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Fluorescence and Electron Microscopic Study of the Tree Shrew Pineal Organ

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With 16 Figures

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Summary

The fine structure of the pineal gland and the pineal innervation in the tree shrew were studied by electron microscopy and glyoxylic acid-induced fluorescence microscopy respectively. The parenchymal cells consist of pinealocytes, glial cells and pigment-containing cells. The pinealocytes are characterized by the presence of granular vesicles, synaptic ribbons, electron-dense bodies and small profiles of rER with dilated cisternae. Glial cells contain light cytoplasmic bodies, lipofuscin granules, bundles of microfilaments, and elongate profiles of rER with flattened cisternae which are often stacked together with light cytoplasmic bodies; the pigment-containing cells are unique in possessing giant pigment granules in the cytoplasm. The pinealocyte/glial cell/pigment cell in tree shrew pineals may be the same cell line of parenchymal cells at different ontogenetic stages. Pigment-containing cells contain pigment granules as a prominent cytoplasmic inclusion, suggesting they are senscent in secretory function. Both pinealocytes and glial cells contain structures suggesting secretory function such as well-developed Golgi complex and granular vesicles. The antigonadotrophic substances may be stored in granular vesicles. The present ultrastructural study supports the conclusion that tree shrew pineal organ is an endocrine gland which is heavily innervated by adrenergic nerves and possibly by cholinergic nerves.

Key words: Pinealocyte, glial cells, pigment, adrenergic innervation, ultrastructure.

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Introduction

The hypothesis that the mammalian pineal organ evolved from photoreceptor cell lines of ancestoral forms is supported by numerous studies. Photoreceptor function is one of the characteristics of the pineal organ in certain lower vertebrates (for review, see *Reiter,* 1977). In some amphibian, reptilian and avian species, the parenchymal cells of the pineal organ exhibit both photoreceptor and endocrine characteristics (*Reiter,* 1977). Evidence obtained from various mammalian species shows that the pineal organ is a glandular tissue (*Anderson,* 1965; *Wolfe,* 1965; *Wartenberg,* 1968) that exerts a significant influence, mainly understood, on reproduction (*Reiter* and *Fraschini,* 1969; *Wurtman et al.,* 1968).

Ultrastructural studies of mammalian pineal organs have been reported in various forms, including cattle and sheep (*Anderson,* 1965), rabbit (*Romijn et al.,* 1977), dog (*Welser et al.,* 1968), rat (*Wolfe,* 1965), and monkey (*Wartenberg,* 1968). To my knowledge, there have been no reports of an ultrastructural study on the pineal organ of the tree shrew, *Tupaia glis,* a subprimate considered to occupy a phylogenetic position somewhere between the insectivora and primitive primate (*Campbell,* 1966). This study is intended to provide a morphological basis for understanding the pineal organ of this rather unique species.

Materials and Methods

Five young adult male tree shrews *(Tupaia glis)* of approximately 150 grams were used for the electron microscopy. They were obtained during the summer from a Florida-based primate center and held in an animal room for several days before sacrifice. They were kept on a normal light cycle (12 hours darkness :12 hours light), and perfused between 9:00 a.m. and 12:00 noon. Each heparinized animal was anesthetized with Nembutal (40 mg/kg body weight), and artificially ventilated with a mixture of 95% O_2 and 5% CO_2, and then the thorax was opened according to a perfusion method of *Williams* and *Jew* (1975). After injection of flaxedil into the heart, the animal was perfused through the aorta with a fixative containing 3% glutaraldehyde in 0.1 M Sorensen's phosphate buffer, pH 7.3. The pineal organ was dissected out and immersed in the same fixative for at least two hours. After osmic acid postfixation, the tissue blocks were dehydrated and embedded in Epon for electron microscopy by routine procedures.

For studying adrenergic innervation at the light microscopic level, the glyoxylic acid-aldehyde method for fluorescence microscopy (*Chiba et al.,* 1976) was used in two animals. In brief, the anesthetized animals were perfused first with 2% glyoxylic acid in Krebs-Ringer solution, pH 7.0, and then with 4% paraformaldehyde—0.5% glutaraldehyde in 0.1 M Sorensen's

phosphate buffer, pH 7.3. After soaking in the fixative containing 10% sucrose, the brains were cut into 18 μ thick sections in the cryostat. The sections were dried under the hair dryer and coverslipped with mineral oil. The sections were then studied under a fluorescence microscope.

Results

The pineal organ of *Tupaia* was attached to the dorsal surface of the diencephalon by two fibrous stalks. Not only did its attachment differ from that in the rat and hamster, but the organ itself did not adhere closely to the overlying dura, as it did in rodents. Close to the pineal stalk, which is shorter than that of rats, some myelinated axons were present as a bundle, but myelinated axons were not seen deep in the parenchyma in this study. The pineal organ itself was highly vascular and heavily innervated.

The pineal parenchyma principally consisted of pinealocytes (Figs. 1–4), glial cells (Figs. 5–9) and pigment-containing cells (Fig. 10); the first two cell types formed the majority of the parenchyma. There was a rich supply of blood vessels which were lined with non-fenestrated endothelia and surrounded by parenchymal cells forming a perivascular space. Connective tissue elements, nerve fibers and sometimes processes of parenchymal cells were observed in the perivascular space.

I. Pinealocytes: This parenchymal cell was irregular in shape; cell processes emerged from the cell body and extended into the interstitial space, which eventually connected to the perivascular space. The nucleus was oval or round; heterochromatin and euchromatin were homogenously dispersed in the nucleus (Fig. 1). Granular endoplasmic reticulum (rER) was abundant in the cytoplasm (Figs. 2, 3). The profiles of rER cisternae were usually small, and more dilated than those of glial cells. Free ribosomes were present in the cytoplasmic matrix, which appeared to be more electron dense than that of glial cells (Figs. 5, 6). A special ER, subsurface cisterna, was encountered and closely apposed to the inner aspect of the plasma membrane of the pinealocyte where nerve endings were occasionally seen (Figs. 12, 13). Golgi complexes (Figs. 1–3) were well developed in association with ER, granular vesicles, Golgi vesicles, Golgi vacuoles and cytoplasmic bodies of different densities. Granular vesicles and pigment granules were also encountered in the cytoplasm (Figs. 1, 2). Synaptic ribbons (SR) (Figs. 1, 4, 5) with different numbers of small clear vesicles clustered around the elongate synaptic rods were seen only in the pinealocytes; these were

