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MORPHOLOGICAL STUDY OF NOCICEPTIVE NEURONS IN THE TRIGEMINAL GANGLION

Sankaran Pk, Sivanandan Ramar. Salameh Bweir Al Dajah

Sankaran Pk,
Associate Professor of Anatomy, Saveetha medical college and hospital, Chennai.

Sivanandan Ramar,
Assistant professor of Anatomy, Majmaah University, Al Majmaah, KSA.

Salameh Bweir Al Dajah
Associate Professor of Physical Therapy, Majmaah University, Al Majmaah, KSA.

Corresponding Author: Sivanandan Ramar

E-mail:
sivanandan.ramar@gmail.com

s.narayanapillai@mu.edu.sa

ABSTRACT

The trigeminal ganglion neurons are responsible for migraine and trigeminal neuralgia so it is important to study the morphology of neurons responsible for nociception. The rats' trigeminal ganglion were collected and stained for crystal violet and immunohistochemical localization for GFAP and CGRP to study the morphology of cells responsible for nociception. The pseudo unipolar neurons were classified into small (<22micron), medium (22-29 micron) and large (>29 micron) based on their size. The CGRP was localized in cytoplasm on neurons especially in small neurons with dense staining indicating those neurons are responsible for nociception. The GFAP was localized in satellite glial cells surrounding each neuron. Upregulation of GFAP and CGRP can lead to pathological conditions like migraine and trigeminal neuralgia.

Keywords: Trigeminal ganglion, Migraine, Neuralgia, immunohistochemical, satellite glial cells

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INTRODUCTION

The trigeminal ganglion is a sensory ganglion that occupies a cavity (Meckel's cave) in the duramater, covering the trigeminal impression near the apex of the petrous part of the temporal bone. It comprises pseudounipolar neurons from the ophthalmic, maxillary, and mandibular divisions of the trigeminal nerve. The pseudounipolar neurons can be classified into different types based on its size in to type A cells that are large, and contain scattered clumps of Nissl substance connected to myelinated nerve fibers. The remaining cells are classified as type B cells which are small sized and contain coarser clumps of Nissl substance connected mainly to unmyelinated nerve fibers [1]. The small sized neurons of trigeminal ganglion modulate pain sensation in migraine and trigeminal neuralgia [2]. Neuropeptides like CGRP [3] and substance P [4] are expressed and released by neurons, which mediate or modulate adjacent neuronal communication by acting on the cell surface receptors. Calcitonin gene-related peptide (CGRP) is a widely distributed neuropeptide in central and peripheral nervous system that has diverse functions as a primary afferent neurotransmitter [5]. This neuropeptide (CGRP) is involved in nociception by producing a slow action potential in the dorsal root ganglion neurons [6]. Any inflammation or injury of peripheral tissue will upregulate the production of CGRP in the neurons innervated by type Aδ and type C-fibres indicating CGRP are an important neuropeptide in nociception [7]

Glial cells which surround the pseudo unipolar neurons directly modulate neuronal function and activity by changing the ionic concentrations in and around the neurons [8]. Interestingly, neuron-glia interactions have been shown to be involved in all stages of inflammation and pain associated with several CNS diseases [9]. Glial cells express characteristic substances in common with immune cells in which they respond to viruses and bacteria, releasing proinflammatory cytokines, which create pathological pain[9]. GFAP is a member of the cytoskeletal protein family, and the principal 8-9 nm intermediate filaments expressed in mature astrocytes of the central nervous system, and satellite glial cells (SGC) of sensory ganglia [10].

So the aim of the study was to classify neurons into large, medium and small size neurons and to localize GFAP and CGRP in the cells of trigeminal ganglions and to identify neurons which are nociceptive.

MATERIALS AND METHODS:

Male albino wistar rats (n=6) of weight ranging from 200g to 250g was used in the present study. The rats were obtained from experimental animal facility of All India Institute of Medical Science after prior approval of the experimental procedure by Institutional Animal Ethics Committee. The animals were kept in cages with not more than three animals in one cage. They were maintained at 12hr:12hr light/dark cycles with water and food available ad libitum.

