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Comparative Light Microscopic Study of Trigeminal Ganglion Neurons in Mammals

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Abstract

Trigeminal ganglion (TRG) consists of collection of primary sensory neurons. The different subsets of neurons have been identified on the basis of morphological and neurochemical characteristics. It remains to be resolved as to whether the various neuronal subsets remain alike across the mammalian species and if there exists some species specific characteristic neurons. The present study was conducted on adult mammals (rat, rabbit, and goat) of either sex. TRG of both sides were procured and fixed in 10% buffered formalin and processed for paraffin embedding. 10 µm thick sections stained with Haematoxylin and Eosin were examined under light microscope and relevant findings were recorded in photomicrographs. It was noticed that the main cellular constituents (neuron and glia) of TRG could be easily identified and features of most of the neurons matched with earlier light microscopic descriptions [1, 2]. However, few neurons in the present study revealed certain additional features. For example – in the medium size neuron, large Nissl granules formed single peripheral ring; in the medium and large sized neurons, coarse Nissl granules formed two concentric (perinuclear and peripheral) rings; and a couple of neurons appeared to share common sheath – a kin to binucleate neurons. In addition, the neuronal somatic size appeared to have direct relationship with the body size of the animal. The number of nucleoli and Cajal bodies per neuron and the number of cells involved in the formation of satellite glial cell (SGC)-sheath could be correlated with the size of neuronal somata. It was concluded that the neuronal subgroups of mammalian TRG remain fairly similar across the species. However some less common neurons with single and double rings of coarse Nissl granules need suitable categorization with respect to their neurochemical and functional characteristics.

Key words: Trigeminal Ganglion, sensory neuron, Nissl body, Cajal body, satellite glial cells.

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Introduction:

Like many other sensory ganglia associated with the cranial nerves, TRG also consists of collection of primary sensory neurons but unlike others, part of its primary sensory neurons in addition are located in the central nervous system as mesencephalic nucleus of trigeminal nerve [3]. It has similarity with the dorsal root ganglia (DRG) in being commonly affected by herpes virus [4, 5] resulting into postherpetic neuralgic pain syndromes and therefore, becoming the focus of large number of studies. Like DRG [6], TRG is also known to consist of different subsets of neurons e.g., two types of neurons based on staining intensity (dark and light) [2]; three types of neurons based on somatic size [7,3], and four types of neurons based on characteristics of Nissl granules [1] have been described. Type I-large neurons have diffusely distributed fine granules, type II-neurons contain coarse sparsely distributed Nissl granules, type III- neurons possess dense Nissl granules of varying size, and type IV-small neurons depict granules concentrated peripherally [1]. In cat the cell innervating meninges are said to be smaller than average [8]. Immunocytochemical studies have shown that to some extent certain morphological subsets of TRG neurons are positive for specific neurotransmitter or its receptors e.g., large TRG neurons are positive to NPY and Peptide-9 [3] and small to medium sized neurons are positive to substance-P, NKA, CGRP [3], NADPH diphorase [9], BDNF ([10]. However, while substance-P positive neurons may be small, medium or large [11] and GABA positive neurons are primarily large but may be medium or small [7] and Glutamate positive neurons are primarily small to medium size but may also be large [7]. In case of Galanin, changing pattern of intensity and somatic sizes of neurons was reported during the course of healing [12]. Four types of neurons based on Nissl granule characteristics [1] have further been defined electron microscopically by length and arrangement of flattened cisterns of granular endoplasmic reticulum and number of neurofilaments Type I- largest and type IV smallest. Type III and type IV lack neurofilaments. Seven types of neurons based on size, shape and electron density of the

neuronal somata have been described [13]. It still remains to be resolved as to what extent the light microscopic features of TRG neuronal heterogeneity (existence of subsets) are maintained across the mammalian species and if there exists some species specific characteristic features of neurons in TRG.