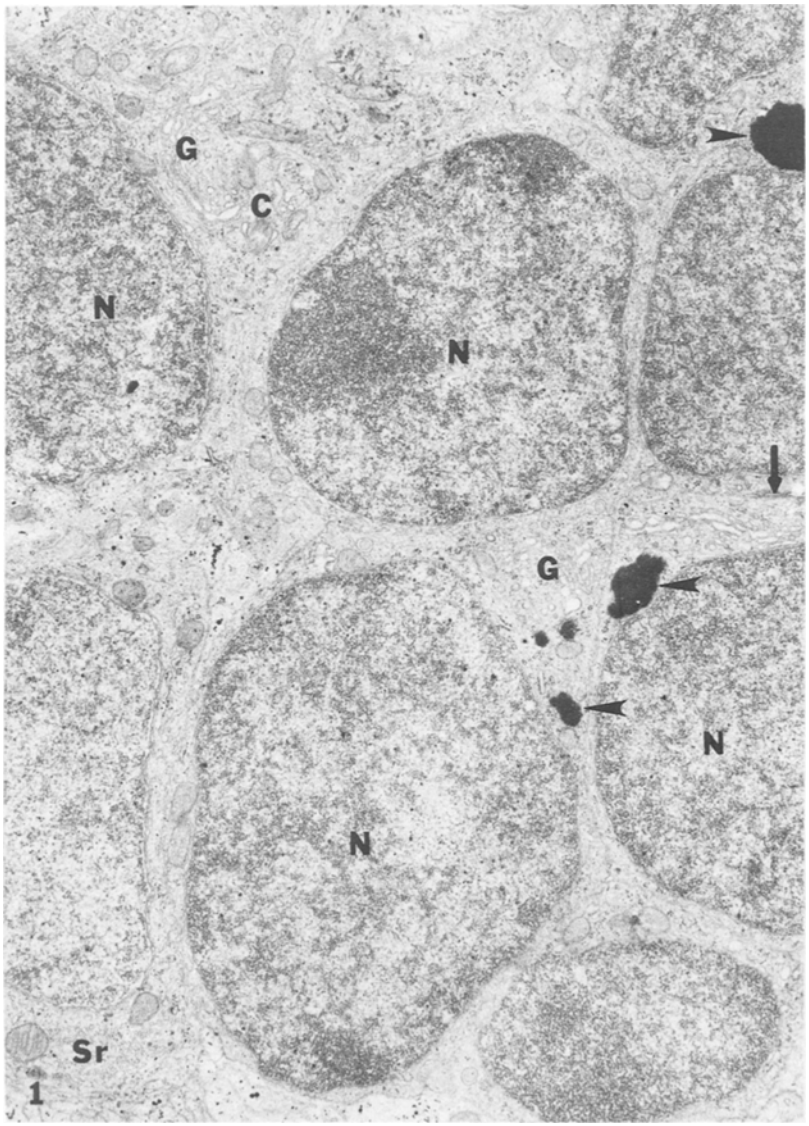

Fig. 1. A cluster of the pinealocytes cutting through nuclei *(N)* which are oval or round in shape. The nucleus contains the heterochromatin and euchromatin usually dispersed homogeneously, although heterochromatin condensation can be seen. Note different organelles in the cytoplasm including well-developed Golgi complex *(G)*, clear vesicles of the synaptic ribbon *(Sr)*, endoplasmic reticulum, mitochondria and even a few pigment granules (arrowheads). A junctional complex is seen between the pinealocytes as indicated by the arrow. Often encountered was the centriole or cilium *(C)* always located close to Golgi complex. ×11,700

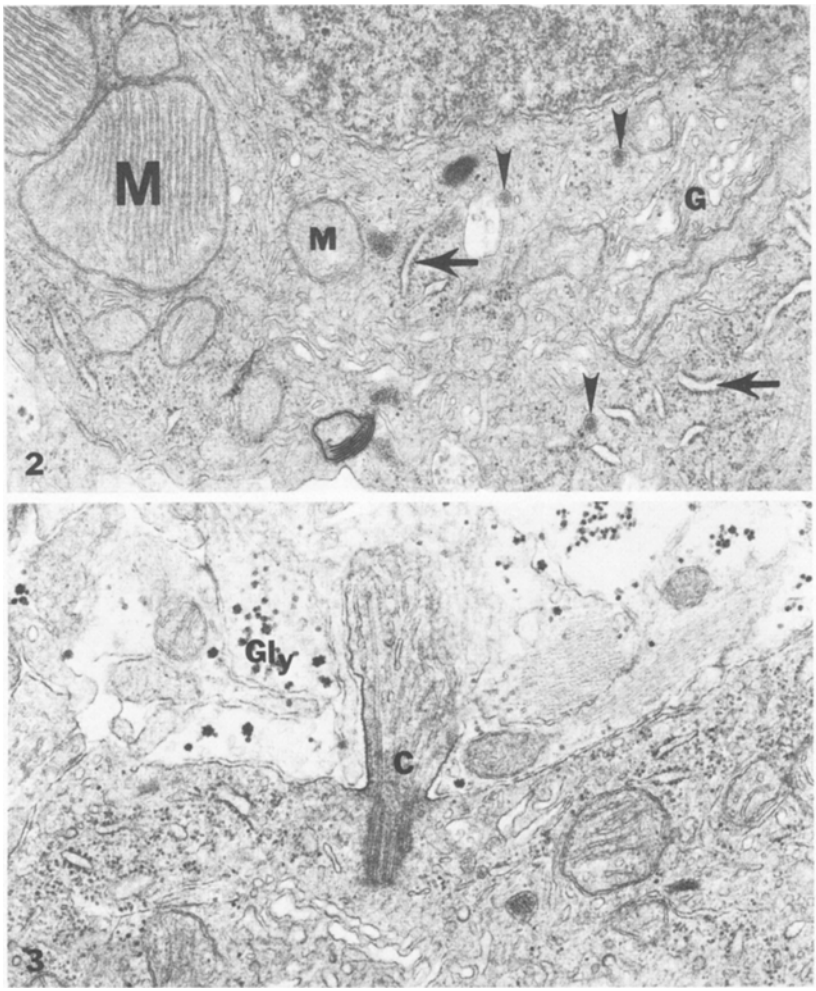

Fig. 2. In the pinealocyte, the giant mitochondrion *(M)* is seen in addition to regular mitochondria. Short profiles of granular endoplasmic reticulum with dilated cisternae are indicated by arrows. Three granular vesicles (arrowheads) appeared in this photograph. Golgi complex *(G)* is also shown in the cytoplasm. ×24,500

