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Ultrastructure of the Pineal Gland in the Adult Dog

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The adult dog pineal gland was studied with the electron microscope. Pineal connective tissue spaces were poorly developed and showed capillaries with nonfenestrated endothelial cells. Two cell types, pinealocytes and astrocytes, could be identified in pineal parenchyma. Dog pinealocytes showed microtubules, centrioles, occasional cilia, and well-developed Golgi complexes. These cells showed thin processes with bulbous endings packed with vesicles. Astrocytes were characterized by the presence of numerous filaments. Their processes finished forming a glial layer bordering connective tissue spaces. The presence of myelinated and unmyelinated nerve fibers was also described.

Key words: dog, pinealocytes

INTRODUCTION

Most of the ultrastructural investigations on the mammalian pineal gland have been performed in laboratory rodents [see review in Vollrath, 1981]. The pineal gland ultrastructure of other mammals is not well known. There are only two studies on pineal gland ultrastructure of the adult dog, both published 20 years ago [Sano and Mashimo, 1966; Welser et al., 1968]. Moreover, the most complete of these studies [Welser et al., 1968], was performed on relatively young dogs (2 to 6 months of age). Since then, only two additional light microscope reports on nerve fibers in the dog pineal gland have been published [Matsuura and Sano, 1983; Matsuura et al., 1983].

Morphological investigations can provide useful knowledge on the pineal gland. In the present paper we describe the ultrastructure of the main cell types of the adult dog pineal gland.

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MATERIALS AND METHODS

Eight mongrel dogs, living under natural-light conditions (approximately 40°N latitude) and clinically healthy were used for this study. Two dogs (male and female) were sacrified at the following age intervals: 9 months and 2, 3, and 4 years. The animals were sacrified under sodium pentobarbital anesthesia at 11:00 A.M. in a period between March and June. The pineal gland was quickly removed and fixed by immersion in cold 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. The samples were then washed in phosphate buffer, postfixed in 1% osmium tetroxide in 0.1 m phosphate buffer, and embedded in Vestopal. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Philips EM 201 electron microscope.

RESULTS

No remarkable differences were found in the ultrastructure of the pineal gland according to the age or sex of the dog, so the results obtained will be described as a whole.

The connective tissue stroma of the dog pineal gland was poorly developed. It mostly consisted of narrow connective tissue spaces surrounding the blood vessels (Fig. 1). The capillaries had nonfenestrated endothelial cells. Connective tissue spaces around blood vessels showed some unmyelinated nerve fibers and very scarce collagen microfibrils and connective tissue cells (Fig. 1).

Two main parenchymatous cell types, pinealocytes and astrocytes, appeared in the adult dog pineal gland. Among them, numerous myelinated and unmyelinated nerve fibers were observed. Myelinated nerve fibers of central type [Peters et al., 1970] were located in proximal regions of the pineal gland (Fig. 2). Unmyelinated axons were seen throughout the gland, mainly in the parenchyma near the basement membrane. These fibers showed many small granular vesicles and some occasional large granular vesicle.

Pineal cells usually appeared forming clusters of variable size (Fig. 2). These cell clusters were separated by regions rich in cellular processes among which relatively wide intracellular spaces could be seen (Fig. 2). Cavities lined by junctional complexes were not found.

The pinealocytes showed a small soma of ovoid or polygonal shape. A large fraction of pinealocyte soma was occupied by a nucleus of round or ovoid shape and smooth surface. Small peripheral clumps of chromatin and one or two nucleoli were seen in these nuclei (Figs. 2, 3). Nucleoli contained a dark nucleolonema surrounding a fibrilar center (Fig. 3).

The cytoplasm showed abundant and scattered free polyribosomes and microtubules (Fig. 4). Few and short cisterns of granular endoplasmic reticulum were seen throughout the cytoplasm. A well-developed Golgi complex was located near the nucleus. It consisted of many dyctiosomes with numerous coated and smooth vesicles associated (Figs. 4, 5). Mitochondria were dispersed throughout the somatic cytoplasm, although they often accumulate near the Golgi complex. A small number of dense bodies appeared in dog pinealocytes (Fig. 6). Some isolated granular vesicles were also found in the Golgi zone or scattered by the soma (Fig. 5).

