Z. Zellforsch. 103, 109—114 (1970)

Ultrastructural Investigation on the Innervation of the Herbst Corpuscle*

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Received September 29, 1969

Summary. Nerve fibres, running longitudinally as well as circularly between the core lamellae in the Herbst corpuscle are described.

These fibres are morphologically different from the central afferent axon. They are most frequently observed in the outer part of the core, and contain inter alia numerous agranular vesicles measuring approx. 450 Å in diameter, dense core vesicles with a diameter approx. 800 Å and microtubuli (250 Å). Occasional specialized junctions are seen between the nerves and the neighbouring lamellae.

Key-Words: Nervous system — Receptors — Herbst corpuscle — Nerve endings — Myelin sheath.

The morphology of the central afferent axon in the Herbst corpuscle has been described in previous papers (Pease *et al.*, 1957; Quilliam, 1966; Munger, 1966; Andersen *et al.*, 1968, and Saxod, 1968). The axoplasmic structures of this terminal seem in many respects to be analogous to the structures described by Andres (1966) in the straight lancet terminals of the vibrissae of the cat, and by Pease and Quilliam (1957) in the central nerve of the Pacinian corpuscle. The central axon is characterized by numerous mitochondria arranged at the periphery of the fibre and a bundle of filaments at the centre. The end of the fibre forms a large spherical head about 8—9 μ in diameter containing more heterogenous axoplasmic structures including a great variety of vesicles, tubules and predominantly in its centre, filamentous material. This central axon is the only nerve type described electron microscopically in the Herbst corpuscle. In a previous work (Andersen *et al.*, 1968), dense core vesicles were sporadically observed in the core lamella. Quilliam (1966) noticed a nerve-like structure containing "semiopaque bodies" in the innermost cell layer of the capsule, only separated from the "fluid lake" by a basement membrane.

Using the light microscope, Goto *et al.* (1961) and Loewenstein *et al.* (1962), in addition to the afferent axon, described a small unmyelinated fibre (C fibre) entering the Pacinian corpuscle.

Using fluorescent microscopy, Santini (1968) observed noradrenergic fibres in the inner core of the Pacinian corpuscle in the cat. On the other hand, Fuxe (1965), using a similar technique, did not observe such fibres.

* This study was supported by the Norwegian Council of Agricultural Research.

