

NEMATOSOMES IN THE RAT TRIGEMINAL GANGLION

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INTRODUCTION

The fine structure of a "new" cytoplasmic inclusion in sympathetic neurons of adult rats has recently been described (2). This inclusion was called a nematosome ("threadlike") and consisted of a convoluted network of electron-opaque strands embedded in a less dense matrix: it averaged 0.9μ in diameter. Morphological similarities between nematosomes, nucleoli, and synaptic material led to the suggestion that a nematosome might be an intermediary in the transfer of nuclear-derived substance to the sub-synaptic specializations occurring beneath the neuronal membrane (2). The present communication describes the presence of a morphologically similar inclusion in the cell bodies of sensory neurons of the rat trigeminal ganglion. This inclusion will also be referred to as a nematosome. The observations were made on tissue examined in the electron microscope during the course of studies of light and dark trigeminal neurons (6) and studies of the importance of fixative osmolality in preserving neuronal fine structure (7).

MATERIALS AND METHODS

Trigeminal ganglia from 150–300 g rats were obtained after fixation by aldehyde perfusion using the procedure described by Palay et al. (5); the fixative was delivered by an apparatus incorporating a pump and flowmeter at a rate of 40 ml/min for the first 3 min and 15 ml/min for the remaining period of time (usually 2 hr) (8). Total fixation times ranged from 2–24 hr and the fixatives used included: (a) a mixture of 1% formaldehyde (prepared from paraformaldehyde) and 2% glutaraldehyde (Fisher Biological Grade, Fisher Scientific Company, Pittsburgh, Pa.) in 0.08 M cacodylate buffer at pH 7.4, containing 0.2% calcium chloride. This was derived from the mixture described by Karnovsky (4); (b) 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2; (c) 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2; (d) 3% glutaraldehyde in 0.1 M phosphate buffer containing 9.2% sucrose, pH 7.2.

After completion of aldehyde fixation, the ganglia were carefully cut into small pieces, washed in the same buffer as used for the primary fixative, and immersed in similarly buffered 1% osmium tetroxide solution for 1 hr, before dehydrating and embedding in Durcupan (Fluka AG, Basel, Switzer-

land). Sections were cut with diamond knives on either a Sorvall MT2 ultramicrotome or on an LKB Ultratome III. Sections mounted directly on grids were stained with lead citrate (13) and with uranyl acetate (11) and examined in an RCA EMU4 electron microscope or an AEI 801 electron microscope.

RESULTS AND DISCUSSION

The bodies identified as nematosomes were observed in ganglia prepared by each fixative. As the tissue was being examined primarily for other purposes the numbers of nematosomes in proportion to the numbers of neurons were not subjected to statistical determination. However the frequency of observation seemed compatible with the view that at least one nematosome could be present in each neuron. The inclusions were morphologically similar to those described by Grillo (2) and this similarity forms the basis for their identification. In the sympathetic ganglion

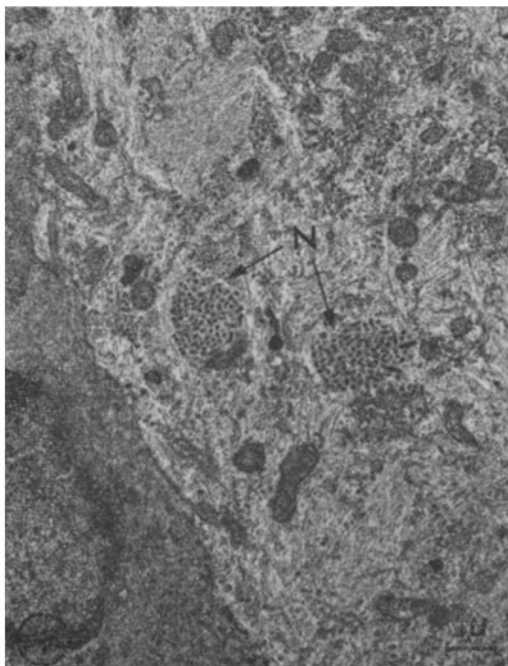


FIGURE 1 Two nematosomes (N) in cytoplasm of trigeminal neuron. $\times 7000$.