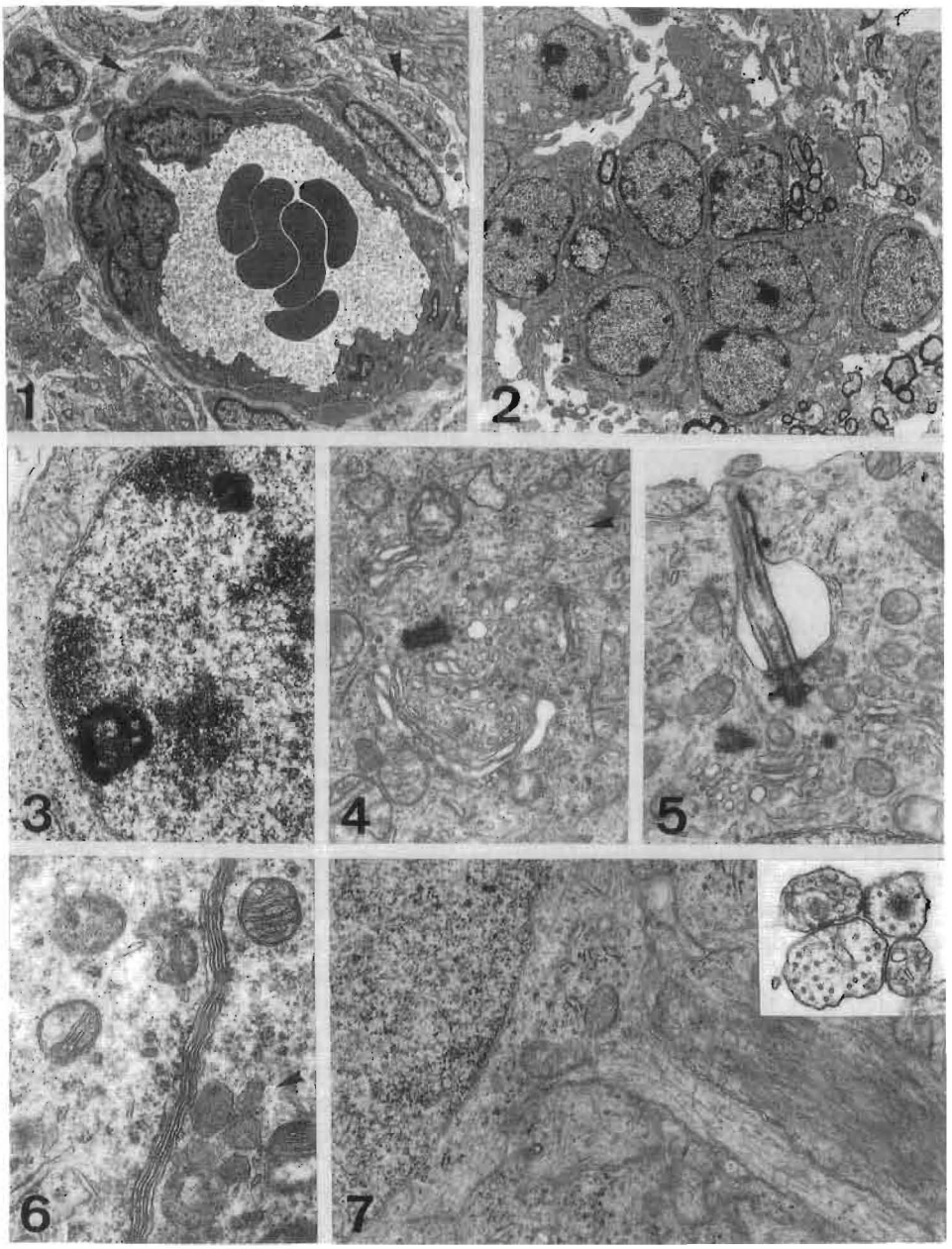

Fig. 1. Connective tissue space containing an arteriole and bundles of unmyelinated nerve fibers (arrowheads). × 31,000.

