Histol Histopathol (2003) 18: 827-836

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Cell populations in the pineal gland of the viscacha (*Lagostomus maximus*). Seasonal variations

R. Cernuda-Cernuda[1], R.S. Piezzi[2], S. Domínguez[2] and M. Alvarez-Uría[1]

[1]Departamento de Morfología y Biología Celular, Universidad de Oviedo, Spain [2]Instituto de Histología y Embriología, Universidad Nacional de Cuyo/CONICET, Mendoza, Argentina and [3]Cátedra de Histología, Universidad Nacional de San Luis, Argentina

Summary. Pineal samples of the viscacha, which were taken in winter and in summer, were analysed using both light and electron microscopy. The differences found between the two seasons were few in number but significant. The parenchyma showed two main cell populations. Type I cells occupied the largest volume of the pineal and showed the characteristics of typical pinealocytes. Many processes, some of which were filled with vesicles, could be seen in intimate contact with the neighbouring cells. The presence in the winter samples of "synaptic" ribbons and spherules, which were almost absent in the summer pineals, suggests a seasonal rhythm. These synaptic-like structures, as well as the abundant subsurface cisterns present in type I cells, appeared as basic differential features which allowed these cells to be distinguished from type II cells. These latter cells, which can be classified as interstitial cells, showed some other distinguishing features, such as irregular-shaped nuclei, abundant deposits of glycogen-like particles and structures of unknown function consisting of concentric cisterns surrounding a dense body. In the summer, interstitial cells displayed numerous large round bodies, which contributed to increase the cellular volume slightly. Regarding other constituents, like glial cell processes, vessels of non-fenestrated endothelium and sympathetic innervation, no qualitative differences were observed between the two seasons studied.

We have presented here some morphological evidences of the circannual rhythm of the viscacha pineal, as well as ultrastructural criteria for distinguishing the main cell populations of this organ, which could be useful for studies carried out in other mammals.

Key words: Pineal, Viscacha, Pinealocyte, Interstitial cell, Synaptic ribbon

Offprint requests to: Rafael Cernuda-Cernuda, Departamento de Morfología y Biología Celular, Facultad de Medicina (8º planta), Universidad de Oviedo, 33071 Oviedo, Spain. Fax: 34 98 5103618. e-mail: rcernuda@correo.uniovi.es

Introduction

The pineal gland is mainly involved in the integration of information about environmental conditions (light, temperature, etc.), and in the measurement of photoperiod length (Pévet, 2000). This gland probably signals the enviromental conditions thus making mammals seasonal breeders (Reiter, 1981). The pineal has been thoroughly investigated; however, the number of species in which its ultrastructure have been studied is a meager 1.5-2% of all mammalians (Bhatnagar, 1992). Previous studies have focused on domestic and laboratory animals housed in artificially controlled conditions. Thus, the study of wild species, which are subjected to natural conditions, will provide new information about seasonal changes in the gland.

The plains viscacha (*Lagostomus maximus*) is a rodent of considerable size (up to 80 cm in total length) that inhabits grasslands and lowland desert scrubs, and also constructs burrow systems in the barren parts of the pampas at an elevation of 2,680 meters. The geographical distribution is relatively wide: extreme southwestern Paraguay, northern and central Argentina, and southern Bolivia (Nowak, 1999).

L. maximus is predominantly nocturnal, emerging from its burrow before dusk to feed. It is a highly social mammal, living in communities of 15-30 and sometimes up to 50 members (Branch, 1993). Given its high cerebral and sensory development and complex social behavior, this species occupies an intermediate position between classically studied rodents and superior mammals, thus constituting a very interesting matter of study.

Since a preliminary report on the viscacha pineal was done in the past (Domínguez et al., 1987), our main objectives were to enlarge this description, giving ultrastructural details that would have been missed then, mainly because of their low frequency of appearance, and to define better the cell populations present in this organ. Special attention was paid to the seasonal changes of some morphological features of these cell types in order to find data representative of the presumed circannual rhythmicity of the gland.

