

Structure of the pineal gland in the adult cat

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Abstract: The ultrastructure of the pineal gland in the adult cat is described and compared with that of other mammals. Connective tissue spaces showed capillaries with nonfenestrated endothelia and numerous unmyelinated nerve fibers. In the proximal region of the gland, myelinated nerve fibers coming from the anterior commissure were also found. Cat pinealocytes showed a nucleus with prominent nucleoli, a well developed Golgi apparatus, centrioles, granular endoplasmic reticulum, ribosomes, abundant microtubuli and enlarged mitochondria. Pinealocytes showed several long processes with bulbous endings filled with clear vesicles and scarce “synaptic” ribbons. Pineal astrocytes and their processes were characterized by the presence of abundant filaments.

Jesús Boya, Jose Luis Calvo, and Dolores Rancaño

Department of Histology, Faculty of Medicine, Complutense University, Madrid, Spain

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Address reprint requests to Prof. Jesús Boya Végu, Department of Histology, Faculty of Medicine, Complutense University, 28040 Madrid, Spain.

Introduction

Our current knowledge of the morphology of the pineal gland is mainly based on descriptions of small mammals, mainly rodents [see review in Bhatnagar, 1992]. Regarding the cat pineal gland, several partial aspects of its morphologic characteristics have been studied with both light [Machado et al., 1965; Kusche, 1966; Nielsen and Møller, 1975; Møller et al., 1981; Korf et al. 1985; Calvo et al., 1992; Boya and Calvo, 1993], and electron microscopy [Wood, 1973; Karasek and Hansen, 1982a; Calvo et al., 1991, 1992; Vigh-Teichmann et al., 1991]. Notwithstanding, our present understanding of the ultrastructure of the cat pineal gland is still based on papers published more than 25 years ago [Duncan and Micheletti, 1966; Wartenberg, 1968]. Given the heterogeneity in the structure of the pineal gland in the species so far studied, it seems pertinent to establish a more detailed description in those species that are more commonly used in experimental studies.

In the present work, we describe the structure and ultrastructure of the adult cat pineal gland.

Materials and methods

Fourteen clinically healthy cats of both sexes living under a natural photoperiod (approximately 40°N latitude) were included in this study. Two cats (one male and one female) were killed at each of the following ages: 6 months, 1 year, and 2 years for the light microscope study; and 7 months and 1, 2, and 4 years for the electron microscopy study. Animals were sacrificed under sodium pentobarbital anesthesia, at 1100 between July and October.

For the light microscope examination, blocks of brain tissue containing the pineal gland were fixed by immersion

in Bouin's solution, embedded in paraffin, and sections 7 μ thick were cut. Blocks were oriented to obtain sagittal sections of the pineal gland that were stained with hematoxylin and eosin.

For the electron microscope study, the pineal glands were quickly removed, cut up, and fixed by immersion in cold 3% glutaraldehyde in 0.1 M buffer phosphate. Tissue samples were then washed in 0.1 M phosphate buffer, postfixed in 1% osmium tetroxide in the same buffer, and embedded in Vestopal. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Phillips EM201 electron microscope.

Results

Light microscopy

The adult cat pineal gland exhibits a lance-head shape in the sagittal section (Fig. 1), and measures 2–2.5 mm in length, 1.5–2 mm in width, and 1 mm in height. The dorsal surface of the gland is flat and is in contact with the suprapineal recess of the III ventricle. Nearly 70 to 80% of the extension of this surface displays an ependymal cover consisting in a single layer of ciliated cubical cells. In the proximal region of the gland a short pineal recess is observed. The anterior commissure is located upon this structure and, in this species, is embraced within the glandular body showing a close contact with the pineal parenchyma (Fig. 1).

Applying routine staining techniques, the pineal parenchyma presents cells that are arranged in irregular, wide cords that are separated by eosinophilic zones containing few nuclei, where the blood vessels are commonly found. At higher magnification, at least two different types of

parenchymal cell types can be observed according to the morphology of their nuclei (Fig. 2).