Fig. 2. Cluster of pinealocyte somata. Myelinated nerve fibers are located around the cluster. × 3,200.

Fig. 3. Pinealocyte nucleus with two nucleoli. One of them shows dark material surrounding a fibrillar center. × 16,400.

Fig. 4. Pinealocyte cytoplasm. Centriole associated to the Golgi complex. The cytoplasm shows numerous microtubules (arrowhead). × 17,500.

Fig. 5. Pinealocyte cilium. The basal body is deeply located in the cytoplasm, associated to the Golgi complex. × 18,200.

Fig. 6. Subsurface cisterns in two contacting pinealocytes. A small group of dense bodies (arrowhead) can be seen. × 29,000.

Fig. 7. Longitudinal section of a pinealocyte process showing abundant microtubules. × 15,300.

A centriole or diplosome was usually associated to the pinealocyte Golgi complex (Fig. 4). Less frequently, a single cilium was found in some pinealocytes (Fig. 5). In transversal sections, the pinealocyte cilium usually lacked the central pair of microtubules. The cilium basal body was placed near the cell surface or more deeply in the Golgi zone (Fig. 5). Only rarely were some very thin ciliary rootlets found associated to basal bodies.

Subsurface cisterns were frequently found along the surface of pinealocytes. Sometimes, very long paired subsurface cisterns were observed in two facing pinealocytes (Fig. 6).

The dog pinealocyte had a small number of thin processes arising from its soma (Fig. 7). These processes, showing numerous microtubules along their trajectories (Fig. 7), finished in bulbous endings packed with clear vesicles (Fig. 8). Endings of pinealocyte processes were located throughout the parenchyma, mainly near the connective tissue spaces. Synaptic ribbons were rarely found in dog pinealocytes, usually in bulbous endings of pinealocyte processes (Fig. 8).

Differences in cytoplasmic and nuclear densities were found among pinealocytes. These differences, however, did not appear to be related to the proportion or type of cytoplasmic organelles.

The second cell type in abundance in the dog pineal gland was the astrocyte. Astroglial cell bodies were located in the cell clusters described above, intermingled with pinealocytes (Fig. 9). Compared with the pinealocyte, the astrocyte nucleus had a more angular contour, more abundant peripheral clumps of chromatin, and a reticulated nucleolus (Fig. 9).

In the cytoplasm, ribosomes, granular endoplasmic reticulum, and the Golgi complex were less developed than in pinealocytes. Also, centrioles and cilia were found less frequently. However, dense bodies and lipofuscin granules were frequently found in astrocyte soma.

The presence of cytoplasmic microfilaments, sometimes fairly numerous, was the most distinctive feature of pineal astrocytes (Figs. 9, 10). However, great variations were observed in the amount of filaments, and some cells presented very few of them. In addition to filaments, pineal astrocytes showed a small number of microtubules.

Processes of pineal astrocytes characterized by the abundance of filaments were usually thicker than those of pinealocytes (Figs. 8, 12, 13). Occasionally, whorled cisterns of endoplasmic reticulum were seen in these processes (Fig. 11). Often, some microtubuli appeared in the periphery of filament bundles (Fig. 13). Astrocyte processes formed a layer in the border between the parenchyma and connective tissue spaces (Fig. 11, 12). This layer was discontinuous in some points. Processes forming this glial layer showed numerous filaments and membrane thickenings similar to hemidesmosomes in the cell surface oriented towards the basal lamina (Fig. 12). Intercellular contacts identifiable as gap junctions were found between processes of glial layer (Fig. 12).

Other very thin lamellar processes were observed arising from the soma of the astrocytes or their processes. These lamellar processes were interposed between pinealocyte somata (Fig. 14). Probably because of the small size of the lamellar processes, no organelles were found in them.