Materials and methods

A total of 7 adult male viscachas were used in this study, 3 of them were captured during the summer and 4 during the winter. The animals were killed in their habitat during the night by shooting with a rifle with telescopic sight. This method avoided stress and prolonged pain to the animal. Special care was taken to aim at the thoracic region, in order to avoid damage of the head, as well as to cause a rapid death and to prevent the animals from getting refuge in their burrows. Within a period of 4-5 minutes, the brain was exposed, the pineal gland dissected "in situ" and fixed in a phosphate buffered (0.1 M, pH 7.3) 3% paraformaldehyde/2.5% glutaraldehyde solution for 2 hours at room temperature. This solution was prepared 1-2 hours before use. The samples were then postfixed in 1% OsO_4 at 4 ºC for 2 hours, washed is distilled water, dehydrated in a series of acetone of increasing concentration and embedded in Epon, following a conventional protocol. 2 blocks of tissue were sampled per gland. Semithin (1 μm thick) and ultrathin (about 70 nm thick) sections were obtained in a Reichert Jung ULTRACUT ultramicrotome. 4 areas separated by 200 μm were sampled per block. Semithin sections were stained with toluidine blue solution and photographed with a Polaroid DMC digital camera. Ultrathin sections were mounted on copper grids and stained with lead citrate and uranyl acetate. Stained ultrathin sections were examined and photographed in a Zeiss EM 109 transmission electron microscope.

Morphometric analysis was carried out at the light microscope level to assess: (1) the volume density of the main cell types and the space occupied by connective tissue, nerves and vessels, and (2) the numerical density of the main cell types per unit area of section

The point-counting method of Weibel (1979) was employed to calculate the relative volume of the gland components. Five semithin sections (1 μm width) separated by at least 100 μm were randomly chosen from each gland. Five fields were randomly selected and photographed at a final magnification of x1000, 0.01 mm^2 being the area of gland tissue examined in each photograph. Measurements were made by placing a transparent screen with a square point lattice spacing 1 cm over each photograph and counting the number of intersections on each component. The volume fraction of a particular component was calculated by dividing the total number of points over that component by the total number of points over the glandular tissue.

The number of cells per unit area of section was estimated by counting their nuclei in 5 random fields of 2500 μm^2 in each of the 5 sections mentioned above.

The data obtained were statistically compared using the Student t test.

Results

The pineal of the viscacha, which can be classified as type AB according to Vollrath (1981), has got a considerable size, with 12 mm in length, 2.5 mm in width, and an average weight of 4.23 mg. The histological features of this gland and the differences observed between the two seasons analysed can be found below.

Light microscopy

The analysis of the semithin sections revealed that pineals of summer and winter showed a fairly similar appearance (Figs. 1a,b). At least, two types of cells could be easily recognized in the pineal parenchyma. Most of the cells possessed a round/oval nucleus with prominent nucleolus (type I cells), but some others presented an irregular-shaped nucleus (type II cells). The type II cells had many dense inclusions in their cytoplasm in the summer samples (Fig. 1a), whereas such inclusions were very scarce or absent in winter (Fig. 1b). Spaces occupied by vessels and connective tissue were intensely stained, probably due to the abundant collagen deposits, and few details could be observed.

No statistical differences were found between the parameters measured in both groups, with the exception of the volume density of type II cells, which was slightly increased ($p<0.05$) in summer (Table 1).

Electron microscopy

Type I and type II cells constituted the main components of the parenchyma of this organ (Fig. 2). In addition, some glial (Type III) cells could also be observed. Nerves and blood vessels penetrated from a connective capsule and were distributed throughout the gland. A detailed description of all of these elements can be found below.

Ultrastructural features of Type I cells

Type I cells, pinealocytes, the most abundant cell type in the pineal parenchyma, were irregular-contoured

Table 1. Morphometric data of the main pineal components. Comparisons between summer and winter samples.

	SUMMER	WINTER
Volume density (%)		
Type I cells (pinealocytes)	70±1.7	71.9±1.5
Type II cells	11.6±1.4	7.2±1.1*
Vessels and perivascular spaces (nerves, connective tissue, etc)	18.4±1.6	20.9±1.4
Numerical density (No. of nuclei/2500 μm^2)		
Type I cells (pinealocytes)	10.1±0.7	11.5±0.5
Type II cells	4.2±0.4	4.0±0.3

Each value is the mean±S.E.M. *P<0.05.