Fig. 3. A cilium *(C)* comprised of microtubules is illustrated extending into the intercellular space near Golgi complex of the pinealocyte. In the pinealocyte, ribosomes abutted onto the endoplasmic reticulum or freely dispersed in the cytoplasm. Glycogen particles *(Gly)* are seen in the process of the glial cell. ×29,000

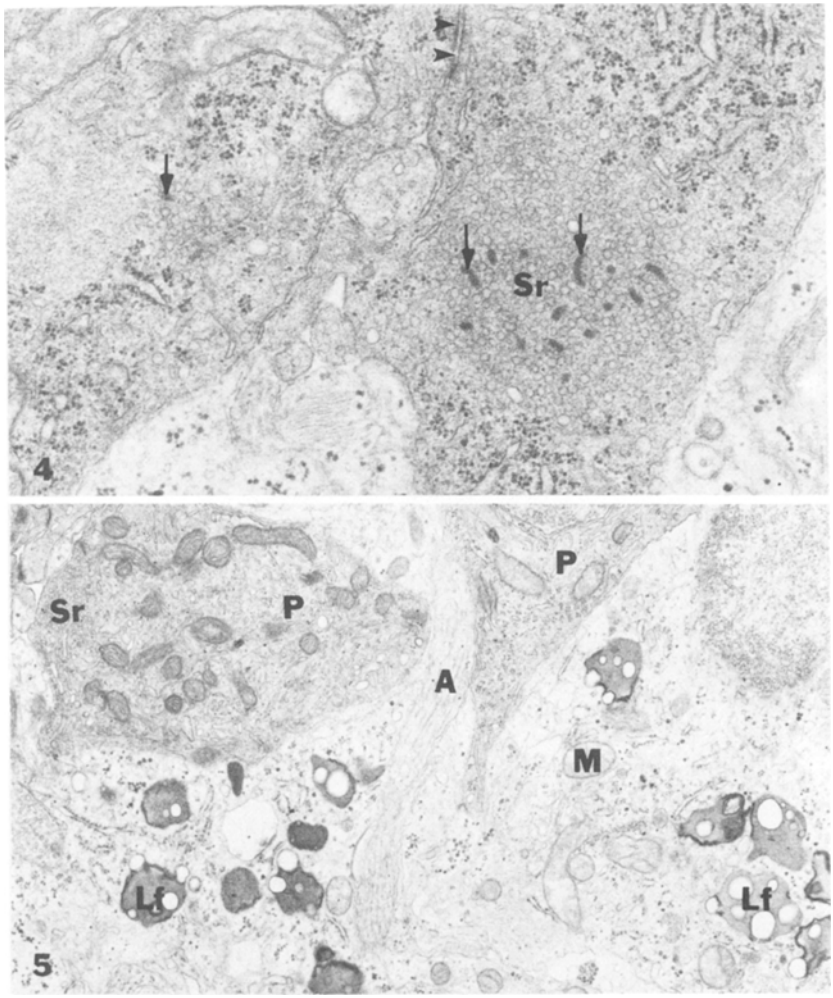

Fig. 4. Profiles of two pinealocytes are shown to have a junctional connection (arrowheads) without synaptic vesicles. Characterized by these two profiles is the presence of synaptic ribbons *(Sr)* containing many clear vesicles and synaptic rods (arrows) in different numbers and length. ×29,000

Fig. 5. One of the pinealocyte profiles was cut through the synaptic ribbon *(Sr)* at the periphery of the cytoplasm. Note the cytoplasmic density of pinealocytes is darker than that of glial cells which possess lipofuscin granules *(Lf)*. Other organelles such as microfilament and mitochondria *(M)* can also be identified in the glial cells. This photomicrograph also shows the intimate relationship between pinealocytes *(P)*, glial cells and nerve *(A)*. ×14,000

encountered in cell processes or in the soma apposed to the cell membrane. Primary SR contained a singular elongate core and several clear vesicles; secondary or tertiary SR consisted of two or more elongate cores and numerous clear vesicles. Such vesicles were sometimes seen in a large aggregation in a tangential plane without showing the rod profile/s. In addition to regular round or slender mitochondria, some giant mitochondria were occasionally encountered (Fig. 2). Microfilaments in the pinealocyte were rare, whereas microtubules were scattered in the cytoplasm. A centriole or cilium (Figs. 1, 3) containing microtubules was often observed close to Golgi complex. Glycogen particles were few in number.

II. Glial cells: The glial cells were more irregular in shape than pinealocytes and exhibited elongate processes often characterized by the presence of a bundle of microfilaments (Figs. 6, 8, 9). Glycogen particles were numerous in the cytoplasm, and there was a tendency for glycogen aggregation in the cell process (Fig. 3). In general, the cytoplasm was more electron lucent (Figs. 5—7) than that of pinealocytes. The nucleus was irregular, frequently horse-shoe or kidney shaped (Fig. 6). Its heterochromatin was usually clustered and became denser just beneath the nuclear envelope, showing a peripheral density. A prominent Golgi complex consisted of arrays of membranous sacs associated with vacuoles and vesicles (Figs. 6, 8). In addition to the small dilated rER profiles similar to those seen in pinealocytes, elongated and flattened cisternae of rER were evident in glial cells; these rER profiles occasionally were stacked together with light membrane-bound cytoplasmic bodies (Figs. 7, 8). Such arrays were unique features of the glial cells. These light cytoplasmic bodies were either closely related to free ribosomes (Fig. 7) or aligned along the rER (Figs. 7, 8). A variety of dense bodies (Fig. 6) larger than the above-mentioned cytoplasmic bodies were also seen; some dense bodies (Figs. 5, 6, 8) possessed myelin figures, lipid droplets, vacuoles and a fine granular matrix. Microfilaments were frequently seen in the perikaryon and in cell processes (Figs. 6, 8, 9). Many of the microfilaments were formed in bundles. Microtubules (Fig. 9), cilia and centrioles (Figs. 7, 8) were present and not morphologically different from those seen in the pinealocytes. The glial cells contained only oval or elongate mitochondria.

