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Ultrastructure of the Rat Pineal Stalk

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Abstract. The ultrastructure of the rat pineal stalk was described. The pineal stalk contained few pinealocytes, glial cells and numerous nerve fibers. The last were mostly non-myelinated axons, although a few myelinated ones were also observed. Glial cells showed many filaments, mostly in the processes which presented a longitudinal orientation. Other more lamellar processes were found enclosing the axons. The pineal stalk became wider as it reached the body of the gland. Ultrastructurally, this wide region resembled more the pineal body. Bundles of non-myelinated nerve fibers were seen around the pineal stalk.

Introduction

The albino rat pineal gland is connected to the roof of the third ventricle by a long and thin pineal stalk [review in *Vollrath,* 1981]. The structure of the pineal stalk has been scarcely studied. According to *Kappers* [1960], the pineal stalk contains pinealocytes, nerve fibers and fibroblasts. *Gregorek* et al. [1977] describe a connective tissue stalk which occasionally presents pinealocytes. According to *Boeckmann* [1980], only 17.7% of rats do have a continuous parenchymal stalk. Until now, no electron-microscopic studies have been carried out on the rat pineal stalk.

In the last decade, studies on pineal innervation have improved our knowledge about the pineal stalk. According to these studies, a nervous connection between the brain and the pineal is established through the pineal stalk. Pineal stalk nerve fibers have been demonstrated with both light and electron microscopy in several rodent species, including hamsters [*Sheridan and Reiter,* 1970; *Vann Veen* et al., 1978] and mongolian gerbils [*Moller and Korf,* 1983a, b]. Lightmicroscopic studies have also shown nerve fibers in the rat pineal stalk. *Bjorklund* et al. [1972] found sympathetic nerve fibers running from the pineal gland to the medial habenular nuclei. More recently, several studies [*Ronnekleiv and Moller,* 1979; *Guerillot* et al., 1982; *Dafny,* 1983] have demonstrated nerve fibers of central origin reaching the pineal gland through the pineal stalk. However, until now, pineal stalk nerve fibers have not been confirmed in the rat with electron microscopy.

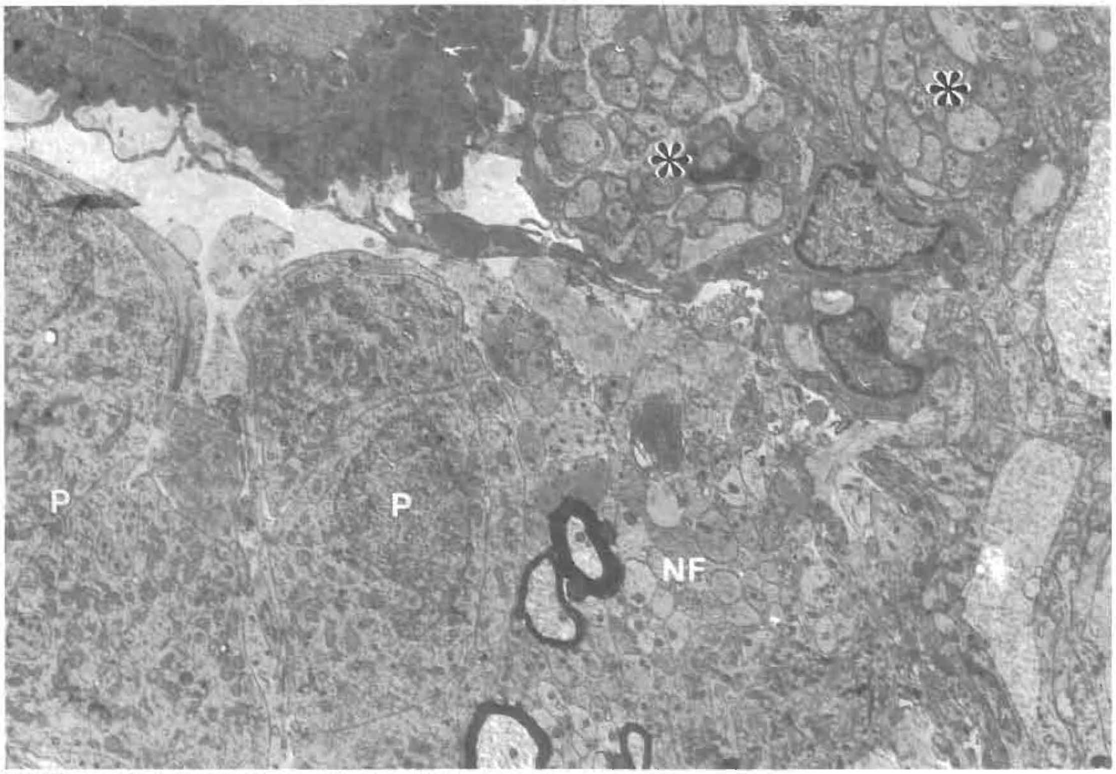
In the present study we describe the ultrastructure of the pineal stalk of the adult albino rat.

