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Frequency and distribution of tubulo-filamentous nuclear inclusions in the celiac ganglion of the cat as revealed by serial sections

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Summary. On sections at random of a cat celiac ganglion we counted 68 sections of nuclear inclusions (NI) for 320 sections of neuronal nuclei, i.e. an 'apparent' frequency of 0.20. As revealed by serial sections the 'actual' frequency is higher since the 5 nuclei entirely explored exhibit 19 NI. Such a study shows that each nucleus may contain at least 3 and up to 5 different tubulo-filamentous NI.

We have previously shown that the nuclei of sympathetic neurons in stellate, superior-cervical and celiac ganglia of the cat contain inclusions similar to the so-called intranuclear rodlets of the light microscopy. These nuclear inclusions (NI) consisting of filaments and/or tubules can be classified in 5 principal types, namely: filamentous spindle-shaped inclusions (type I), tubular spindle-shaped inclusions (type II), tubulo-filamentous spindle-shaped inclusions (type III), crystalloid inclusions formed only by filaments (type IV), crystalloid inclusions composed by filaments and tubules (type V)[2-4].

One of the most important problems dealing with these nuclear structures is to know whether they are common or uncommon, i.e. to fix their frequency of occurrence according to species and loci. In the sympathetic ganglia of the cat we have shown that these inclusions are encountered in all adult animals studied, with variations in frequency of occurrence from animal to animal. This frequency has been shown strongly increased following electrical stimulation[5] or local perfusion of cAMP analogs and theophylline in treated stellate ganglia compared to controlled ones[6]. These results suggest that filaments and tubules are normal components of the nucleus of sympathetic neurons and reflect the level of neuronal activity[2,6]. This frequency has been fixed by counting the number of sections of NI in 320 sections of neuronal nuclei on tissue sections taken at random in the ganglia, named in this work 'apparent' frequency. The 'apparent' frequency varies from 0.008 to 0.20 in the controlled ganglia[5,6].

The problem is to know which is the actual distribution of the NI for a given 'apparent' frequency, otherwise to know whether the phenomenon occurs in the whole neuronal population or only in some neurons of sympathetic ganglia. Such an answer should be found in a statistical processing of the quantitative data as far as the volume and size of these inclusions could be identified to a simple geometric pattern. So that it is the distribution of the nuclear bodies taken as spheres could be determined by some authors[7].

Cell	Type I Filamentous spindle-shaped inclusions	Type II Tubular spindle-shaped inclusions	Type III Tubulo-filamentous spindle-shaped inclusions	Total number of inclusions by neuron
A	4	1	0	5
B	2	1	0	3
C	3	1	0	4
D	3	1	0	4
E	2	0	1	3
Total number of inclusions	14	4	1	
Percentage (serial sections)	74	21	5	
Percentage (sections at random)	59	15	24	