III. Pigment-containing cells: This type of cell was sparse and usually found in the dorsal part of the pineal rather than in the central parenchyma. They were oval and possessed prominent and round nuclei (Fig. 10) that occupy a large portion of the cytoplasm. The homogenous appearance of the chromatin in the nucleus was similar to that seen in the pinealocyte. Large pigment granules

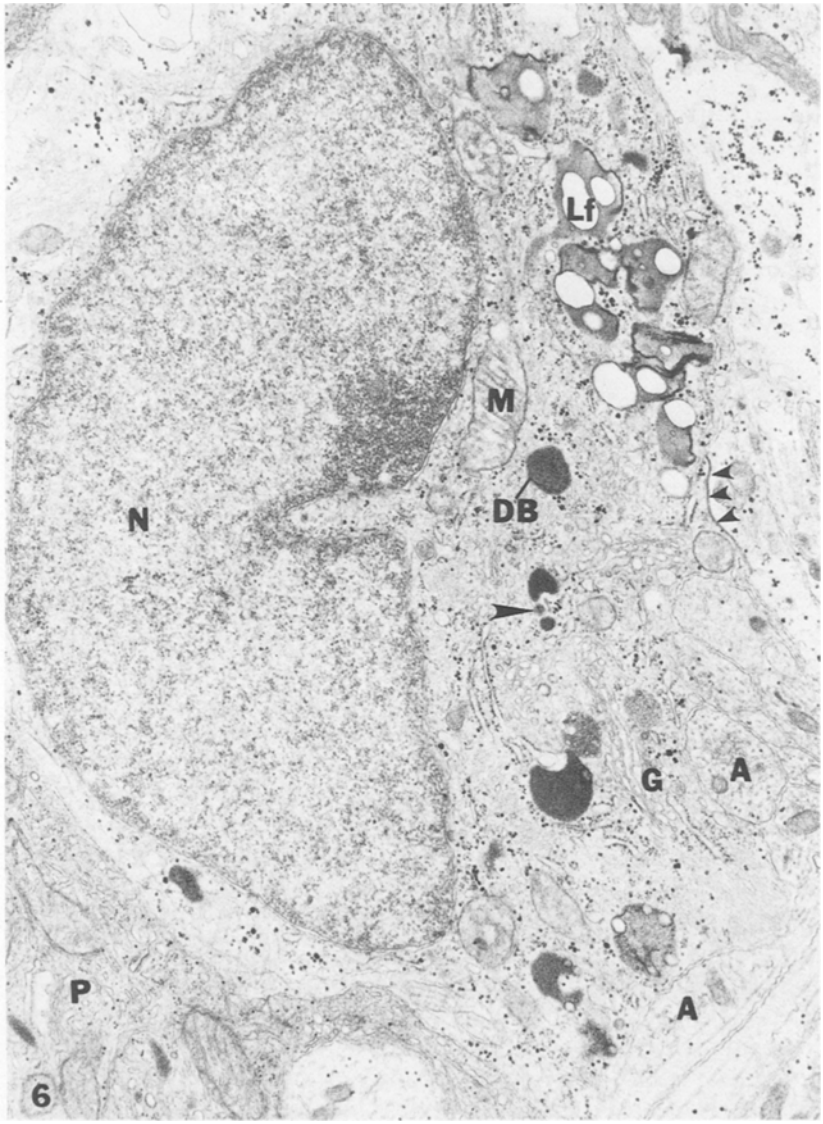

Fig. 6. The nucleus *(N)* in the glial cell of the Tupaia pineal gland is horseshoe- or kidney-shaped, and it possesses a peripheral cluster of the heterochromatin just beneath the nuclear envelope, showing heterochromatin condensation. The glial cell is characterized by the presence of the lipofuscin granule *(Lf)*, granular vesicle (larger arrowhead), well-developed Golgi complex *(G)*, different kinds of dense bodies *(DB)* and microfilaments in the cytoplasm. The cytoplasm of the glial cell is lighter in density, compared to that of the pinealocyte *(P)* at the lower left corner. A gap junction (smaller arrowheads) is seen between the glial soma and process. ×17,500