TISSUE COLLECTION:

Fixation was done using 500ml of 4% paraformaldehyde in 0.1 M phosphate buffered saline, through transcardiac perfusion. Then the skull was cut open, trigeminal ganglion was identified and removed (Fig 1). Tissues were sectioned (40>m thick) serially with cryostat using OCT (optimum cutting temperature) as medium and stained with cresyl violet.

MORPHOMETRIC ANALYSIS OF TRIGEMINAL GANGLION NEURONS:

The cresyl violet stained sections were visualized using ProgRes image capture from JENOPTIK ProgRes Capture Pro 2.7 (Germany) in 20X objective in an E-600 Nikon compound light microscope. The diameters of the neurons from every fifth section were measured using ProgRes image analysis software. The measured diameters were then divided into three types small sized, medium sized and large sized using SPSS software.

IMMUNOHISTOCHEMISTRY:

For free floating immunohistochemical localization the antibodies for **CGRP** and GFAP was obtained from Sigma laboratories (USA). The standard dilution ratio for CGRP (1:1000) and GFAP (1:400) was determined after repeated histochemical localization at various dilution ratios. The stained slides were captured by ProgRes image capture using JENOPTIK ProgRes Capture Pro 2.7 (Germany) in an E-600 Nikon compound light microscope.

RESULT:

Morphological study of neurons:

The diameters of the neurons measured were analysed statistically using SPSS software and the values were divided into three groups (Table 1 and 2)

TABLE 1: Results of Statistical Analysis Using Spss

Statistical Measures	**right trigeminal ganglia neurons (µm)**		**left trigeminal ganglia neurons (µm)**
Mean	26.18		26.29
Median	25.5		25.39
Standard Deviation	7.44		7.49
Std Error Of Mean	0.18		0.18
Range	48.74		66.31
Minimum	10.56		10.27
Maximum	59.30		76.58
Percentiles	33.3	22.18	22.28
	66.66	29.1	28.88

Localization of GFAP and CGRP:

GFAP was localized in satellite glial cells (Fig 1) (black arrow) surrounding all neurons and CGRP was localized in cytoplasm (Fig 2a) of neurons. Based on staining patern of neurons, they can be classified into type A – fine, less dense, lightly stained large neurons(Fig 2b blue arrow) and type B - dense, coarse, darkly stained small neurons(Fig 2b yellow arrow). The maximum diameter of coarse, darkly stained CGRP neurons of trigeminal ganglion showed small and medium sized groups. The primary afferent nerve fiber also showed staining for CGRP (Fig 2c white arrow).