Electron microscopy

The pineal parenchyma is made up by cell somata clustered in compact groups that are separated by zones plentiful of cell processes (Fig. 3). These zones are dispersed throughout the parenchyma, showing a tendency to surround the blood vessels. Pineal blood vessels show nonfenestrated endothelial cells with occasional pericytes. Some blood vessels are surrounded by a layer of unmyelinated fibers that, occasionally, can become very thick (Fig. 4). Nerve fibers have abundant small clear vesicles, and more sparse dense core vesicles with larger diameters (Fig. 5). Bundles of nerve fibers frequently penetrate within the pineal parenchyma through disruptions of the parenchymal basal lamina (Fig. 6). Other blood vessels -mainly thin capillaries- are almost in contact with the parenchymal basal lamina. When this occurs, the perivascular spaces are very tiny and contain very sparse connective tissue fibers, mainly collagen. Disruptions of the parenchymal basal lamina can also be observed at this level.

The dorsal surface of the pineal gland is lined by a single layer of ependymal cells with many cilia in their luminal surface. There is not basal lamina between the ependymal cells and the pineal parenchyma (Fig. 7). One special feature of the ependymal cells is the presence of glycogen both in the soma and the basal processes.

The anterior commissure, as described in the previous section, is located in the depth of the pineal parenchyma in this species. At the ultrastructural level, the commissural myelinated nerve fibers are also in direct contact with the pineal parenchyma (Fig. 8). Thus, in the limit between these structures, myelinated fibers and pinealocyte processes are intermingled without any boundary between them. Close to this area, isolated myelinated fibers—probably arising from the anterior commissure—are commonly found within the pineal parenchyma. Finally, an outstanding feature of the cat pineal gland is the presence of cells containing melanin granules. These cells have been extensively described previously [Calvo et al., 1992].

Pinealocytes

Pinealocytes show a small soma of round or ovoid shape where the nucleus is located (Figs. 9–12). Occasionally a cell process arising from the soma can be observed (Fig. 12). The nucleus is also round or ovoid with a smooth surface, and the chromatin appears in small clumps within a dense nucleoplasm. They exhibit a characteristic nucleolus with a very dense nucleolonema surrounding a fibrillary core (Figs. 9, 10).

The cytoplasm at the soma displays a certain degree of polarization with a small cytoplasmic accumulation where the Golgi apparatus is located (Figs. 11, 12). The cyto-

plasm contains very abundant intermingled microtubules, free polyribosomes, and sparse, short cisterns of rough endoplasmic reticulum. Subsurface cisterns are also observed. The Golgi complex is located near the nucleus and is composed of dilated cisterns and abundant associated vesicles. Transformed centrioles can be seen in the Golgi zone (Fig. 12), as previously reported [Calvo et al., 1991]. Mitochondria also display characteristic features; they are very large, few in number, and appear dilated. Dense bodies or lysosomes are occasionally found in the vicinity of the Golgi area (Fig. 11).

Pinealocyte processes made up large clusters among the somata, representing a significant component of the pineal parenchyma (Figs. 8, 13). In areas rich in cell processes, small bundles of axons showing granular vesicles are commonly found. The diameter of the pinealocyte processes is very heterogeneous. These structures contain predominantly large numbers of microtubules (Figs. 13, 16, 18), and among them are some mitochondria and dispersed elements of the reticulum. The processes end, forming very large bulbous endings that are stuffed with clear vesicles (Fig. 14). Some mitochondria can also be detected, as well as “synaptic” ribbons (Fig. 14). The bulbous endings are located in the pineal parenchyma, mainly in the vicinity of the connective spaces, intermingled with nerve fibers.

Astrocytes

Pineal astrocytes are less abundant than pinealocytes. They display an ovoid nucleus, occasionally with an indented surface. Chromatin appears granular within a clearer nucleoplasm (Fig. 15).

The soma is very rich in filaments with very few organelles among them (Fig. 15). Lipofuscin is commonly observed. The cell processes are almost filled with filaments (Figs. 16, 18). The presence of a layer of glial cell processes in the boundary of the vascular spaces is a characteristic common finding (Figs. 4, 17). In some occasions, mainly in the wide perivascular spaces that contain abundant nerve fibers, astrocyte processes cross the parenchymal basal lamina and intermingle with these nerve fibers (Fig. 17).