Material and Methods

In the present study TRG from both sides of 5 adult healthy animals of either sex (rat, rabbit, and goat) were included. Those from rat and rabbits were procured by euthanising the animals with over dose of general anaesthesia, followed by fixation with intracardiac perfusion of 10% buffered formalin. TRG from goat were collected from slaughter house within 15 to 20 minutes of sacrifice of the animals and was immersion fixed in 10% buffered formalin. TRG from each animal were processed separately for paraffin embedding. 10 µm-thick sections cut with the rotary microtome and stained with Haematoxylin and Eosin were observed under light microscope. Salient findings were recorded in photomicrographs taken at final magnification of X400 and X1000 (Olympus BX40).

Observations

The TRG contained two main types of cells namely neurons and glia. Neurons were larger and more prominent and less numerous while glia cells were smaller in size, more numerous and surround each neuron. Clusters of neurons were interspersed among nerve fascicles (Fig. 1 and Fig. 2). In cross sections, the nerve cell clusters appeared to assume different size and shapes. Almost all nerve cell bodies were circular to oval in outline and revealed a wide range of size variation both in an individual and across the species. The neuronal somatic sizes in rat, rabbit and goat ranged from 12 to 30 µm, 15 to 75 µm and 25 to 120 µm respectively. Each nest of neurons possessed cells of different sizes and there appeared to be no specific pattern in their manner of arrangement. In all animals, most of the neurons were lightly stained with some small and medium sized neuron took dark staining. Neuronal cell was characterized by large centrally placed

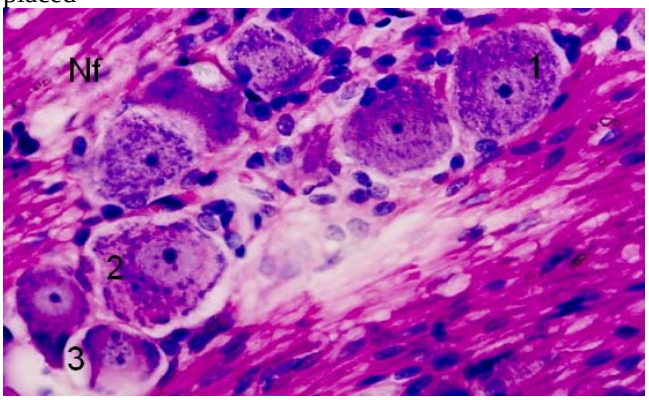

Figure 1. *Rat TRG: Cluster of neurons along with SGCs among fascicles of nerve fibres (Nf). Note typical neuron with single nucleolus and three Cajal bodies (1); single nucleolus, three Cajal bodies and Nissl granules in two concentric rings (2); medium sized neuron with double nucleoli (3). H & E; X 400.*

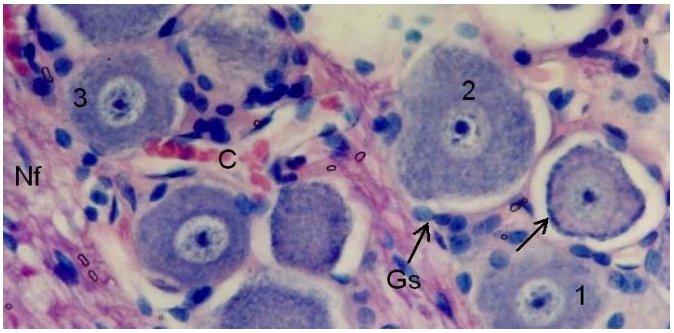

Figure 2. *Rabbit TRG: Neurons of different size interspersed among nerve fascicles (Nf) which is permeated by capillaries (C). Large Nissl granules forming prominent*

peripheral ring (↑), neurons 1, 2, and 3 have Cajal bodies. Also note the marked perinucleolar halo in neuron -2; and satellite glial cells (Gs) forming sheath. H & E X400.