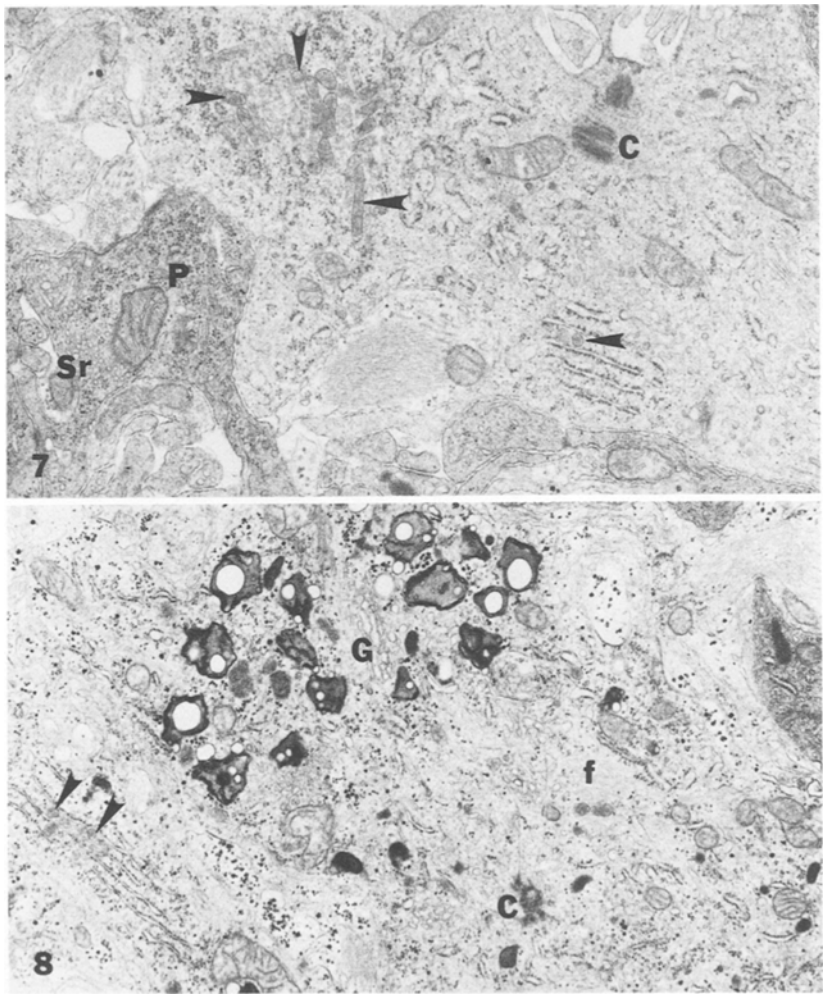

Fig. 7. The light cytoplasmic bodies (arrowheads) in different lengths and shapes are one of the most unique organelles seen in the glial cell. These cytoplasmic bodies are seen to aggregate near the ribosomes or to align along the granular endoplasmic reticulum. The centriole *(C)* is shown in the glial cell. The process of the pinealocyte *(P)* containing the synaptic ribbon *(Sr)* is intimately apposed to the glial cell. Note the difference of the cytoplasmic density between these two types of cells. ×17,500

Fig. 8. This glial cell is shown to have Golgi complex *(G)* which is surrounded by lipofuscin granules and various dense bodies. Microfilaments *(f)* cut through different angles, and centrioles *(C)* are also demonstrated. Note that several light cytoplasmic bodies (arrowheads) are seen to align themselves between the cisternae of the granular endoplasmic reticulum. ×11,900

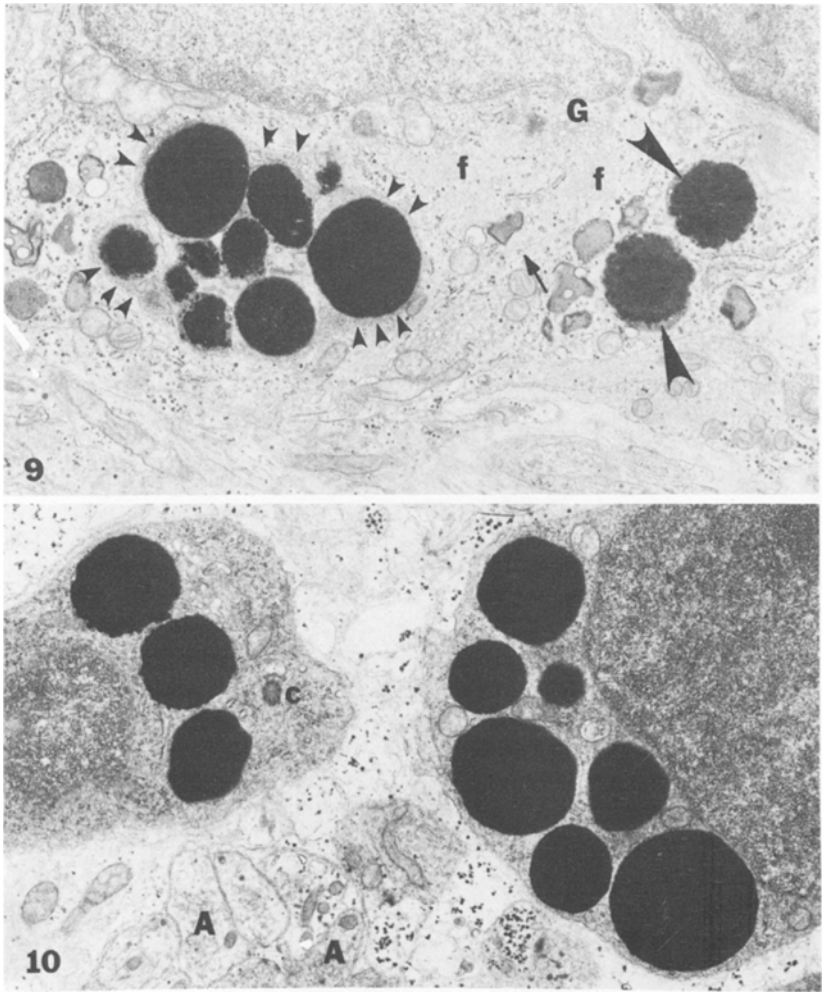

Fig. 9. The profile of a pigment-containing cell (circled by smaller arrowheads) possessing many pigment granules protrudes into a glial cell, and is isolated in the glial cell after sectioning. The glial cell is shown to have Golgi complex *(G)*, microfilaments *(f)*, microtubule (arrow), lipofuscin granules and two dense bodies (larger arrowheads) which are morphologically intermediate between the lipofuscin granule and pigment granule. ×12,600

Fig. 10. Two pigment-containing cells are characterized by the occurrence of large pigment granules, which are a prominent inclusion in the cytoplasm. Compared with the glial cell in Fig. 9, the cytoplasm of the pigment-containing cell is darker. A centriole *(c)* is cut through in one of the cells. The cross section of a nerve bundle *(A)* is also shown at the lower middle portion. ×11,900

(Figs. 9, 10) were most conspicuous in the cytoplasm. Other organelles such as ribosomes, ER, Golgi complex, mitochondria and centriole were also observed. However, intermediate forms of cell types among the parenchymal cells were found. A cell type intermediate between glial cells and pigment-containing cells was illustrated in Figs. 9 and 16.

IV. Innervation: Fluorescence histochemical study showed an intense network of green fluorescent fibers (Fig. 11) in the parenchyma and around blood vessels, indicating an adrenergic innervation of the pineal. Electron micrographs showed that nerve profiles (Fig. 12) contained mitochondria and a variety of vesicles, including small and large granular vesicles, while some nerve profiles were filled mainly with clear vesicles (Figs. 13, 14). Many of the nerve endings were seen to intrude (Fig. 13) into the parenchymal cells. Synaptic connections were observed in the tree shrew, although such classic synaptic connections had never been observed in rats (*Wolfe,* 1965). In the perivascular space, elongate profiles (Fig. 15) full of large secretory-like granules and small clear vesicles were occasionally encountered during the course of this study.