The amount of filaments varies between astrocytes. In those glial cells with less abundant filaments, the development of cell organelles is much higher, with a notable presence of large cisterns of rough endoplasmic reticulum with numerous ribosomes attached, and a very well developed Golgi apparatus. Lipofuscin is also a common component of this type of astrocytes.

Discussion

Following the original description at the light microscope by Zach [1960], the adult cat pineal gland has been considered as corresponding to the type AB of Vollrath [1981]. Zach [1960] described the cat pineal gland as

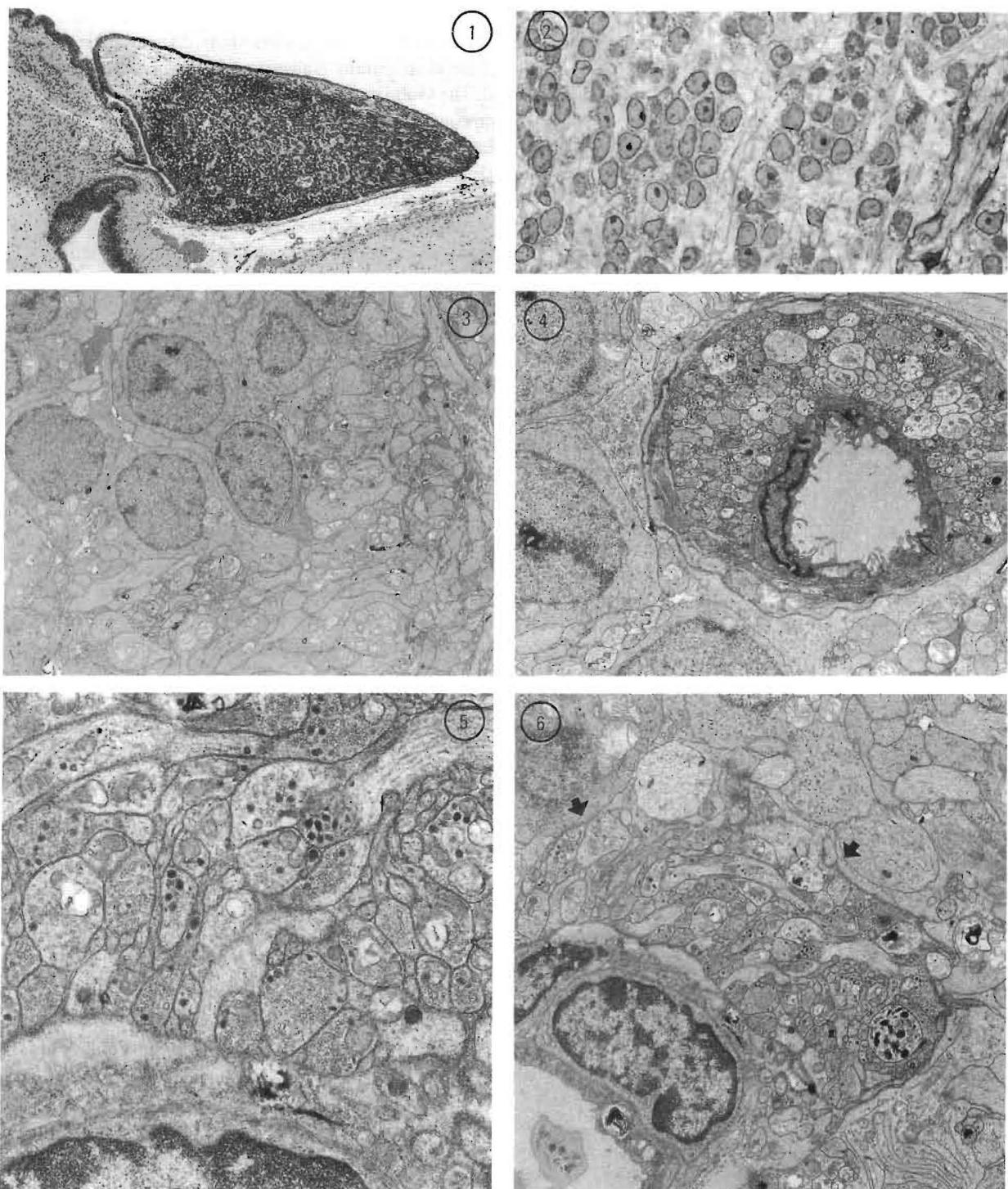


Fig. 1. Sagittal section of the pineal gland of a 1 year-old male cat. Hematoxylin-eosin. $\times 35$.