only one per cell was found (2) and this was usually the case in the trigeminal neurons. However, occasionally two were present (Fig. 1). They usually appeared as small circular aggregations of dense material ($\sim 1 \mu$ in diameter) (Fig. 1), but occasionally elongated forms ($\sim 4 \mu$ in length) were found (Fig. 2). The possibility exists that the circular profiles represent sections through a curved elongated body. However, as circular profiles were much more frequent, a spherical shape seems to be the more usual form. The nematosome was not membrane bounded and consisted of many coarse strands, $\sim 400 \text{ \AA}$ thick, each of which was made up of a finely filamentous material (Fig. 3). Depending on the plane of section, the coarse strands had varying lengths from 0.1μ to 4μ , and possessed a central electron-opaque core. The strands were embedded in a matrix which showed evidence of a finely fibrillar character but did not differ markedly from the rest of the neuronal cytoplasm. This was probably due to the initial fixation in aldehyde followed by osmium tetroxide. The material possessed a

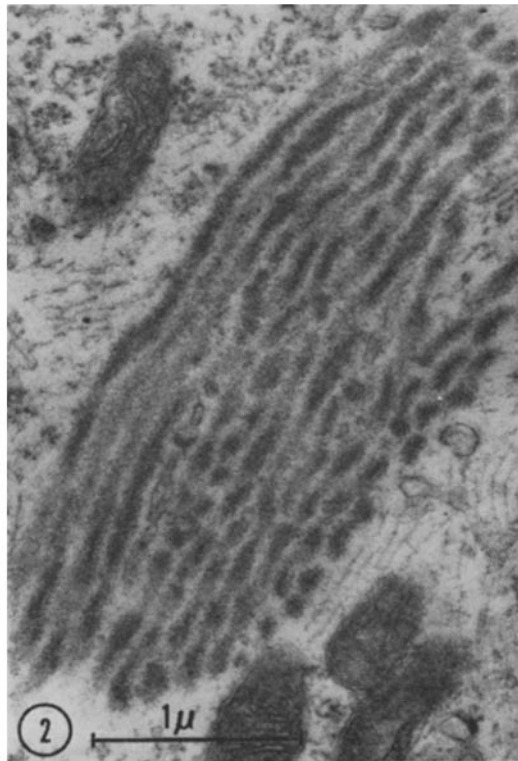


FIGURE 2 Elongated nematosome. $\times 28,000$.

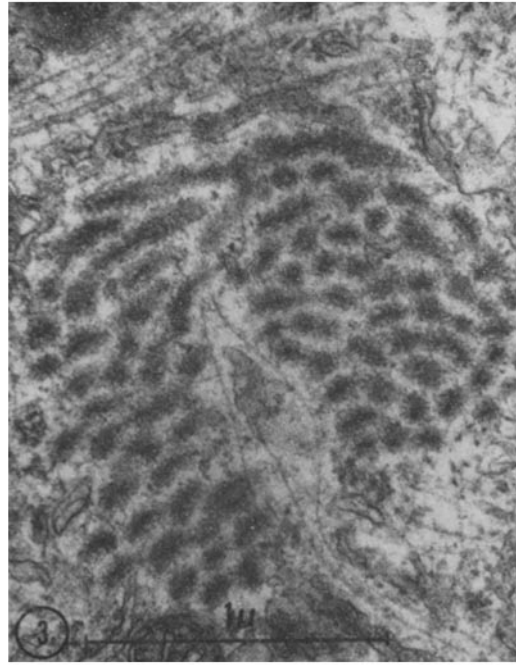


FIGURE 3 Higher magnification of nematosome: association with cytoplasmic organelles. $\times 40,000$.

greater density in the sympathetic neuron when fixed in osmium tetroxide alone (2).

The nematosomes tended to occur near the periphery of the trigeminal neurons. They were observed only in the larger, light nerve cell bodies of the trigeminal ganglion; they were not seen in the smaller, dark nerve cells, in satellite cells, or in the neuronal processes. The apparent absence from the dark neurons of the ganglion may not be significant. These cells are less numerous and have a more homogeneous compact cytoplasm (9) thus making the nematosome less readily detected, if present within their cytoplasm. More extensive searches might reveal its presence within the small dark cell. Although some have claimed that the dark cells may be fixation artifacts (12) evidence has been presented elsewhere that this is not the case (9).