Discussion

The present observations indicate that the pineal organ of *Tupaia glis,* consisting of pinealocytes, glial cells and pigment-containing cells, is a glandular structure which is heavily innervated by adrenergic nerves and possibly by cholinergic nerves. These observations are parallel to other reports (for review, see *Reiter,* 1977) made in other mammals. In rats, the pineal parenchyma essentially consists of pinealocytes (*Wolfe,* 1965). In addition to pinealocytes, a number of glial cells are present in the pineal organs of all mammals studied. The glial cells have also been termed by different authors as interstitial cells (*Wolfe,* 1965), dark cells or astrocytes (*Sheridan* and *Reiter,* 1973). One population of pinealocytes has been reported in the sheep and cow (*Anderson,* 1965), the seal (*Cuello,* 1973) and the rat (*Wolfe,* 1965), although two populations have been described in the rabbit (*Pévet,* 1977) and the dog (*Welser et al.,* 1968). *Sheridan* and *Reiter* (1973), however, believed that light pinealocyte and dark pinealocyte in the gopher are the same line of parenchyma, yet are in a different functional status, whereas *Wolfe* (1965) concluded that there is only one population of the pinealocytes in the rat after osmic acid perfusion. By using an improved perfusion protocol (*Williams* and *Jew,* 1975), we found essentially one population of pinealocyte in *Tupaia.*

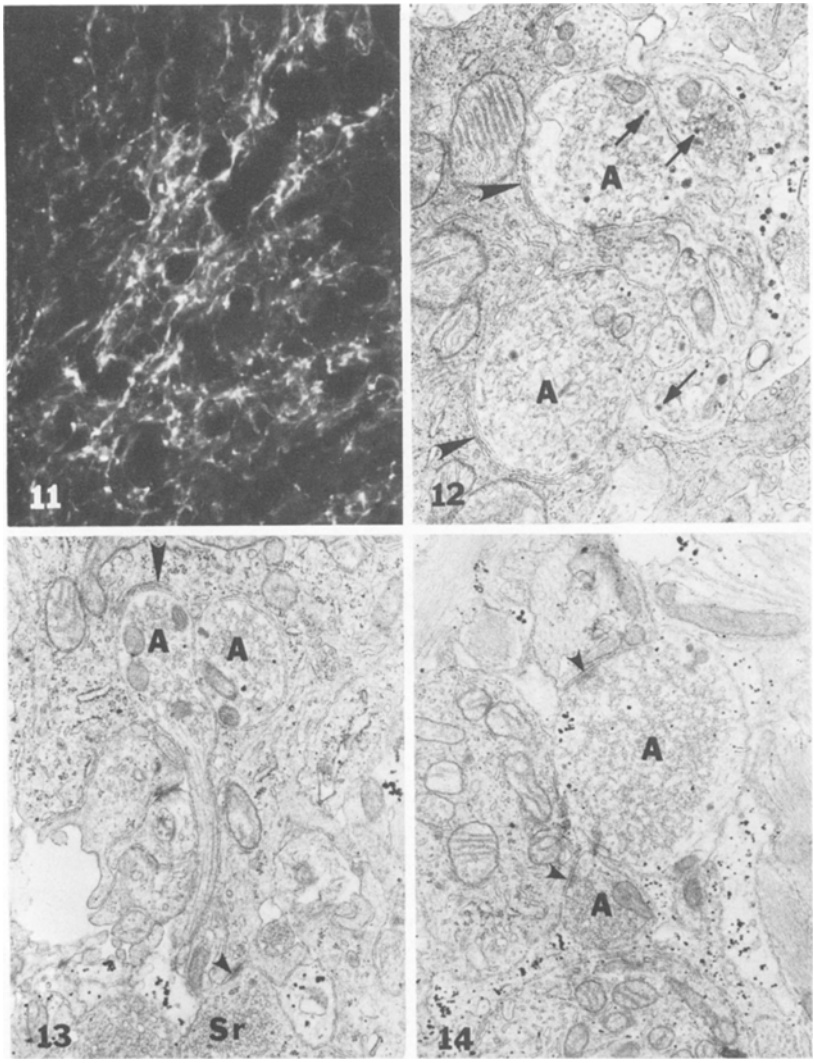

Fig. 11. The adrenergic nerves distributing over the pineal gland, as revealed by the glyoxylic acid-induced fluorescence method are shown as white axons or varicosities on this microphotograph. ×330

Fig. 12. The electron micrograph shows several profiles of presumable adrenergic endings *(A)* which bear small granular vesicles (arrows), characteristic of adrenergic terminals. The endings (marked with *A*) may form synaptic contacts with the pinealocyte. Note there are subsurface cisternae (arrowheads) close to the inner aspect of the pinealocyte plasma membrane. ×17,000

Fig. 13. Two nerve endings *(A)* containing clear synaptic vesicles and mitochondria are apposed to the pinealocyte, without cutting through the synapse. Note the subsurface cisterna (large arrowhead) in the pinealocyte. A junctional complex (small arrowhead) is seen between processes of pinealocytes, one with the synaptic ribbon *(Sr)*. ×15,400

Fig. 14. Nerve endings *(A)* containing clear synaptic vesicles are found to have synaptic contacts (arrowheads) with pinealocytes. ×16,000

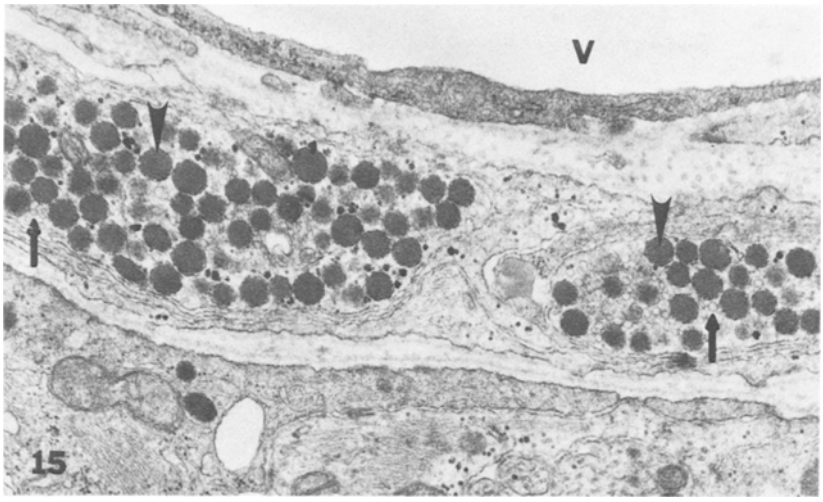

Fig. 15. Profiles of cells with an unknown origin containing many neurosecretory-like granules (arrowheads) and small clear vesicles (arrows) are occasionally seen just outside the blood vessel *(V)* in the perivascular space. ×24,500

One of the best known functions of the mammalian pineal gland is its antigonadotrophic effect (*Cheesman,* 1970; *Moszkowska et al.,* 1974; *O'Steen,* 1966; *Reiter,* 1973, 1977; and *Fraschini,* 1969). This effect is presumably caused by the release of antigonadotrophic substance/s from the parenchymal cells into the hypothalamus-hypophysis-gonad axis. Melatonin (*Wurtman et al.,* 1968) was suggested to be one of the candidates for antigonadotrophic substances in the pineal and stored in the granular vesicles (*Karasek,* 1974). This is further supported by autoradiographical study (*Meiniel,* 1976) that tritiated 5-hydroxytryptophan was localized over the granular vesicles, suggesting that serotonin which is the precursor of melatonin is actually stored in the granular vesicles (*Sheridan* and *Keppel,* 1971; *Karasek,* 1974; *Romijn* and *Gelsena,* 1976).