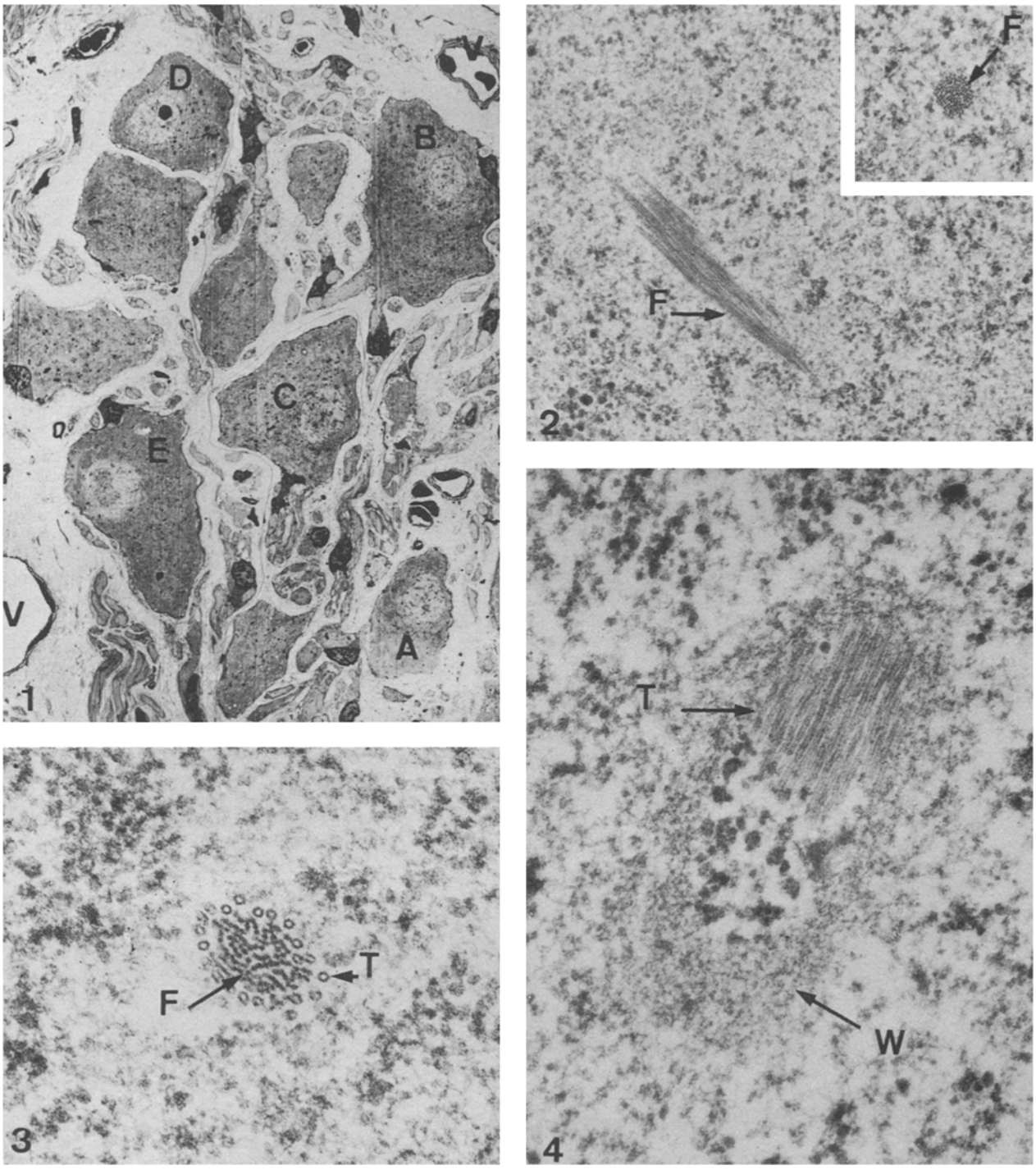

Fig. 1. Photographic mounting of the 105th section showing the cells cluster (ABCDE) in serial sections with reference-marks (vessels: V). × 800. Fig. 2. Longitudinal section of a filamentous spindle-shaped inclusion. This type is formed only by filaments (F) in close contact with the nucleoplasm. × 40,000. Inset: the same inclusion after tilting experiment. × 46,000. Fig. 3. Transversal section of a tubulo-filamentous spindle-shaped inclusion. This kind of inclusion is the longest and is formed by parallel filaments (F) surrounded by a layer of tubules (T). × 120,000. Fig. 4. Oblique section of a tubular spindle-shaped inclusion whose tubules (T) are isolated from the nucleoplasm by a granulo-fibrillar material wrapping (W). × 80,000.

Recent studies in serial sections have shown us that the shape and size of the NI are highly variable[4].

Therefore, to know the distribution of the NI in a given ganglion, it becomes necessary to cover completely the neuronal nuclei by serial sections. Here, we have determined a) the 'apparent' frequency of neuronal nuclei in a celiac ganglion in the cat and b) the frequency of occurrence of NI in each nucleus, i.e. the 'actual' frequency by examining the whole serial sections of 5 neurons of the same ganglion. The questions were as follows:

Do all the neurons contain such NI for a given 'apparent' frequency?

Is it possible to find several distinct NI in one given nucleus and, if so, are the NI the same or a different type?

Methods. After a short induction of anesthesia with fluothane, an adult male cat was given 0.25 mg/kg sodium pentobarbital solution i.v. The left celiac ganglion was removed, cut into small fragments, fixed in a 2.5% glutaraldehyde solution (pH 7.4) for 1 h, postfixed in a 2% osmium tetroxyde solution (pH 7.4) for 1 h and embedded in Epon. We have taken at random 4 blocks of ganglion from ten. White sections were made on an LKB ultramicrotome and taken at random on grids (mesh 200). One grid was examined for each block, then, counted 80 sections of nuclei on each grid obtaining 320 sections by ganglion. For serial sections, white-coloured serial sections obtained from one of these blocks have all been collected on one-hole grids (copper grids with a rectangular $2 \times 0{,}6$ mm hole, Janning) covered with 0.25% formwar membrane. The sections were stained with uranyl acetate and lead citrate then examined in a Siemens Elmiskop 101 with a goniometer stage.