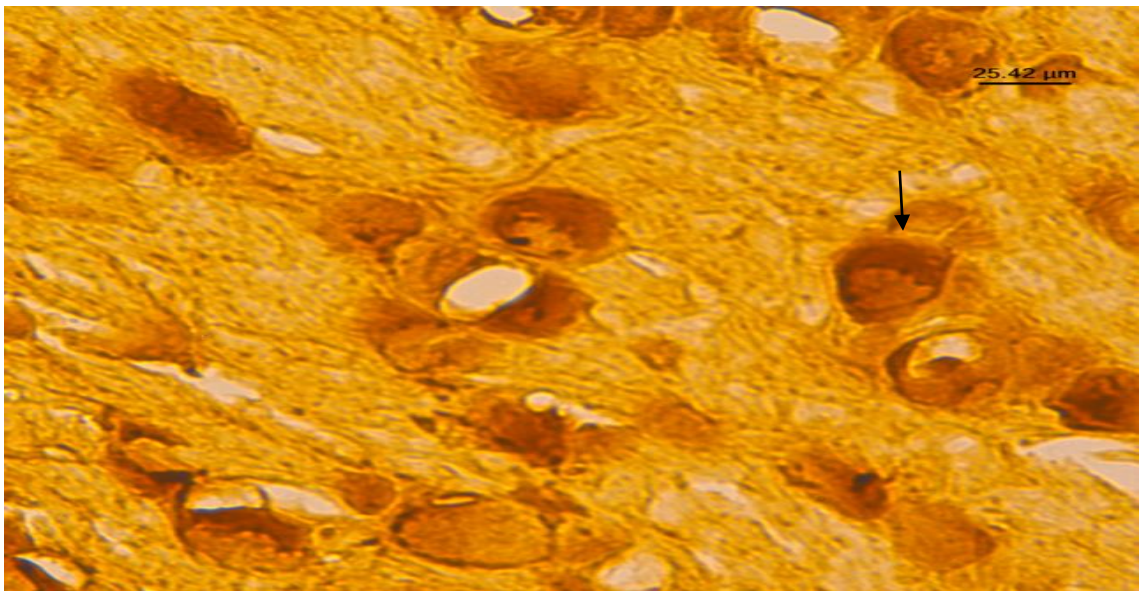

Fig 1: immunohistochemical localization of GFAP in satellite glial cells (black arrow)

TABLE 2: Classification Of Neuron Based On Size

Types Of Neurons	Right Trigeminal Ganglia Neurons (µm)	Left Trigeminal Ganglia Neurons(µm)
Small Neurons	<22.18	<22.28
Medium Sized Neurons	22.18-29.1	22.28-28.88
Large Neurons	>29.1	>28.88

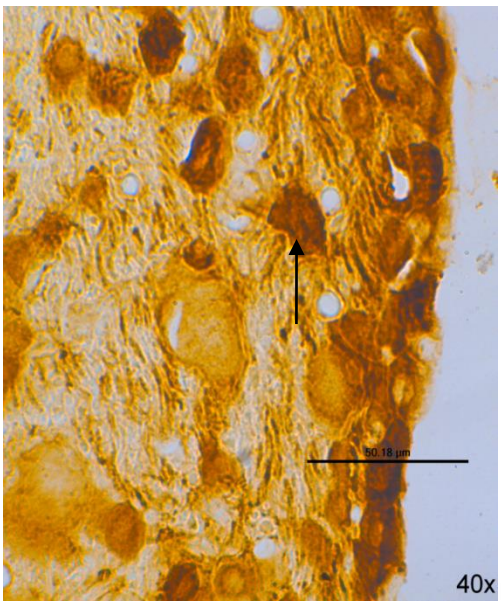

Fig 2a: immunohistochemical localization of CGRP in cytoplasm of neurons (black arrow).

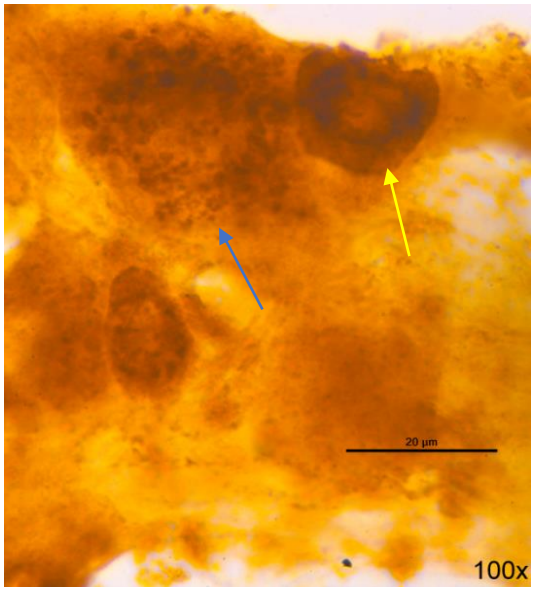

Fig 2 b:Immunohistochemical localization of CGRP in large neurons (blue arrow) and small neurons (Yellow arrow)

DISCUSSION:

Dorsal root ganglia (DRG) neurons had been classified in three main types (A, B, C) on the basis of their size. Type A neurons are large neurons (40–75 >m in diameter), type B cells correspond to medium neurons (20–40 >m in diameter), whereas type C neurons are the smallest cells with a diameter of less than 20>m [11]. In the present study, neurons in the trigeminal ganglion which is also a sensory ganglion homologues to DRG were classified based on its diameter into small (approx <22>m), medium (approx 22 - 29>m) and large sized neurons (approx >29>m). Each type of neurons is concerned with different sensation for e.g. small and medium sized neurons are mainly concerned with pain and temperature which are called nociceptors. Most nociceptors are polymodal respond to mechanical (touch and pressure), thermal (cold and heat), and chemical stimuli [12]. Also type A and B neurons have myelinated fibers that are mainly mechanoreceptive whereas type C gives rise to largely unmyelinated fibers that are mainly nociceptive [13]. In the present study 70 percent of neurons in the trigeminal ganglion consist small neurons (<22 >m) and medium neurons (22 - 29 >m) which may mediate nociception by synthesising neuropeptides, neurotransmitters in any pathological conditions.

In the present study there was intense localization of CGRP in the smaller and medium sized neurons and also there was weak localization of CGRP in the large sized neurons. The small sized neurons are innervated by type Aδ and type C-fibres which receive pain perception is carried by the small sized neurons of trigeminal ganglion [7]. Thus calcitonin gene-related peptide appears to have an important role in nociception [14]. The rat and human CGRP gene expression is stimulated in response to cAMP, a secondary messenger generated in response to CGRP receptor activation.

Thalakoti *et al* 2007[15] confirmed that activation of trigeminal neurons leads to changes in adjacent glial cells through gap junctions[16] and paracrine signalling by activating one branch of trigeminal nerve resulted in activation other branches[17]. Based on their findings, it is likely that neuronal–glial communication via gap junctions and paracrine signalling i.e., by release of CGRP and substance P are involved in the development of peripheral sensitization within the trigeminal ganglion and thus, are likely to play an important role in the initiation of migraine.

Each neuron in the ganglia is completely surrounded by several satellite glial cells which form a sheath or envelope thus forming a distinct morphological and functional unit. The group of neurons are separated from each other by satellite glial cell sheath with minimal connective tissue between them [18]. This complete glial sheath formed by satellite glial cells around the sensory neuron is an unique feature and is not found in CNS [19]. The satellite glial cell envelope usually consists of flat processes that lie close to the neuronal plasma membrane. The distance between glial cell and neuronal surface is about 20nm and therefore extracellular space between the neuron and satellite glial cell is minimal [19]. GFAP is important in modulating astrocyte motility by providing structural stability to astrocytic processes[20]. Glial fibrillary acidic protein (GFAP) the archetypal marker for astrocytes is reported to be present at either low levels in the normal satellite glial cells or in activated satellite glial cells [21]. Following peripheral nerve injury, satellite glial cells undergo changes similar to those found in CNS and show marked increase in glial fibrillary acidic protein (GFAP)[22]. there is increasing evidence that glia within the spinal cord dorsal horn contribute to the maintenance of pathological pain[23]. In chronic constriction injury of the sciatic nerve there was up-regulation of TNF-alpha leading to over expression of GFAP in the dorsal root ganglion neurons. Also, it was noted that this TNF-alpha signalling pathway is the major pathological change leading to inflammatory pain [24].

Increased GFAP immunostaining was observed in the gray matter of the spinal cord ipsilateral to the lesion and specific to spinal segments in which the sciatic nerve is distributed. Elevated GFAP staining density was attributed primarily to hypertrophy of astrocytes rather than their proliferation or migration since counts of astrocyte profiles demonstrated no significant difference when comparing the lesion to the control side. The magnitude of the increase in GFAP staining correlated with the degree of hyperalgesia [25].

CONCLUSIONS:

Thus the neurons in the trigeminal ganglion can be classified into small, medium and large neurons based on their shapes. The small neurons which express dense CGRP and innervated by unmyelinated nerve fibres' are responsible for nociception in painful conditions. Also the satellite glial cells surrounding the pseudounipolar neurons express GFAP which may upregulate in painful conditions leading to triggering of inflammatory process.

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