Fig. 2. Differences in the morphology of the nuclei of the pineal parenchymal cells. Pinealocyte nuclei show prominent nucleoli (2-year-old female). Semithin section stained with toluidine blue. $\times 720$

Fig. 3. Pineal parenchyma showing a cluster of pineal cell somata and a region with numerous cell processes (1-year-old female). $\times 3,700$.

Fig. 4. Perivascular space filled with unmyelinated nerve fibers (7-month-old male). $\times 5860$.

Figs. 5, 6. Unmyelinated nerve fibers showing abundant clear and dense-core vesicles (Fig. 5. $\times 16,600$). The parenchymal basal lamina is missing in the region between the two arrows (Fig. 6. $\times 9,200$) (2-year-old female).

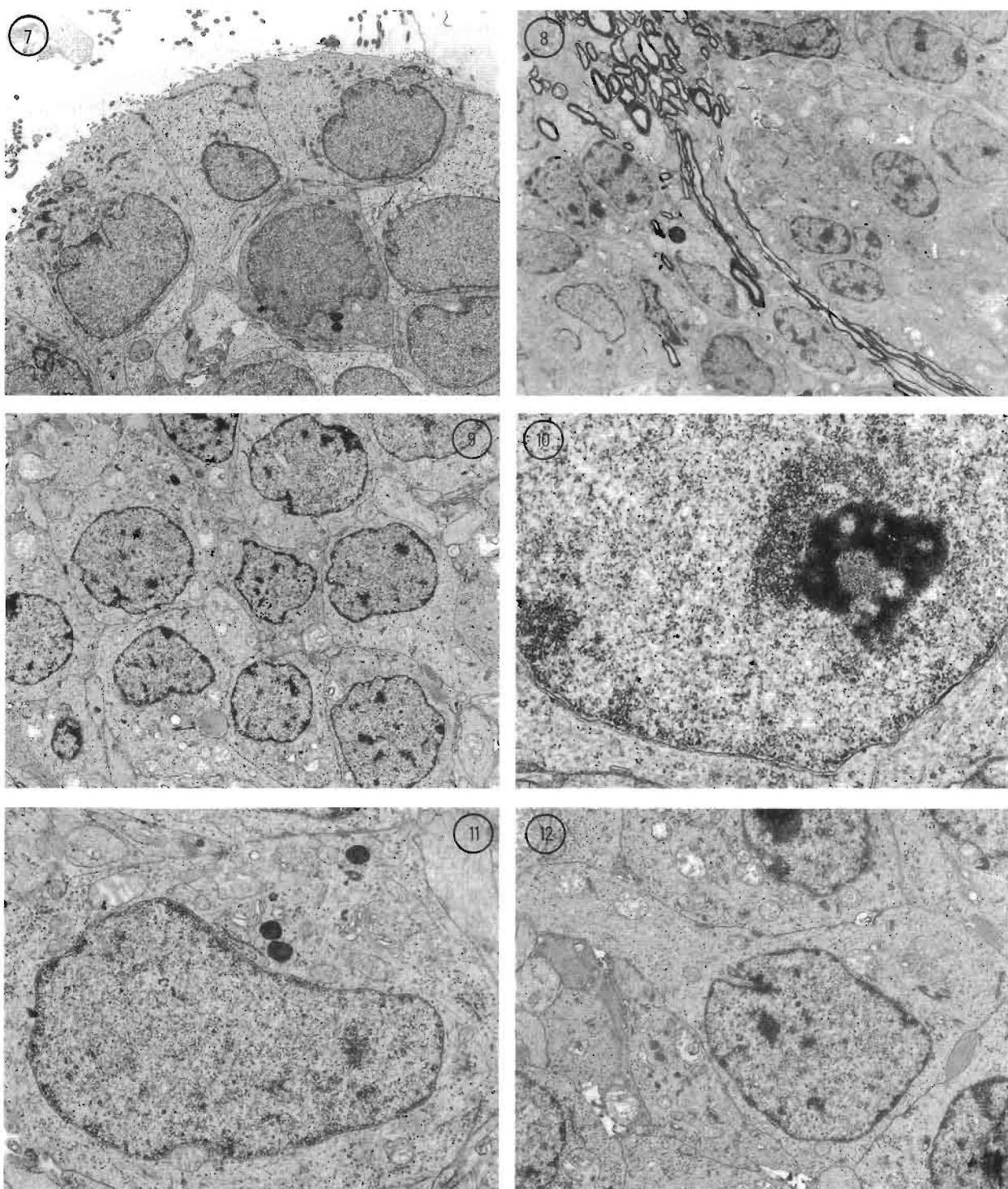


Fig. 7. Ependymal layer of the dorsal surface of cat pineal gland. Ependymal cells are in direct contact with the pineal parenchyma (7-month-old male). $\times 4,100$.

Fig. 8. Myelinated nerve fibers of the anterior commissure penetrating within the pineal gland (2-year-old male). $\times 2,700$.

Fig. 9. Cluster of pinealocyte somata (4-year-old female). $\times 3,700$.

Fig. 10. Pinealocyte nucleus with a prominent nucleolus (2-year-old male). $\times 21,900$.

Fig. 11. Detail of the somatic cytoplasm of a pinealocyte (7-month-old female). $\times 10,700$.

Fig. 12. A process arising from a pinealocyte soma (1-year-old female). $\times 6,400$.

having a pear shape and measuring 3.2 mm in length and 1.4 mm in width. We were not able to confirm these findings in our material. In the adult cat, the pineal gland has a characteristic lanceolated or almond shape, measuring 2–2.5 mm length, less than the figure given by Zach [1960]. According to our findings, the cat pineal gland should be included in the type A of Vollrath [1981]. In the work of Zach [1960], the pineal parenchyma is described as lobulated and showing follicles, above all at the periphery of the gland. She also emphasized the presence of ependymal-derived cysts. Again, in our material, we did not find any lobules or follicles; furthermore, there were no differences in the distribution of the pinealocytes at the periphery and the center of the gland. On the other hand, cysts lined by ependymal cells located in the vicinity of the recess were an exceptional finding in the cats we studied. Finally, the presence of pigmented cells and cells identified as astrocytes by immunocytochemical techniques have been extensively described elsewhere [Calvo and Boya, 1992; Boya and Calvo, 1993].