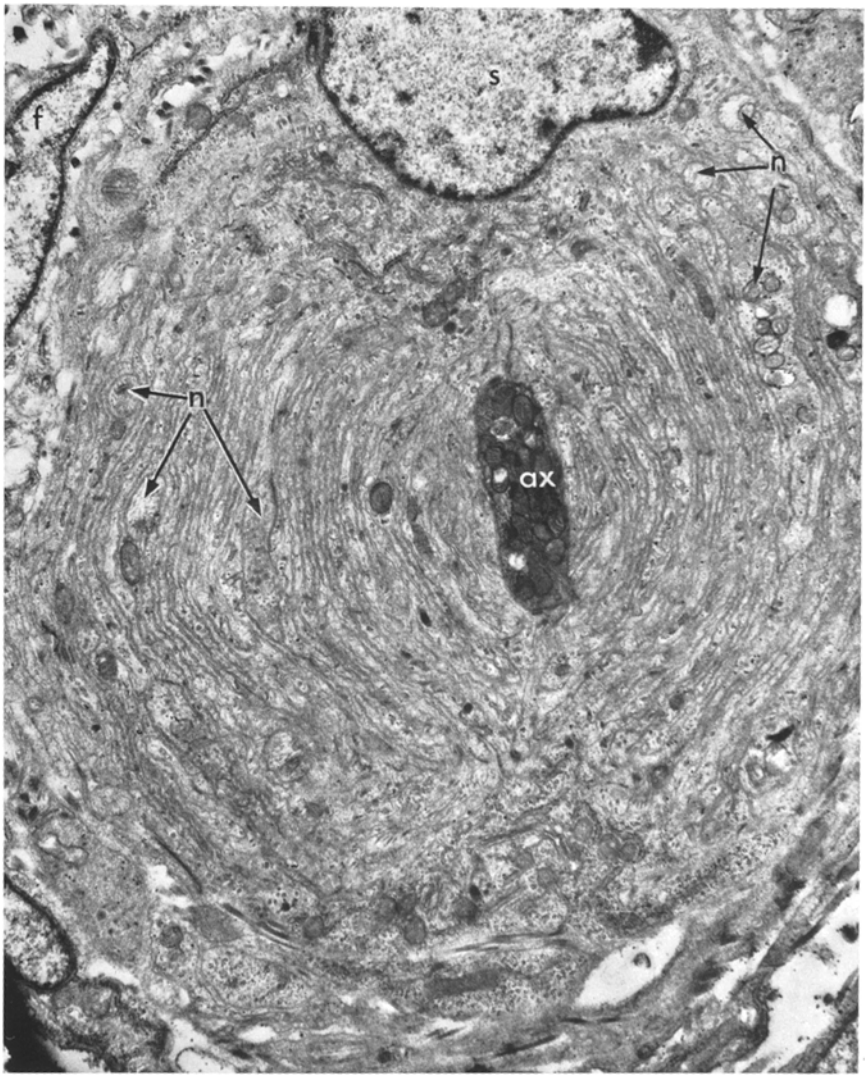

Fig. 1. Electron micrograph. Cross section of a Herbst corpuscle, showing the core. In addition to the dark central afferent nerve, nerve fibres are visible in the outer part of the core. *ax* central afferent axon, *f* outer core fibrocyte nucleus, *n* nerve fibres, *s* sentinal cell nucleus. × 10,000

Materials and Methods

3 month old chicks were used. Before preparation they were anaesthetised with phenemal sodium.

Some of the material was prepared in the same way as described in the paper of Andersen *et al.* (1968), i.e. perfusion with 3% glutar-aldehyde in Millonigs Phosphate buffer and 3% Macrodex, postfixation for about $1^1/_2$ hour in 2% OsO_4, aceton dehydration and Araldite embedding. In some chicks, immersion fixation with the same fixatives was carried out. The fixation time in the glutaraldehyde solution was about 2 hours. Finally, perfusion as well

as immersion fixation with 3% potassium permanganate was performed. The ultra-thin sections were double stained with aqueous uranyl acetate and lead citrate (Reynolds, 1963).

In this study, only Herbst corpuscles situated in the frontal part of the hard palate were examined.

The material included 47 animals, from which a various number of serial sections were studied. About 3 μ thick toluidine blue stained sections were examined under the light microscope to localize the Herbst corpuscles. Ultra-thin serial sections were then carried out, and it was possible to examine about 3 corpuscles at the same time in each of the series.

Results

In the immersion fixed material, nerve-like fibres were observed, mainly in the outer core (Fig. 1). Serial sections indicated that the fibres are beaded and occasionally flattened. They run circularly as well as longitudinally between the core lamellae. The dilatated parts are rich in vesicles, mitochondria and glycogenlike granules. Two kinds of vesicles can regularly be observed. Roughly estimated, about 80% of them are agranular with a diameter approx. 450 Å. The remainder are granular or dense core vesicles, with a diameter approx. 800 Å (Fig. 2). Desmosome-like junctions can be observed between these dilatations and the core lamellae (Fig. 2, inset). These junctions do not seem to be significantly different from the interlamellar desmosomes described earlier, but occasionally the electron-dense material in the axonal cytoplasm is lacking (Fig. 2, Inset).

In the constricted part of the fibre, the vesicles and mitochondria are less numerous, and microtubules are the most conspicious structure (Fig. 2). The tubules, which run parallel with the fibre, have a constant diameter approx. 250 Å.

Nerve-like structures were also observed in the "fluid lake" which surrounds the core of the Herbst corpuscle (Fig. 3C). They are enveloped in cytoplasmic sheats limited by a basal lamina. The axoplasma of these fibres are characterized by microtubuli and mitochondria.

During this work we succeeded in obtaining continous serial sections of the myelinated entry nerves. They were usually found to be coiled, at the site of demyelination (Fig. 3), and they showed varying numbers of myelin diverticuli. In single sections, this morphology is easily misinterpreted, and gives an appearance of more than one myelinated entry nerve (Fig. 3B). In earlier material based on interrupted serial sections, an attenuation of the fibre in this region is described (Andersen *et al.*). In serially sectioned material, however, the diameter was found to be constant throughout the entry region.