Materials and Methods

Twenty adult male and female Wistar rats, 4–6 months old 300–400 g body weight) kept under routine laboratory conditions (light: dark 14:10) were decapitated under ether anesthesia. The brains with the pineal gland left in situ were fixed by immersion in 2% glutaraldehyde–2% paraformaldehyde in 0.1 *M* phosphate buffer, pH 7.4. The cerebral hemispheres were dissected in order to allow rapid fixation of the pineal stalk. After fixation, a block was cut with the pineal complex resting on the corpora quadrigemina. The blocks were washed in phosphate buffer, postfixed for 2 h in 2% osmium tetroxide in 0.1 *M* phosphate buffer and embedded in Vestopal. The blocks were oriented in order to obtain transversal sections of the pineal stalk. Ultrathin sections obtained every 100 μm were stained with uranyl acetate and lead citrate and examined in a Philips 201 EM.

Results

Along most of its length, the pineal stalk is a very thin cord with a diameter generally below 100 μm. The



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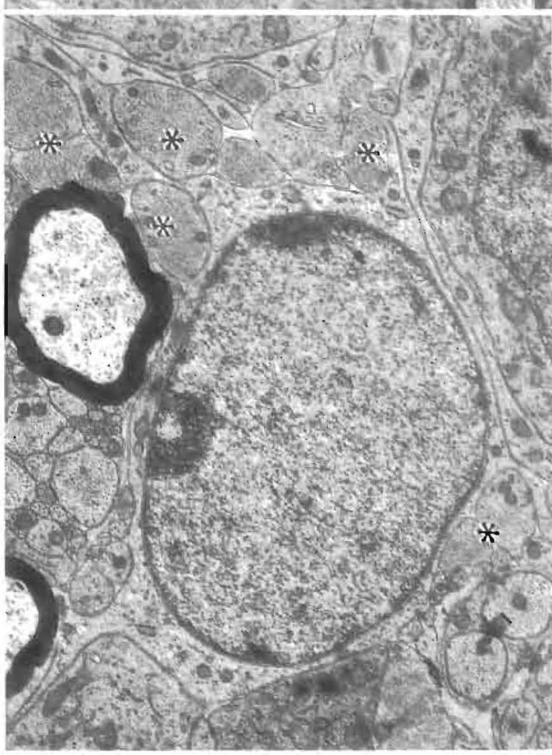

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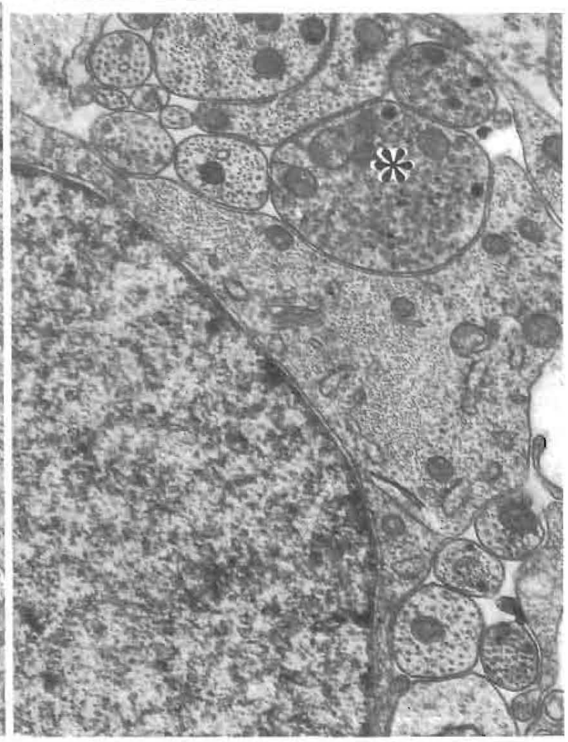