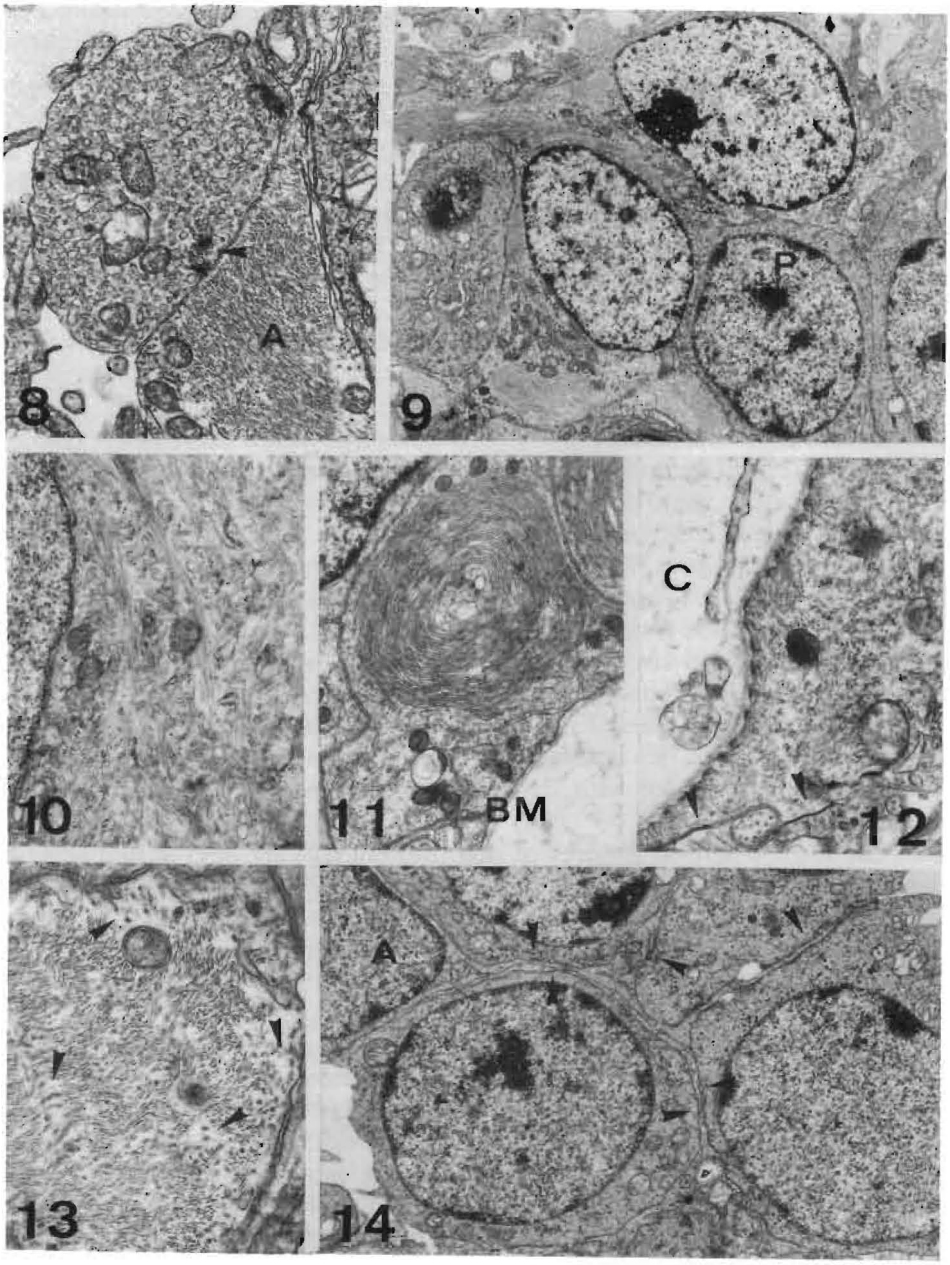

Fig. 8. Bulbous ending full of clear vesicles. Arrowhead points to two possible synaptic ribbons. A, Astrocyte process rich in filaments. × 18,400.