Electron-dense cytoplasmic bodies in pinealocytes of *Tupaia* are commonly seen. Some of them are probably lysosomes, since their morphological feature is similar to that of acid phosphatase-positive electron-dense bodies in rat pinealocytes (*Hwang,* 1972). Such lysosomes in rat pinealocytes are involved in the disposal of cellular elements including some secretory granules (*Hwang,* 1972). A similar disposal of cellular material was also detected in pinealocytes of the hamster after a removal of the superior cervical ganglia (*Lin et al.,* 1975). Furthermore, lysosomes in the rabbit pinealocytes were found to participate in the autophagocytosis of glycogen particles and then

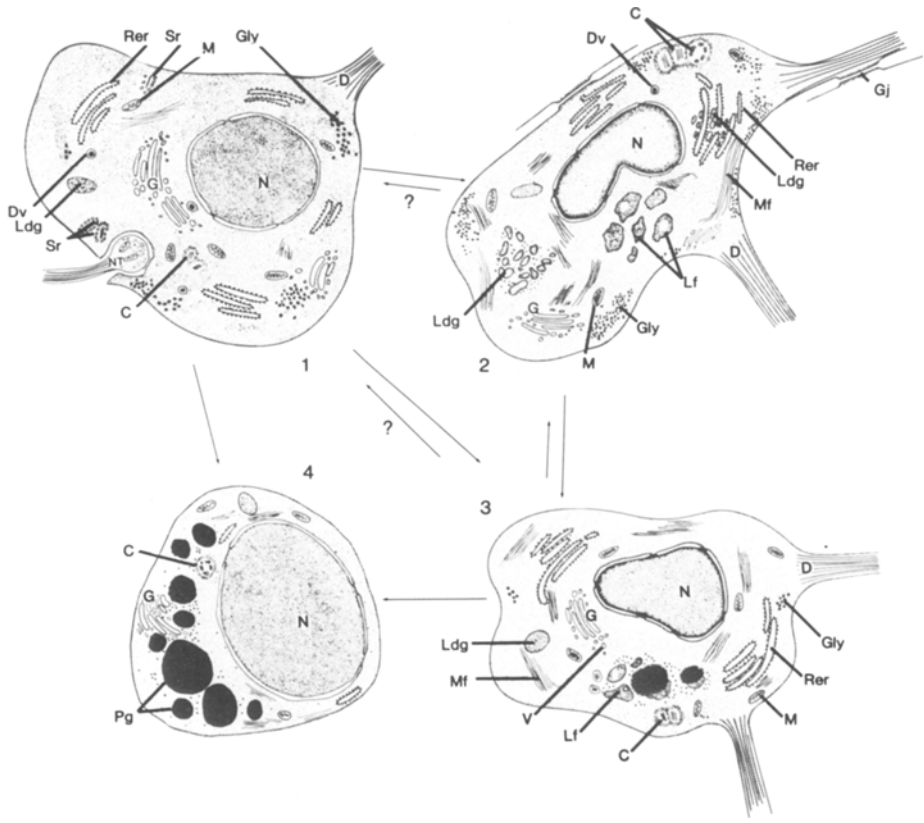

Fig. 16. This schematic drawing illustrates the possible derivation of the parenchymal cells in the *Tupaia* pineal gland. The plausible consequence of the cellular changes during the ontogenic maturation in adulthood is indicated by the direction of the arrows. This speculation is based upon (a) the observation of intermediate forms of parenchymal cells, and (b) the occurrence of common organelles including granular vesicles, pigment granules, and the centriole or cilium. An intermediate cell *(3)* between the glial cell *(2)* and pigment-containing cell *(4)* is shown. From the presence of the well-developed Golgi complex, granular vesicles and endoplasmic reticulum, the pinealocyte *(1)* and glial cell *(2)* are possibly secretory cells. From the viewpoint that pigment granules are the prominent inclusion in the cytoplasm, the pigment-containing cells are more likely senescent forms of parenchymal cells. *1* pinealocyte; *2* glial cell; *3* intermediate form; *4* pigment-containing cell; *C* centriole or cilium; *D* dendrite; *Dv* granular vesicles; *G* Golgi complex; *Gj* gap junction; *Gly* glycogens; *Ldg* light cytoplasmic bodies; *Lf* lipofuscin granules; *M* mitochondria; *Mf* microfilaments; *N* nucleus; *Pg* pigment granules; *Rer* granular endoplasmic reticulum; *Sr* synaptic ribbon; *V* Golgi vesicles

in the conversion of lysosomes into pigment granules (*Romijn et al.*, 1977). The present ultrastructural study suggests that electron-dense bodies in *Tupaia* pinealocytes are most likely to be lysosomes which may engage in the autolysis of certain substances and finally produce pigment granules. Thus, few pigment-like granules have ever been encountered in *Tupaia* pinealocytes.

The synaptic ribbons (SR) may play an important role in the functioning of the mammalian pinealocytes, since an increase in number of SR is found in the pinealocytes of the rabbits which have had sympathectomy and continuous illumination (*Romijn*, 1975). It is noteworthy to mention that there is a dramatic increase in number of SR within pinealocytes while the number of granular vesicles is remarkably decreased in rabbit pineal organ culture after norepinephrine application (*Romijn* and *Gelsena*, 1976). As to the contents of SR, *Kristic* (1976) described that the vesicles of the rat SR may contain GABA. In *Tupaia* pinealocytes, the contents of SR remain unknown at the present time; but they may contain transmitters relating to conduction or secretion, because (i) SR are often seen near the junctional complex (Figs. 4, 13) between pinealocytes, or (ii) SR are found at the peripheral cytoplasm of the pinealocytes toward the intercellular space. In addition, gap junctions (Fig. 6) are often found between glial cells, suggesting that there is intense conductivity or coupling between parenchymal cells.