euchromatic, vesicular nucleus and prominent nucleolus. The number of nucleoli per neuron ranged from 1-3 (Fig. 1). In addition some neurons depicted variable number (1-5) of Cajal bodies of varied size, staining intensity and arrangement with respect to the nucleolus (Figs. 1, 2, 4) and are believed to reflect a distinct transcription-dependent organization of nucleolus splicing machinery of TRG neurons [17]. Occasionally, a perinucleolar halo could also be visualized (Fig. 2, neuron-2). The perikaryon was filled with Nissl substance which assumed different appearance in terms of its overall amount, distribution pattern, size of granules, and intergranular space. Features of most of the TRG neurons matched with light microscopic features mentioned in the literature [1, 2]. However, few neurons in the present study revealed certain additional interesting features. For example – in the medium sized neuron, large Nissl granules formed single peripheral ring while fine granules occupied rest of the soma (Fig. 2), and medium and large sized neurons, coarse Nissl granules were arranged in the form of two concentric (perinuclear and peripheral) rings (Fig. 1). Normally, each neuron was surrounded by SGCs which actually formed a sheath and thus each individual neuron along with its SGCs could be identified as isolated units. But sometimes two medium sized or small neurons were placed so closely that part of SGC-sheath between adjacent neurons remained invisible even at higher magnification and it appeared that both neurons shared a common sheath (Fig. 3) or else represented a case of binucleated sensory neuron. As seen in the cross section, the number of SGCs involved in the formation of perineuronal sheath varied with the size of neuronal cell body ranging from just 3 around small neuron to 25 around large neurons. In other words, number of glial cells taking part in the formation of SGC-sheath seems lower in rats as compared to rabbit and goat. The cytoplasm of SGC was revealed only occasionally (Fig. 4).

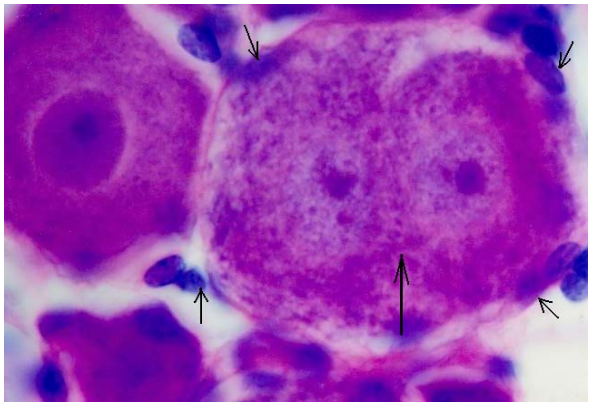

Fig. 3*. Rabbit TRG: Three neurons with their SGC-sheaths. Two neurons within common sheath (small arrows). No trace of connective tissue between two neurons (vertical long and thick arrow) thus giving appearance of a binucleate neuron. H & E; X 1000.*

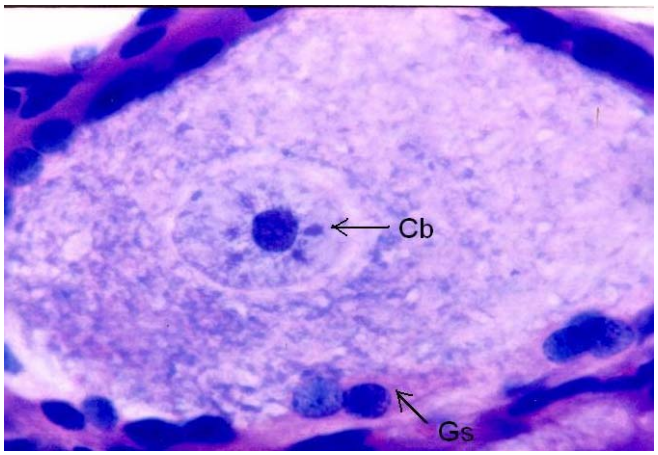

Fig. 4. *Goat TRG: Large oval neuron. Uniformly distributed Nissl granules, Single prominent nucleolus and five Cajal bodies (Cb). Perineuronal glial cell sheath (Gs) can easily be identified. H & E; X 400.*