Vigh-Teichmann et al. [1991] have demonstrated the presence of neurons within the cat pineal parenchyma. A subpopulation of these neurons are stained with immunocytochemical techniques for the detection of GABA. In our material, we have not found any cell with the ultrastructural features of neurons. According to Vigh-Teichmann et al. [1991], in the cat pineal gland these neurons are scarce and are confined to the proximal portion of the gland, close to the posterior and habenular commissures. It would be likely that without the application of specific immunocytochemical techniques and/or a deeper study of the proximal portion, the population of neurons could be easily unnoticed in this species.

Besides the findings at the light microscope level, our study was mainly focused on the ultrastructural description of the gland. Although the domestic cat is a species extensively used in physiological, pharmacological, and biochemical investigations, our knowledge of its ultrastructure is still based in the works published by Duncan and Micheletti [1966] and Wartenberg [1968]. The work of Duncan and Micheletti [1966] was performed using a very early electron microscopic technique (fixation in osmium solutions), and it is mostly dedicated to nerve fibers. On the other hand, Wartenberg [1968], applying more recent fixation techniques, compared the ultrastructure of the pineal gland in cats and monkeys, although his description is more focused on the latter species. More recently, partial aspects of the ultrastructure of the cat pineal gland that were not included in the two previous papers have been published [Karasek and Hansen, 1982a; Calvo et al., 1991, 1992].

Our present results confirm that there are common characteristics in the morphology of the pineal gland in carnivore species. The adult cat pineal gland shares many

morphologic data with the dog pineal gland [Calvo et al. 1988a,b; 1990a,b], and it is extremely similar to the fox pineal gland [Karasek and Hansen, 1982b].

