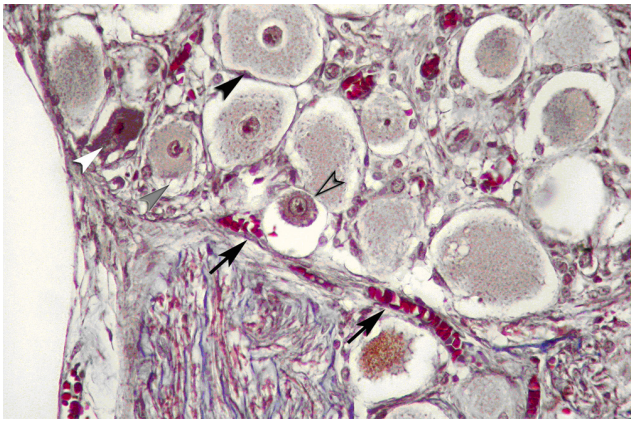

Figure 6. A capillary (short arrows) running between a group of neurons of various sizes and their fibers, forming the second branch of the trigeminal nerve in the human TG. The different neurons are marked with arrowheads of different colors, the black arrowhead indicates a large light neuron, the gray one shows a medium-sized light neuron, the white one denotes a medium-sized dark fusiform neuron, and an open arrowhead indicates a small dark neuron. Human TG. Azan x 150.

Discussion

Disclosing the cytoarchitecture of the TG is directly dependent on the methods used. Despite the numerous studies on the ganglion, performed by using the Nissl staining method [12,13,14,15], and other methods less often used for visualizing pseudounipolar neurons, such as the Golgi method [16,17,18,19,20], a full knowledge about the TG cytoarchitecture still requires additional data. For this purpose, we applied a standard approach and routine methods for its staining. Preparations of human material from both sexes from fertile to senile age were used in forensic studies. The great quantity of studied material is the grounds for our claim of the comprehensiveness and credibility of the present research. In general, the experimental data on the cytoarchitectonic characteristics of the human TG, obtained in this study, correlate with the results of other authors who worked on sensory ganglia in animal species. Here we report considerable differences in the arrangement of the represented neuron groups, their classification and characteristics as follows.

Large light neurons. Neurons of a large size are described in the works of several authors, studying the TG in various animal species and the human [21,22,23,24,25]. This type of pseudounipolar neurons prevails in the peripheral part of the ganglion near the point of origin of the three main branches of the trigeminal nerve [26,27], and also at the most dorsal edge of the trigeminal ganglion in man. However, in most animals they are scattered in separate agglomerations throughout the ganglion [26]. We have very rarely observed the accumulation of pigment and its content increases with age in the human TG. The perikaryal shape of this neuronal type is typically oval and characteristic of pseudounipolar neurons, but neurons with an elongated and fusiform shape, with an oval nucleus and a compact nucleolus, also occur.

Medium-sized neurons. This is the most common neuronal type of pseudounipolar neurons observed in this study, in the three agglomerations, giving rise to the respective main branches of the trigeminal nerve [26], especially in the human TG. Despite the numerous cytoarchitectonic studies of the TG in cats, rodents and other mammals, performed by a number of authors [27,28,29], there is no classification that defines them as a separate group. The size of this type of neurons ranges from 18 to 25 μm. We did not observe a significant difference in their size and diversity, associated with the pigmentation of their cytoplasm, i.e. between neurons with lighter, and those with darker cytoplasm, which is consistent with the findings of other authors, working on the topic [30,31,32]. They classify them as a group of small ones. Neurons with darker pigmented cytoplasm prevail in sites, specifically at the origin of the three main trigeminal branches.

Small neurons. Small neurons are scattered throughout all the agglomerations, from the ventromedial to the dorsolateral pole of the human TG [27]. Such a description of their localization is also given in other previous studies [28,30,31]. As already noted, we can divide them into neurons with a lighter and darker cytoplasm [26,27,33]. In morphometric measurements we have found that in comparison to the lighter ones, the darker are with a smaller diameter of their perikarya [27]. Often the darker-stained neurons have an irregular, and in some cases, a fusiform cell body. Small neurons are the best impregnated cell group of the pseudounipolar neurons, according to the above mentioned authors, a statement we are in agreement with. Under the light microscope, in the human TG, a significant difference between small and dark neurons may be evidenced, when compared to light neurons [31,33].

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