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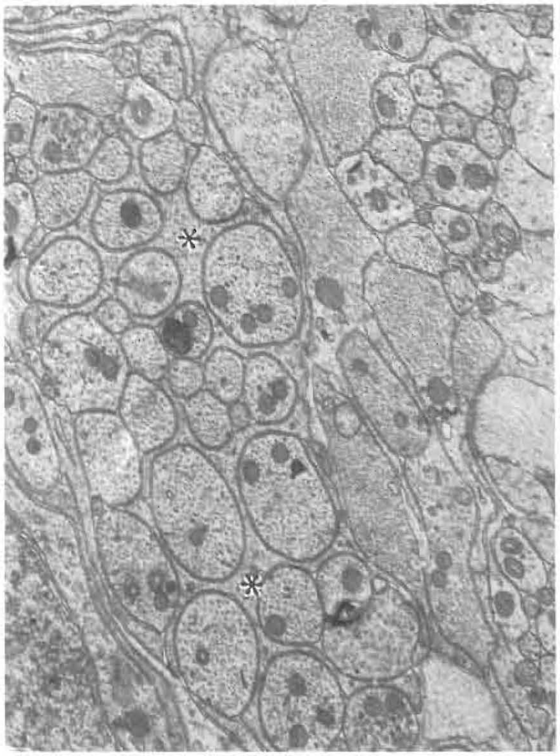
Fig. 1. Low-magnification image of the pineal stalk. Several type I pinealocytes (P) and numerous nerve fibers (NF), some of which are myelinated, are shown. Close to the stalk there are bundles of amyelinic nerve fibers (asterisks) and an arteriole. ×5,600.

Fig. 2. Glial cell with a small soma from which several processes emerge. The cell is located among nerve fibers and filament-rich glial cell processes (asterisks). ×12,800.

Fig. 3. Glial cell cytoplasm with abundant microfilaments. Close to the cell there are axons, some of which contain granular vesicles (asterisks). ×24,600.



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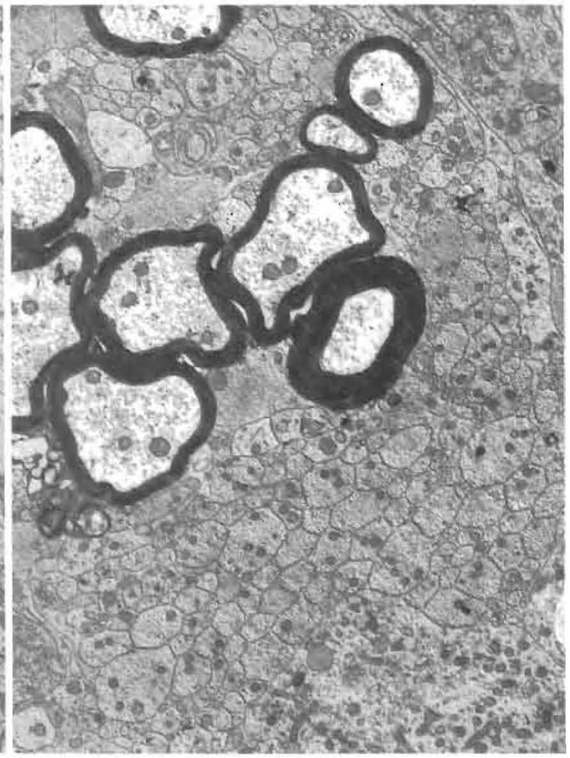

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Fig. 4. Axons and glial cell processes. The latter show a more irregular contour and contain numerous microfilaments. Other lamellar glial cell processes (asterisks) are enclosing axons. ×19,200.

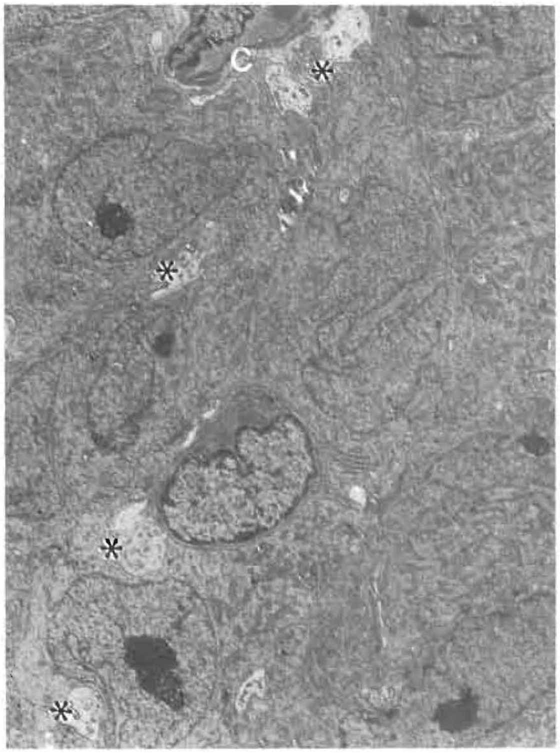
Fig. 5. More proximal section of the stalk in figure 1. It shows nine myelinated fibers among many amyelinic ones. In more distal sections (fig. 1), the number of myelinated fibers is lower. ×8,900.

pineal stalk showed pinealocytes, glial cells and numerous nerve fibers. Pinealocytes were few in number but they appeared along the entire stalk. They were all type I pinealocytes [*Calvo and Boya,* 1983, 1984] which had a lesser development of cytoplasmic organelles than those found in the pineal body (fig. 1). The scarcity of lipid droplets and dense bodies mostly stands out.