Fig. 9. Astrocytes with numerous cytoplasmic filaments. P, Pinealocyte. × 5,500.

Fig. 10. Astrocyte cytoplasm full of filaments oriented in several directions. × 16,600.

Fig. 11. Whorled endoplasmic reticulum in an astrocyte process. BM, membrana basal. × 12,800.

Fig. 12. Astrocyte process full of filaments in contact to a connective tissue space (C). Arrowheads point to two possible gap junctions. × 18,600.

Fig. 13. Astrocyte process with numerous filaments and some microtubules (arrowheads). × 25,400.

Fig. 14. Astrocyte lamellar processes located between pinealocyte somata (arrowheads). One of these processes arises from an astrocyte soma (A). × 7,300.

DISCUSSION

According to our results, the dog pineal gland shows a poorly developed connective tissue stroma containing nonfenestrated capillaries. These results confirm previous descriptions of Sano and Mashimo [1966] and Welser et al. [1968]. Both features may be related to the deep anatomical position of dog pineal gland in the brain. Fenestrated capillaries appear to be restricted to species with a superficial pineal gland. [Wolfe, 1965; Sheridan and Reiter, 1968; Ito and Matsushima, 1968]. With regard to the connective tissue spaces, the relation is less evident. In species with a superficial pineal gland, large connective tissue spaces have been found [Rodin and Turner, 1966; Calvo and Boya, 1984], although these spaces are narrow in some cases [Wartenberg and Gusek, 1965].

According to Hartmann [1957], numerous myelinated nerve fibers coming from the posterior commissure penetrate the dog pineal gland where they form loops and leave the gland contralerally. The abundant central myelinated nerve fibers found in this study in proximal regions of the pineal gland are probably these commissural fibers. The dog pineal gland also contains sympathetic nerve fibers coming from the superior cervical ganglia [Hartmann, 1957; Matsuura and Sano, 1983], as well as serotoninergic [Matsuura and Sano, 1983] and peptidergic fibers [Matsuura et ala., 1983]. We have found unmyelinated nerve fibers in connective tissue spaces and pineal parenchyma, mostly close to the connective tissue spaces. Several types of vesicles, either clear or granular, were found in these fibers. However, our findings are insufficient to permit a correlation with the fiber types described with fluorescence and immunohistochemical techniques.

The ultrastructural features of dog pinealocytes are very different from those described for other mammal species. Thus, the dog pinealocyte nucleus has a smooth surface, peripheral clumps of chromatin, and small nucleoli. In other mammals, the nucleus of the pinealocyte has deep infoldings of its nuclear envelope, lacks peripheral heterochromatin, and exhibit large nucleoli [Anderson, 1965; Wolfe, 1965; Arstila, 1967; Calvo and Boya, 1983, 1984; Cozzi, 1986]. In dog pinealocytes, the amount of cytoplasm and number of organelles are scarce compared to rodent pinealocytes [Vollrath, 1981; Karasek, 1983]. Supporting the previous findings of Welser et al. [1968], the Golgi complex is relatively well developed and shows numerous associated vesicles. In contrast to other mammals, in most of the adult dog pinealocytes a centriole or diplosome is found in the Golgi zone. The presence of a cilium is also a frequent finding in dog pinealocytes. In other mammals, both organelles can be seen during fetal [Clabough, 1973; Calvo and Boya, 1981] and neonatal period [Zimmerman and Tso, 1975; Calvo and Boya, 1983]. With increasing age, however, centrioles and cilia disappear or become disrupted [Lin, 72; Calvo and Boya, 1983, 1984]. According to Welser et al. [1968], dog pinealocyte cilium has a $9+2$ pattern. Transversal sections of cilia found in this study showed, however, a $9+0$ pattern. The presence of cilia with a $9+0$ pattern in the mammalian pinealocytes may be considered as an evidence of their phylogenetical evolution from photoreceptor cells [Vollrath, 1981].