Results. For 320 sections of neuronal nuclei we found a total number of 68 sections of NI, accordingly an 0.20 'apparent' frequency, as below:

- 40 sections of type I inclusions (roughly 59% of all the inclusions observed),
- 10 sections of type II (15%),
- 16 sections of type III (24%),
- 2 sections of type IV and V (2%).

In order to determine the 'actual' frequency of NI, we studied 400 serial sections of the same ganglion from a zone which we could identify by capillaries (figure 1). These capillaries were taken as reference-marks and were again discovered on the whole thickness of the examined tissue (30 µm). In this area we completed a study of 5 neurons designated as ABCDE (figure 1). All of the 5 nuclei were examined at a 40,000 magnification. We observed 19 NI clearly identified with the help of a goniometer stage (figures 2–4). It must be pointed out that no crystalloid inclusion in these serial sections were found. The table summarizes the repartition and the frequency of these NI in the 5 neuronal nuclei that were studied.

Discussion. Several remarks can be made about these results. For an 0.20 'apparent' frequency, all the examined neurons contain NI. This result still includes too few a number of cells to be extrapoled to the whole neuronal population. Indeed, one can think that the presence of NI is a specific feature of a group of adjacent neurons such as those that have been studied. However this hypothesis is unlikely as our previous studies carried out on sections taken at random on the ganglion have shown that NI occur in all parts of a sympathetic ganglion[2,5,6].

The most striking fact is that up to 5 different NI may occur in a single nucleus, a possibility which did not appear clearly by observing sections taken at random. Indeed out of 68 sections of inclusions counted by this means only one case showing simultaneously 2 sections of NI in the same nucleus occurred. It appears clearly that one same nucleus may contain different types of NI (see table).

However a difference appears in this work between the 'apparent' and the 'actual' frequency of the type III (see table). This difference is explained by our previous studies. If one considers the 3-dimensional pattern of these NI: this kind of NI is the longest (up to 10 µm) when the type II never exceed 4 µm[4]. The probability for the level of a section to cut through type III NI is consequently much greater and explains the difference between the 'apparent' and 'actual' frequency.

Conclusion. Serial sections show clearly that the 'actual' frequency of tubulo-filamentous NI in sympathetic neurons is more higher than previously claimed since a single nucleus may contain up to 5 different inclusions. The present observations confirm our previous ones according to such structures are normal components of the healthy nucleus[2,6].

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Prefrontal cortex of the cat: Evidence for an additional area

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Summary. Direct projections from the mediodorsal nucleus of the thalamus to ventral parts of the insular region of the cat's cortex were demonstrated by using the horseradish peroxidase technique.

The extent of the prefrontal cortex of the cat has been defined differentially in various behavioral[1] and anatomical[2–6] studies. However, in nearly all studies, there is conformity with respect to at least 2 basic positions: 1. The extent of the cat's prefrontal cortex is defined as the projection area of the mediodorsal nucleus of the thalamus (MD). 2. The extent of the cat's prefrontal cortex consistently is limited to the frontal pole[7,8].

Topologically therefore, the cat's prefrontal cortex is similar in position to the respective delineation of the monkey's prefrontal region, but differs from that of the rat: Both the monkey's and cat's prefrontal cortices are situated around the frontal pole, whereas the rat's is divided into 2 areas of which none extends to the frontal pole[9,10].

Our experiments show evidence that also the cat's prefrontal cortex topologically consists of 2 separable regions. After having detected horseradish peroxidase (HRP) labelled cells within the cat's insular region after injections into the frontal pole[11], we injected HRP in different parts of the insular cortex and of neighboring regions, using Mesulam's modification of the HRP technique[12,13]. In 11 cats, small amounts of HRP (Boehringer, Grade I) were injected into parts of the orbital, sylvian and ectosylvian gyri, as is schematized in figure 1. 8 of the cats received unilateral