The glial cells were classified in the *Tupaia* pineals, because this type of cell possesses lipofuscin granules and microfilaments that are unique structures found in the glial elements of the CNS particularly the astrocytes. Bundles of microfilaments in *Tupaia* glial cells may be components of the cytoskeleton. This morphological aspect of the glial cells suggests that they have a supportive function among the pineal parenchyma cells.

In glial cells, electron-dense bodies in various forms are prominent; their diversity may suggest different functional states of lysosomes. Different lysosomes including lipofuscin granules (secondary lysosomes) appear in the glial cytoplasm, reflecting that they play a role in the disposal of certain elements.

One of the most striking observations in *Tupaia* glial cells is that there are many light cytoplasmic bodies in an aggregate or in an array found either among the ribosomes or between rER cisternae. This topographical relationship between light cytoplasmic bodies and ribosomes/ER indicates the former are a sort of secretory granules which are synthesized by ribosomes. The nature of contents in these cytoplasmic bodies and their function needs to be further studied. But it is very plausible that they are not lysosomes, based upon the

facts that (i) primary lysosomes are derived from Golgi complex, and (ii) similar cytoplasmic bodies found in the rat pineal gland, have been seen to be autophagocytized by acid phosphatase-positive lysosomes (*Hwang*, 1972). Thus from these points of view, it is conceivable that the light cytoplasmic bodies in glial cells of *Tupaia* are secretory granules which may be "digested" by lysosomes.

Glycogen particles are abundant in *Tupaia* glial cells. Yet *Tupaia* pinealocytes contain relatively few glycogen particles. In contrast, numerous glycogen particles have been reported in pinealocytes of the bat (*Pévet*, 1977), mole-rat (*Pévet et al.*, 1976) and rabbit (*Romijn et al.*, 1977). This may indicate there is species difference in glycogen storage.

Although pigment-containing cells have been found in the gopher pineal gland (*Sheridan* and *Reiter*, 1973), ultrastructural studies of pineal morphology in other mammals have not stressed the occurrence of pigment-containing cells (*Anderson*, 1965; *Hwang*, 1972; *Lin et al.*, 1975). The present study on the pineal gland of *Tupaia* reveals that not only pigment-containing cells but also some pinealocytes and glial cells bear pigment granules. These giant pigment granules which are likely derived from lipofuscin granules are membrane-bound and thus not lipids. The morphological features of pigment granules found in this study are similar to those elements containing melanin.

A variety of cytoplasmic inclusions such as dense bodies and pigment granules has been observed in the *Tupaia* pineals. Now, in light of (i) pinealocytes possessing numerous lysosome-like bodies and a few pigment granules, (ii) glial cells possessing many lipofuscin granules as well as pigment granules, and (iii) pigment-containing cells possessing numerous pigment granules, it is likely that pinealocytes/glial cells/pigment cells in *Tupaia* pineals are the same cell lines of parenchyma, but at different functional stages during the ontogenesis which is maintained in adulthood. Furthermore, all three cell types also possess the solitary cilium or centriole which is not easily encountered in other cell lines, implicating a further link among pinealocyte/glial cell/pigment cell. However, pinealocytes in transformational forms have been found in bats (*Pévet et al.*, 1977) and in rats (*Zimmerman* and *Tso*, 1975). *Pévet* and *Collin* (1976) also reported that the presence of principal types of pinealocytes, and of intermediary types in the mole pineal gland can be explained as the result of different stages of cell differentiation during ontogenesis.

From a morphological basis, the pinealocyte containing well developed Golgi apparatus, granular vesicles, synaptic ribbons, and rER might be the first stage of active cells, glial cells containing light

cytoplasmic bodies, lipofuscin granules and microfilaments the intermediate stage of cells to produce the pineal hormone, and pigment cells the third stage of cells as shown in Fig. 16. Pigment cells would be senescent in secretory functions because large pigment granules are the only prominent cytoplasmic inclusion in the perikaryon. The first two cell types, namely pinealocytes and glial cells, are secretory cells, because they contain similar secretory structures such as endoplasmic reticulum, well-developed Golgi complex and granular vesicles. In other species, the antigonadotrophic substances have been proposed to be stored in granular vesicles.

It has been well established in recent years that the pineal function is mainly controlled by the light via sympathetic nerves (*Wurtman et al.,* 1964; *Reiter,* 1977) originating from the superior cervical ganglia (*Kappers,* 1960). The adrenergic nerves were demonstrated by the fluorescence histochemistry in *Tupaia* pineals and appeared as green varicosities and fibers. Further, the electron microscopy revealed some terminals, in perivascular spaces, characterized by the presence of small granular vesicles, suggesting that they are adrenergic nerve endings. Other endings, characterized by the presence of small clear vesicles accompanying one or more large granular vesicles, were also observed throughout this study; they are probably cholinergic in nature. However, a dual innervation (sympathetic and parasympathetic control) has been found in some mammals including the rabbit (*Romijn,* 1975 a) and human (*Møllgaad* and *Møller,* 1973). These previous studies are parallel to the claim of the present study that *Tupaia* pineal gland receives a dual innervation. Interestingly, synaptic connections were observed in *Tupaia* pineals, although classic synaptic connections had never been reported in rat pineals (*Wolfe,* 1975). In addition, subsurface cisternae are occasionally seen in the pinealocytes adjacent to the nerve endings. It is unclear whether or not subsurface cisternae have functional significance in synaptic transmission (*Peters et al.,* 1976). Also encountered within the perivascular space were profiles of unknown origin containing neurosecretory-like granules resembling those found in the brain of different animals studied by *Sterba et al.* (1979) who described those granules to be peptidergic in nature.

This ultrastructural study revealed that *Tupaia* pineal organ contains secretory granules, and possesses endocrine features. In conclusion, *Tupaia* pineal organ is therefore considered to be an endocrine gland, not a photoreceptor organ. The nature of contents in secretory granules such as granular vesicles and light cytoplasmic bodies remains to be determined.

Acknowledgements

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