Discussion

This study clearly indicates the presence of two different nerve types in the Herbst corpuscles. It is unlikely that the fibres described in the core represent branches of the central afferent axon. In a few cases the central axon was seen to divide into two main branches. They retained, however, the surrounding lamellar organisation and the axoplasmic characteristics of the central nerve. Further, serial sections revealed no continuity between these fibres and the 1—2 μ long thorne-like protrusions of the central fibre.

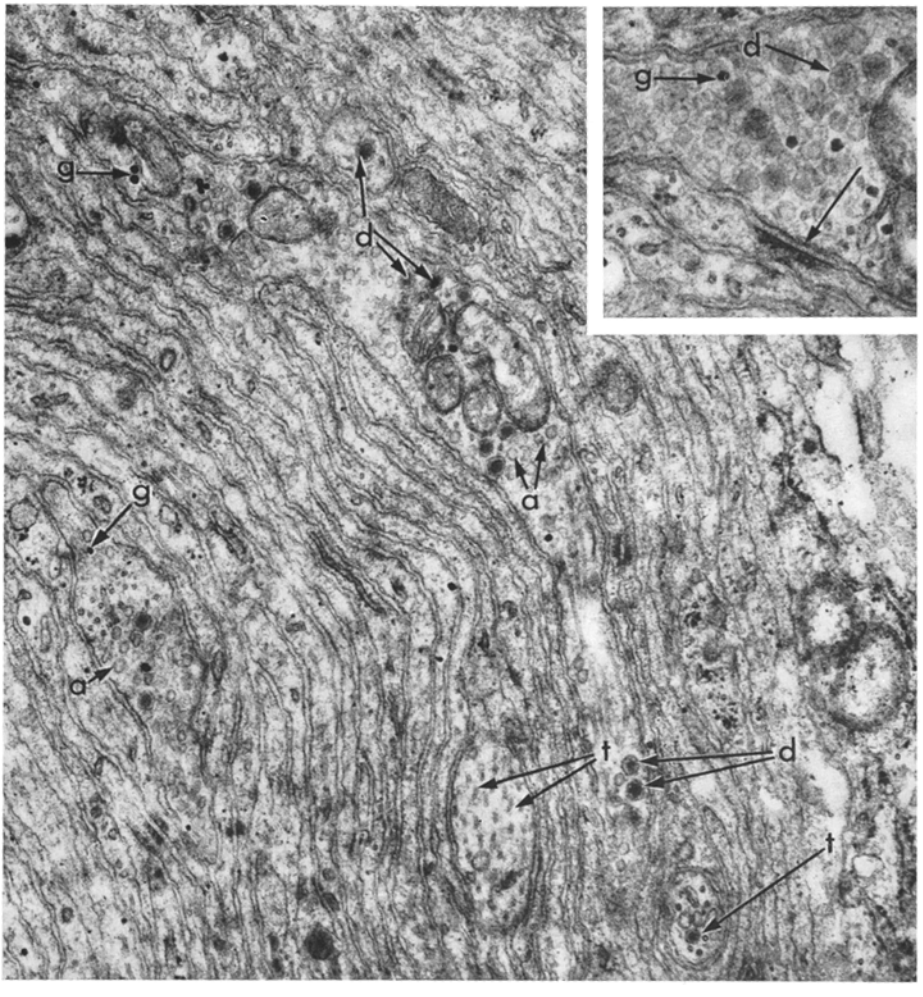

Fig. 2. Nerve fibres in the laminated core of a Herbst corpuscle. Microtubuli are most frequently observed in the constricted parts of the fibres, while the dilatations are rich in vesicles. The inset demonstrates a synapse-like junction between a nerve dilatation and the neighbouring lamella (arrow). *a* agranular vesicles, *d* dense core vesicles, *g* glycogen-like granula, *t* microtubuli. × 30,000, inset × 45,000

The profiles of the fibres are sometimes flattened in a similar way to the outer core lamellae. We tried to find continuities between the fibres and the cytoplasma of the twin rows of cells forming the core (sentinal cells). Such continuity could prove a relationship between these cells and nerve cells. No convincing continuity was seen, and ribosomes were not observed in the fibres. Ribosomes, however, are numerous in the "sentinal cells" and especially in the outer core lamellae.