Lipid droplets are frequent in mammal pinealocytes [Vollrath, 1981;

Karasek, 1983] and especially abundant in the rat pinealocytes [Wolfe, 1965; Arstila, 1967; Calvo and Boya, 1984]. According to our results, the adult dog pinealocytes lack lipid droplets. Sano and Mashimo [1966] and Welser et al. [1968] described some osmiophylic bodies as lipid droplets. However, judging by the micrographs of these studies, these osmiophylic bodies seem to be lysosomal dense bodies or rather pigment granules which appear in some dog pineal parenchymal cells (unpublished results). In this study, some lysosome-like bodies appeared in dog pinealocytes, although their number was smaller than that described for other mammal species [Vollrath, 1981; Karasek, 1983].

Processes of the dog pinealocytes finished in bulbous endings packed with clear vesicles similar to those described in other mammals [Vollrath, 1981]. Although, according to Sano and Mashimo [1966], the endings of pinealocyte processes are located in perivascular connective tissue spaces, all bulbous endings found in this study were located in the pineal parenchyma. Synaptic ribbons are an infrequent finding in the dog pineal gland. Synaptic ribbons found in this study were located in bulbous endings of pinealocyte processes. In several mammals, the number of synaptic ribbons in the pinealocytes and the amount of adrenergic nervous endings in the pineal gland have been described as inversely correlated [Karasek et al., 1983]. According to Matsuura and Sano [1983], the dog pineal gland shows a dense network of adrenergic nervous fibers. The scarce number of synaptic ribbons we could find in dog pinealocytes is thus in keeping with the inverse correlation described by Karasek et al. [1983].

The functional significance of the cellular organelles of mammalian pinealocytes has been revised by Pevet [1979]. Our study largely confirms the presence in the dog pinealocytes of functionally important cell structures such as granular vesicles, subsurface cisterns, bulbous endings packed with vesicles, and synaptic ribbons [Pevet, 1979; Vollrath, 1981]. Nevertheless, other cellular organelles (lipid droplets, annulate lamellae, proteinaceous material in cisterns of granular endoplasmic reticulum) can hardly be identified or are absent in dog pinealocytes. At present, it is not feasible to assign a functional significance to these morphological features of dog pinealocytes given the lack of biochemical and functional studies on the dog pineal gland.

Glial cells have been examined with the electron microscope in the pineal gland of several mammal species [Duncan and Micheletti, 1967; Anderson, 1965; Sheridan and Reiter, 1970, 1973]. Glial cells have also been identified in mammalian pineal gland by using immunohistochemical techniques for astrocyte antigenic markers [Moller et al., 1978; Lowenthal et al., 1982; Huang et al., 1984; Schachner et al., 1984; Cozzi, 1986]. In the dog, only Welser et al. [1968] have mentioned the presence of a few glial cells, but without describing them. In our material, the abundance of filaments and the tendency of these cells to form a glial layer in the border between the pineal parenchyma and connective tissue spaces suggest that they can be identified as astrocytes. Moreover, our results show that astrocytes are a frequent cell type in the adult dog pineal gland, supporting the light microscope results of Hulsemann [1967]. This frequency may be related with the deep location of the dog pineal gland. Astrocytes have been described in species whose pineal gland is placed deeply in the brain, lacking or being scarce in those with a superficial pineal gland

[Hulsemann, 1967; Sheridan and Reiter, 1973]. In the hamster, astrocytes can be only found in deep pineal [Sheridan and Reiter, 1970]. In the albino rat, astrocyte-like cells have been described in the pineal stalk [Luo et al., 1984; Calvo and Boya, 1985]. Pineal astrocytes of species other than dog show some antigenic markers, mostly vimentin, taken as a characteristic of immature astrocytes [Huang et al., 1984; Schachner et al., 1984]. Our finding of microtubules in dog pineal astrocytes supports this observation as the presence of microtubules is considered as an evidence of astrocyte immaturity [Peters et al., 1976].

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