In carnivores, such as dog, fox, and cat, the pineal gland appears to be located deeply (type A or AB of Vollrath). The few pineal connective spaces appear narrow, and are mainly occupied with capillaries with a nonfenestrated endothelium, in clear contrast with the findings in rodents. Three types of parenchymal cell types can be distinguished: pinealocytes, pigment-containing cells (type II pinealocytes according to Karasek and Hansen [1982b]), and cells rich in filaments and immunopositives for the glial fibrillary acidic-protein [Boya and Calvo, 1993], categorized as astrocytes. In those species with more superficial pineal glands, as in rodents, the differentiation of the pineal glial cells towards astrocytes is less complete [Schachner et al., 1984; Calvo et al. 1988c; Lopez-Muñoz et al., 1992]. In carnivores, the pinealocytes and the astrocytes exhibit large cell processes that lead to the presence of large zones within the pineal parenchyma occupied exclusively by these processes and lacking cell somata. These areas are much less evident in rodents, perhaps because the cell processes are less developed. The parenchymal cell processes show a poor organelle endowment, and contain mainly microtubules (pinealocytes) or filaments (astrocytes). In contrast with many rodent species, the presence of lipid droplets in the cytoplasm of the pinealocytes is a rare finding in carnivores. Korf et al. [1985], using immunocytochemical techniques for the detection of retinal S-antigen at the light microscope level, have described a stronger immunolabeling of the periphery of the cat pineal gland, further distinguishing two different cell types according to their immunolabeling pattern. At the electron microscopic level, although some differences can be observed in the endowment of cell organelles and cytoplasmic electrodensity among pinealocytes, we are not able to detect any special characteristic that allows one to distinguish different pinealocyte types. It is not clear whether glial pineal cells [not considered in the study of Korf et al., 1985] could be affecting the immunostaining pattern described in this paper.

There are also differences in the relationships between the bulbous endings of the pinealocytes and the nerve fibers. In rodents, very small bundles of sympathetic nerve fibers are found in the large perivascular connective tissue spaces. The pinealocyte processes cross the basal lamina and end within the connective space, very close to the sympathetic nerve fibers. According to the data presented here on the cat pineal gland, sympathetic nerve fibers make up relatively large bundles that almost fill the narrow perivascular space. These nerve fibers end penetrating the parenchyma and forming small bundles that are intermingled with the bulbous endings of the pinealocytes that, in this case, stay within the parenchyma, although with a clear