Discussion

Sensory neurons in TRG are structurally, functionally and neurochemically heterogeneous [1,2,3,7,9,10,11,12,13]. In the present study only light microscopic morphological criteria have been taken for identification of different subsets of TRG neurons. The features of almost all TRG neurons fell within one or the other e.g., dark and light [2]; type I, II, III and IV [1]. However, few neurons revealed features which did not match with those described previously (Fig 1, 2 and 3). Neuron -2 in figure 1 is large and possesses double rings of coarse Nissl granules-and hence different from those describe earlier. The neuron in fig. 2 with single ring of coarse Nissl granule is akin to type IV neuron [1] but here it is of medium size neuron rather than small size. Thus these neurons present certain atypical features analogous to those described recently in the cervical DRG neurons of rabbit [15]. How these minor features can be related with some known characteristics of certain subsets of neurons in health and disease remains to be resolved.

In one study [7] it was noticed that around 20-24 % of TRG neurons were GABA positive while 64-66% were glutamate positive and was speculatively concluded that these amino acids act not only as neurotransmitter but also act as neuromodulators of sensory information. In other study [3] it was noticed that neurons showing immunoreactivity with glutamate, substance P, neurokinin, CGRP, CCK and SOM had small and medium sized somata, while those with NPY, and Peptide-19 had large sized somata, but nitric oxide synthase and paralbumin immunoreactive cells fell within all somata sizes. Thus, because of only partial correlation between somatic size and neurochemical characteristics, in the present study it will not be appropriate to comment on the neurochemical nature of these atypical neurons. Differential susceptibility of TRG neurons to herpes virus [16] and their subsequent atrophy or apoptosis [17 and demyelination of nerve fibres may be the morphological basis for the development of trigeminal neuralgia [18].

Santiago Ramón y Cajal described a new organelle in the nuclei of vertebrate neurons. When viewed with the electron microscope, these subnuclear 'organelles' appear to consist of a tangle of coiled threads and were hence also referred to as coiled bodies. Small Cajal body-specific RNAs (scaRNAs) are a class of small nucleolar RNAs (snoRNAs) which specifically localise to the Cajal body, a nuclear organelle involved in the biogenesis of small nuclear ribonucleoproteins (snRNPs) [19, 20]. In the present study these bodies are commonly noticed in large neurons of all species. Their number appears to be directly related to the size of neuronal somata e.g., their number is commonly 2-3 in rat and rabbit and may be 5-6 in case of goat (Fig. 4). Recent studies indicate that the Cajal body has numerous roles in the assembly and/or modification of the nuclear-transcription and RNA-processing machinery [21].

Neurons of TRG are anatomically isolated from one another and are not synaptically interconnected. And as such they are classically thought to function as independent sensory communication elements. In the present study a couple of neurons appear to share a common sheath formed by SGCs (Fig. 3). And the interneuronal sheath element though not visible cannot be ruled out with certainty and significance of such intimate association between certain neurons remains unclear. On one hand absence of interneuronal sheath element makes its appearance akin to a binucleate neuron, similar to one shown recently in DRG [15]. In DRG most neurons are transiently depolarized when axons of neighboring neurons of the same ganglion are stimulated repeatedly [22]. Such cross-depolarization contributes to mutual cross-excitation. Thus it appears that such type of intimate association (Fig. 3) may provide a suitable morphological substrate for the intraganglionic communication [22,23]. Although much less is known about SGCs in sensory ganglia, it appears that these cells share many characteristics with their central counterparts. Like Schwann cells, SGCs cytoplasm contains peroxisomes which may influence oxygen levels in the vicinity of' perikarya [24], and in adult TRG responds by proliferation to the mitogenic protein from explanted sensory neuron [25]. In the present study the SGCs number seems to positively correlate with the size of neuronal cell somata and this is in agreement with the finding in other species showing the volume of SGC-sheath to be directly proportional to both the volume and surface area of the related neuronal cell body [26].

Conclusion

Basic neuronal subtypes in TRG are similar across the species. Neurons with single or double rings of coarse Nissl granules require suitable categorization with respect to their neurochemical and functional characteristics.

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