Glial cells showed a small cell body and many thin processes (fig. 2). These glial cells were characterized by the presence of microfilaments, which were sometimes very numerous (fig. 3). The ovoid nucleus presented a thin peripheral rim of heterochromatin and a clear nucleoplasm (fig. 2, 3). The perinuclear cytoplasm was generally scarce, although large cytoplasmic profiles were sometimes found. Among other organelles found were prominent cisterns of rough endoplasmic reticulum, small mitochondria with a dense matrix and occasional lipofuscin granules. A few microtubules were also observed scattered among the microfilaments in the perinuclear cytoplasm (fig. 3). Most glial cell processes tended to adopt a longitudinal orientation with respect to the stalk axis. These processes contained numerous microfilaments (fig. 2, 4).

The pineal stalk showed abundant nerve fibers. Most were amyelinic axons not enclosed by Schwann cells (fig. 1, 5). Nevertheless, small bundles of fibers and also single axons were sometimes enclosed by glial cell processes (fig. 4). The axons displayed prominent microtubules, microfilaments and few small mitochondria (fig. 3, 4). In the transversal sections studied, glial cell processes showed more irregular contours than the more rounded profiles of axons (fig. 4). A few myelinated nerve fibers were also observed, mainly at the most proximal level of the pineal stalk (fig. 1, 5). The number of these fibers decreased in the more distal areas until they finally disappeared before reaching the medial third of the stalk.

Although the pineal stalk lacked the large connective tissue spaces typical of the pineal body, it presented small irregular connective tissue spaces arising from its



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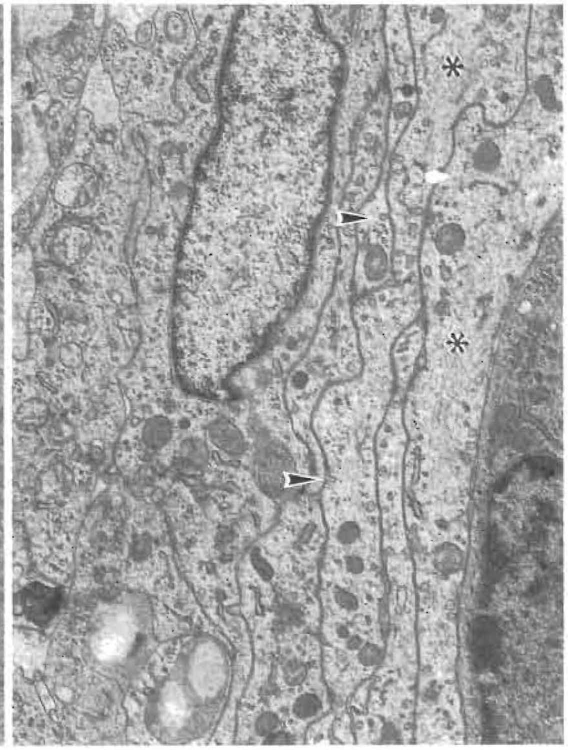

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Fig. 6. Wide region of the pineal stalk. The parenchyma has a compact appearance showing type I pinealocytes with poorly developed organelles and a type II pinealocyte. There are also some clear processes of glial cells (asterisks) and a capillay (C). ×3,800.

Fig. 7. Layer of lamellar clear cells which surrounds the pineal stalk. In the clear cytoplasms there are filaments (asterisks) and pinocytic vesicles (arrowheads). ×12,800.

external surface (fig. 1). These spaces contained abundant collagen fibers. The basal lamina was often incomplete or absent in these spaces.

The pineal stalk widened as it reached the body of the gland. This wide region of the stalk is short and displayed an ultrastructural appearance resembling the pineal body, although there were still differences between them. Except for the occasional presence of some blood vessels, there were no large connective tissue spaces, which gives a compact appearance to this region of the stalk, different from the cord-like structure observed in the pineal body (fig. 6).

Most cells observed in this wide stalk region were type I pinealocytes, although there were also some type II pinealocytes [*Calvo and Boya,* 1983, 1984] but fewer than in the pineal body. Type I pinealocytes displayed poorly developed cytoplasmic organelles, as described for the thin region (fig. 6).