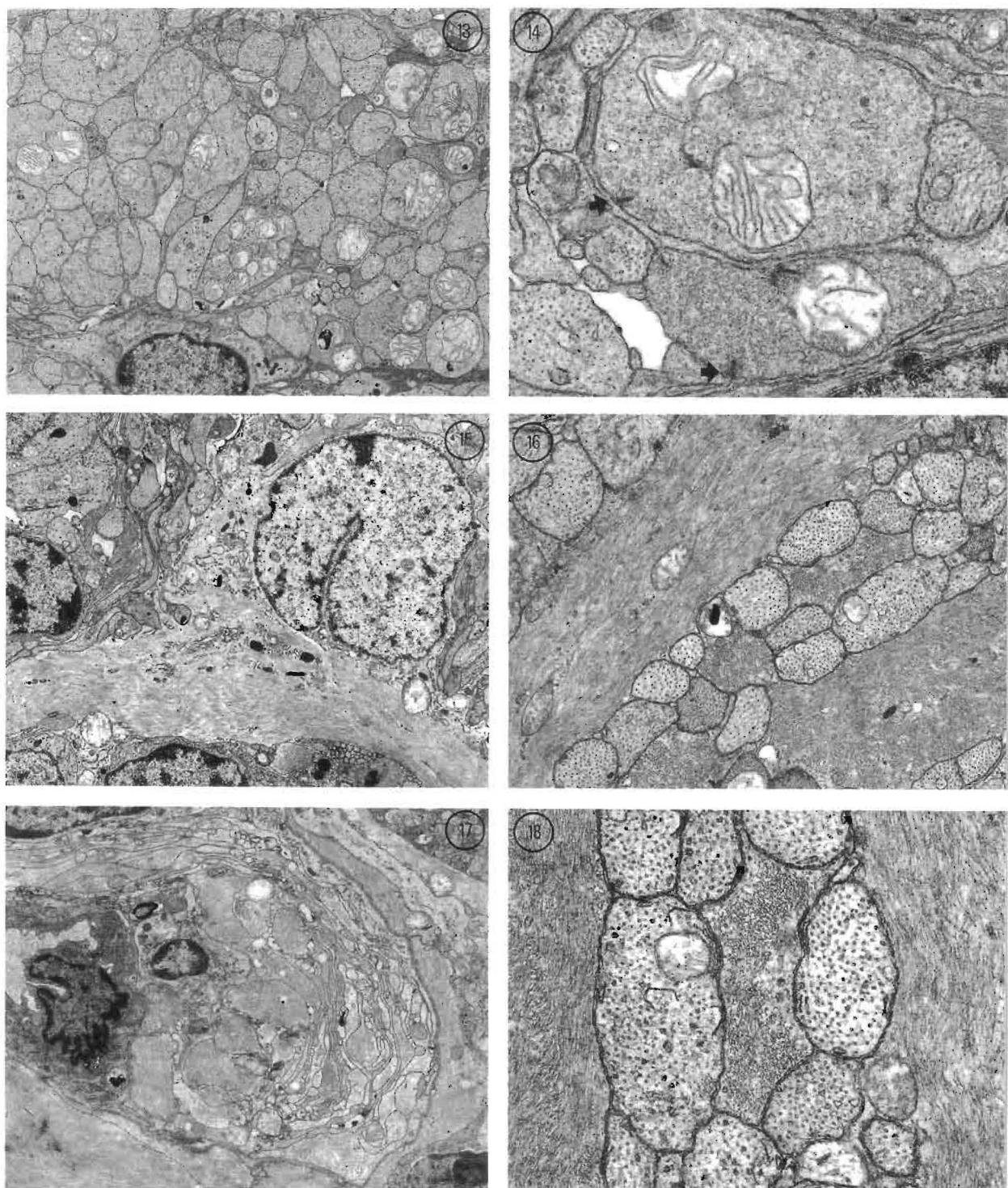


Fig. 13. A region with numerous pinealocyte processes (2-year-old male). $\times 6800$.

Fig. 14. Bulbous endings of pinealocyte processes showing abundant clear vesicles and some "synaptic" ribbons (arrows) (7-month-old male). $\times 19,700$.

Fig. 15. Pineal astrocyte showing abundant filaments (1-year-old female). $\times 13400$.

Fig. 16. Processes of pinealocytes and astrocytes (4-year-old male). $\times 6,400$.

Fig. 17. Presence of cell processes filled with filaments in a perivascular space (2-year-old male). $\times 7,100$.

Fig. 18. Higher magnification of Figure 16 showing the presence of filaments in the astrocyte processes and microtubules in the pinealocyte processes (4-year-old male). $\times 25,500$.

tendency to be located in the vicinity of the connective tissue spaces. Nerve fibers can be easily distinguished from the pinealocyte processes because the former contain abundant dense core vesicles. Although in the first studies [Duncan and Micheletti, 1966; Wartenberg, 1968] the presence of dense core vesicles in the cat pinealocytes was not mentioned, Karasek and Hansen [1982a] have demonstrated the presence of these type of vesicles, although few in number. Similar results regarding the nerve fiber endings and the localization of the bulbous endings of the pinealocytes have been described in other carnivores [Duncan and Micheletti, 1966; Wartenberg, 1968; Calvo et al. 1988a, 1990b; Karasek and Hansen, 1982b]. The relationships that are established between the bulbous endings and the sympathetic nerve fibers in the cat pineal gland, are very similar to those described by Redecker [1993] in the Mongolian gerbil. Another characteristic of the pineal gland in carnivore mammals [Calvo et al. 1988a, 1990b; Karasek and Hansen, 1982b], confirmed in the present work, is the dearth of "synaptic" ribbons in the bulbous endings. This was interpreted by Karasek and Hansen [1982b] as a possible inverse relationship between the numbers of "synaptic" ribbons in a given species and the development of the network of sympathetic nerve fibers in this species.

According to our results, the cat pineal gland possesses several morphologic singularities. Although some of them are also found in other carnivores, several other characteristics are exclusive of this species [Calvo et al. 1991, 1992]. Further studies are needed to find out whether the morphologic differences observed between carnivore and rodent pineal glands are translated in functional special features. This histophysiological correlation may contribute to important clues for the knowledge of this exciting organ.

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