In a great number of Herbst corpuscles examined in this study, the nerve structures described could not constantly be observed. In aldehyde and osmium fixatives, eruptions and discontinuities in the membranes of the outer core and

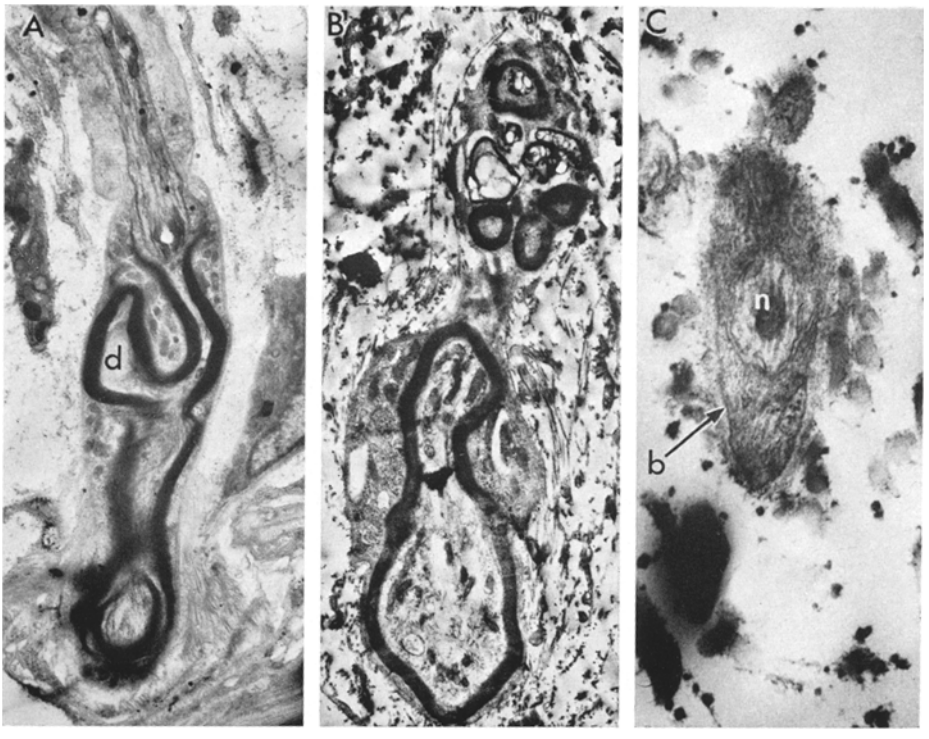

Fig. 3. A illustrates the afferent axon at the site of demyelination, showing diverticula in the myelin sheath. In single sections these diverticula may be misinterpreted as tiny myelinated "accessory" fibres as demonstrated in B. C shows an unmyelinated fibre situated in the "fluid lake", presumed to be the entry nerve of the core fibres described in this paper. *b* Schwann cell basal lamina, *d* myelin diverticula, *n* unmyelinated fibre. A and B $\times$ 4,600, C $\times$ 30,000

capsular lamellae, are constantly present (Fig. 1). Some of these artifacts may be residues of the outer core and capsular nerves. Our sporadic registrations might thus be due to fixation sensitivity of this type of fibre. In potassium-permanganate fixed material the artifacts were less frequently observed. However, the paucity of intracytoplasmic structures seen using this fixation method, makes it difficult to identify the cytoplasmic profiles seen in the sections. Dense core vesicles, on the other hand, were observed more constantly in some of the profiles by this method, which is in agreement with the postulation of Richardson (1966).

Recently, Andres (1969) described replacement of the supporting cells and the apical part of the olfactory cells in the olfactory mucosa of cat, particularly in young animals.

During this investigation, Herbst corpuscles showing an immature pattern, were regularly observed. They are characterized by an indistinct "fluid lake", and there is no clear differentiation between the core and capsular structures. Mitotic figures could be observed sporadically in the cells surrounding the central nerve ending.

That Herbst corpuscles also have a limited lifetime cannot be discounted in this study. If this is the case, it is possible that our inconstant registration of doubly innervated corpuscles, was due to some extent, to variations in the maturity of the organs.

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