The wide region contained few glial cells. These cells were identified by their chromatin pattern and the presence of microfilaments and cisterns of rough endoplasmic reticulum. Nevertheless, the glial cells of the wide region showed a clearer cytoplasm with fewer organelles (fig. 6). There were also fewer microfilaments in the soma and processes than in the thin region. However, processes filled with microfilaments were sometimes found. Glial cell processes tended to be related with connective tissue spaces, although they did not form a continuous layer around them.

The wide region of the pineal stalk lacks the large bundles of nerve fibers found in the thin region. It instead presented scattered nerve fibers which appeared isolated or formed very small groups, generally located near a connective tissue space and frequently related with glial cell processes. Myelinated nerve fibers were no found in this region.

The pineal stalk is surrounded by a layer of clear cells (fig. 7) located outside the basal lamina which limits the pineal tissue, with a narrow connective tissue space between these two elements. This cell layer was practically continuous in the wide region and more incomplete in the thin one. These cells were characterized by their lamellar shape. They showed macula-adherens type interdigitations and junctional complexes. Their clear cytoplasms contained pinocytic vesicles and microfilaments. (fig. 7). Similar cells were seen in the arachnoid trabeculae located outside the pineal stalk. Small connective tissue spaces which contained abundant collagen fibers were frequently observed among the clear cells of this layer.

Small bundles of amyelinic fibers, enclosed by typical Schwann cells were constantly found around the pineal stalk along all of its length (fig. 1).

Discussion

According to our results, the ultrastructural appearance of the pineal stalk differs markedly from that described for the pineal body. Some of the previously described light-microscopic data on the rat pineal stalk must be revised. Thus, the pineal stalk is not a connective tissue structure since it shows pinealocytes at all its levels. These are mainly type I pinealocytes with a characteristic poor development of cytoplasmic organelles. We have not been able to find discontinuities in the pineal stalk with the electron microscope. According to *Boeckmann* [1980], more than 80% of rats have a discontinuous pineal stalk. Although we cannot absolutely exclude this, the presence of nerve fibers extending throughout the stalk makes the existence of real discontinuities very unlikely.

The filament-rich glial cells found in the thin region of the pineal stalk are morphologically similar to central nervous system astrocytes. Owing to the neuroectodermal origin of the pineal gland and close spatial relation between pineal stalk and central nervous system, we believe that these cells can be considered astrocytes. It is noteworthy that the region rich in glial cells lacks type II pinealocytes, whereas the inverse is true for the pineal body. Although the morphology of type II pinealocytes is very different from that of glial cells, some filaments have been described in the former cells [*Wolfe* 1965; *Calvo and Boya,* 1984]. All this could suggest a glial nature, although very modified, for the type II pinealocyte.

Nerve fibers are probably the most important functional component of the pineal stalk. The ultrastructural aspect of stalk nerve fibers suggests that most, if not all, are passing nerve fibers. This supports previous light microscopic studies which show fibers passing from the pineal to the brain through the stalk [*Bjorklund* et al., 1972; *Wiklund,* 1974; *Nielsen and Moller,* 1975] and from the brain to the pineal [*Ronnekleiv and Moller,* 1979; *Guerillot* et al., 1982; *Dafny,* 1983]. Nerve fibers are especially numerous in the thin portion of the stalk. In the wide region, nerve fibers are more scattered, although, owing to the increased surface of stalk transversal section, the total number of fibers may be similar to that of the thin region.

Most nerve fibers lack a myelin and Schwann cell envelope. There is only a tendency of enclosure of nerve fibers by glial cells. Outside the pineal stalk there are several typical bundles of amyelinic nerve fibers enclosed by Schwann cells. These fibers are similar to conarii nerve branches. As cited above, light microscopic studies demonstrate a coexistence of both afferent and efferent nerve fibers in the pineal stalk. This may be related to two types of nerve fibers observed in this study. If this were so, amyelinic fibers located outside the stalk may correspond to sympathetic fibers of the conarii nerve which run from the pineal to the brain. Due to the small diameter of the stalk and the close proximity of these fibers, it is easy to believe on light-microscopic inspection that they are inside the stalk. Fibers seen inside the stalk in this study may then correspond to fibers of central origin passing to the pineal body.

Very few stalk nerve fibers are myelinated in the rat. These fibers decrease in number and finally disappear in more distal sections. Disappearance of myelinated fibers may suggest that they are the aberrant commissural fibers described by *Kappers* [1960]. A more probable explanation would be a loss of the myelin sheet, which would make them indistinguishable from the other fibers. However, their low number suggest that the functional role of myelinated fibers must be small.

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