The nematosomes showed frequent, but apparently random, associations with many cellular organelles such as neurofilaments, rough and smooth endoplasmic reticulum, and mitochondria. Examination of the cytoplasm of many nerve cell bodies did not reveal the presence of structures that might be considered derivatives

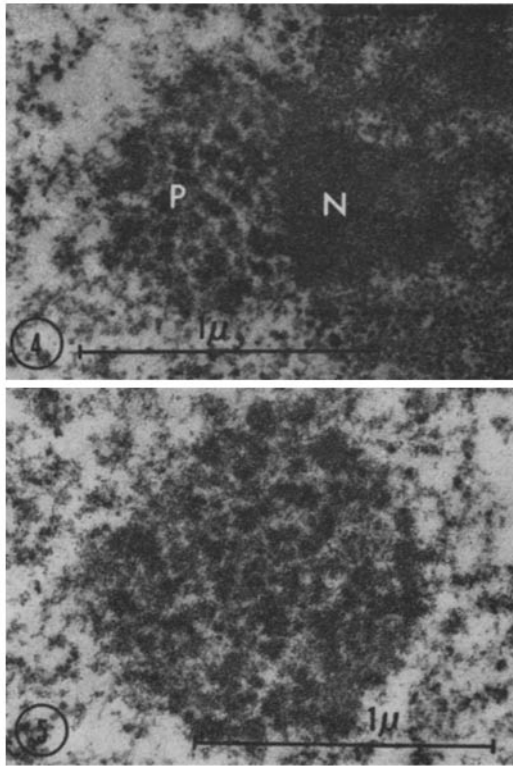


FIGURE 4 Paranucleolar body (P). N, nucleolus. Trigeminal neuron. $\times 40,000$.

FIGURE 5 Accessory body of Cajal from nucleus of trigeminal neuron. $\times 40,000$.

or precursors. However, some support was provided to the view that nematosomes may be derived from material of nucleolar origin (2). Bodies somewhat similar to nematosomes were found within the nuclei of the trigeminal neurons. The paranucleolar body (Fig. 4) and the accessory body of Cajal (Fig. 5) bore some resemblance to the nematosome in size, shape, and general appearance. These two nuclear inclusions differed from the nematosome in that their constituent strands were present only as very short lengths in the plane of section and presented a granular rather than a fiber-like appearance. They were identified on the basis of the descriptions provided by Hardin et al. (3), who demonstrated that in the rat trigeminal ganglion the accessory body may be derived from the nucleolus via the paranucleolar body. However these investigators did not consider the accessory body to be transported to the cytoplasm as it was never observed in contact with the nuclear membrane.

CONCLUSIONS

When originally describing the nematosome in the sympathetic neurons of the rat, Grillo (2) commented on the need for additional information concerning the distribution of nematosomes and their occurrence in other neurons whose synapses do and do not have a subsynaptic formation. The presence of nematosomes in trigeminal neurons is significant because synaptic contacts have never been observed on these cells during the course of numerous investigations (1, 6, 7, 9, 12). Thus subsynaptic specializations in the cell body have not been found and are unlikely to occur. The latter qualification is imposed by the sampling problems inherent in electron microscopy. However it is clear, at least, that any such specializations occur much less frequently than does the nematosome. Thus it seems unlikely that the nematosome represents subsynaptic material en route between nucleus and synapse as originally suggested (2).

Other situations in which bodies resembling nematosomes have been found are in a neuron of the cerebral hemisphere of the adult toad¹ and as structures designated "glomerular" bodies in the syncytial trophoblast of the mouse and rat placenta (14). The significance of their presence in the latter situation is not clear.

The functional role of the nematosome in the trigeminal neurons remains unknown. Continuing studies of the cells, their growth in tissue culture, and their embryonic development (10) may provide further information of value in interpreting the functional significance of the nematosome.

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¹ Rosenbluth, J. Quoted by Grillo (2).

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