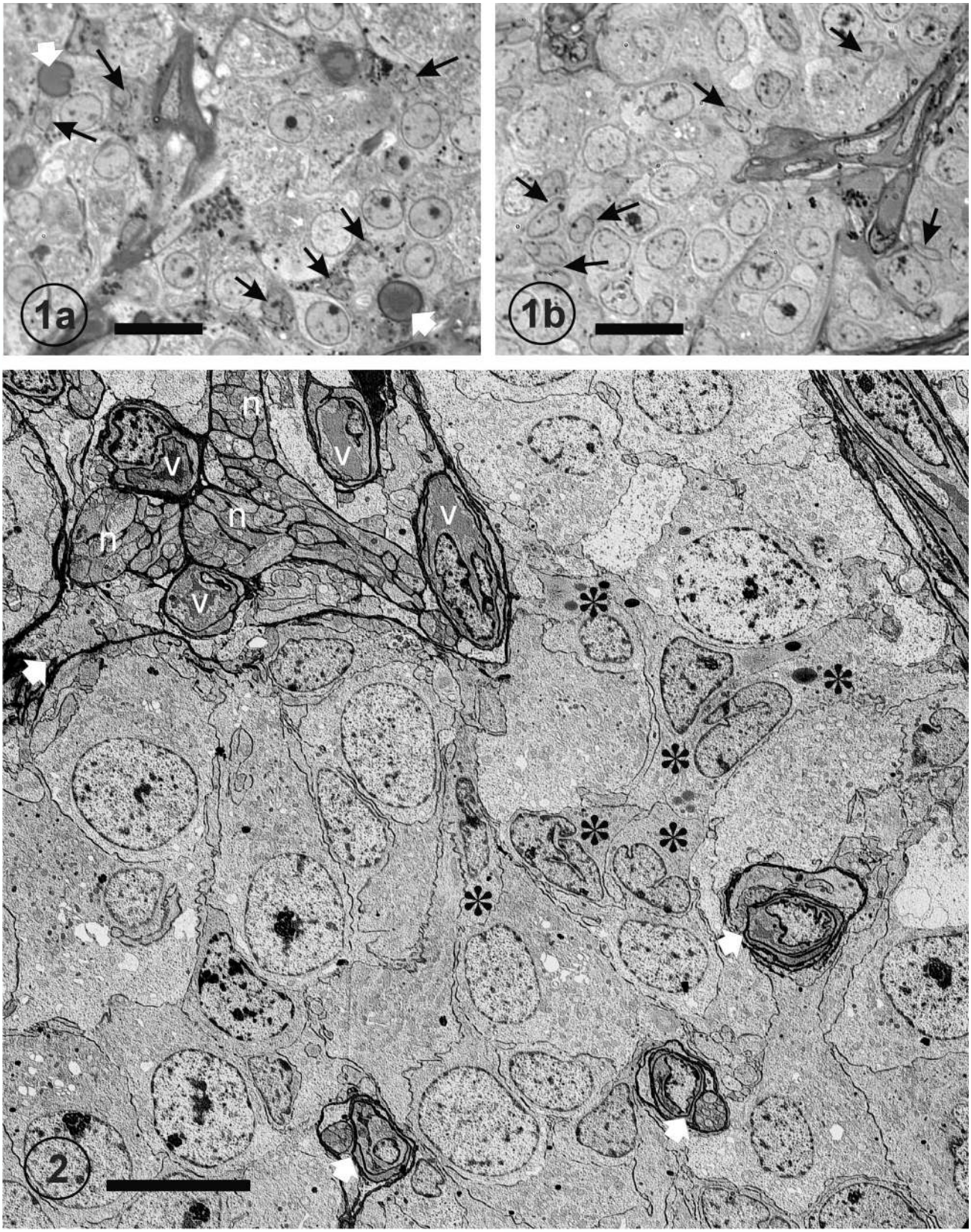


Fig. 1. Semithin sections of pineal taken in summer **(a)** and in winter **(b)**. The general appearance is fairly similar in both of them. Small black arrows point to cells with irregular-shaped nuclei (type II cells). Note that in the summer pineal **(a)**, these cells possess numerous dense inclusions (white arrows point to two large-sized bodies). x 750. Calibration bar: 20 μm.

Fig. 2. A low magnification micrograph of the pineal taken in winter shows a general appearance of this organ. Pinealocytes or type I cells, which constitute the main cellular population, have large round nuclei and their cytoplasm can be of variable electron density. Type II cells (asterisks) occupy a smaller volume and have a more electron-dense nuclei with an irregular contour. Vessels (v) and nerves (n) are grouped in septa (white arrows) that penetrate from the outer connective capsule and branch into the pineal parenchyma. A dark basal membrane surrounds these structures. x 2200. Calibration bar: 10 μm.

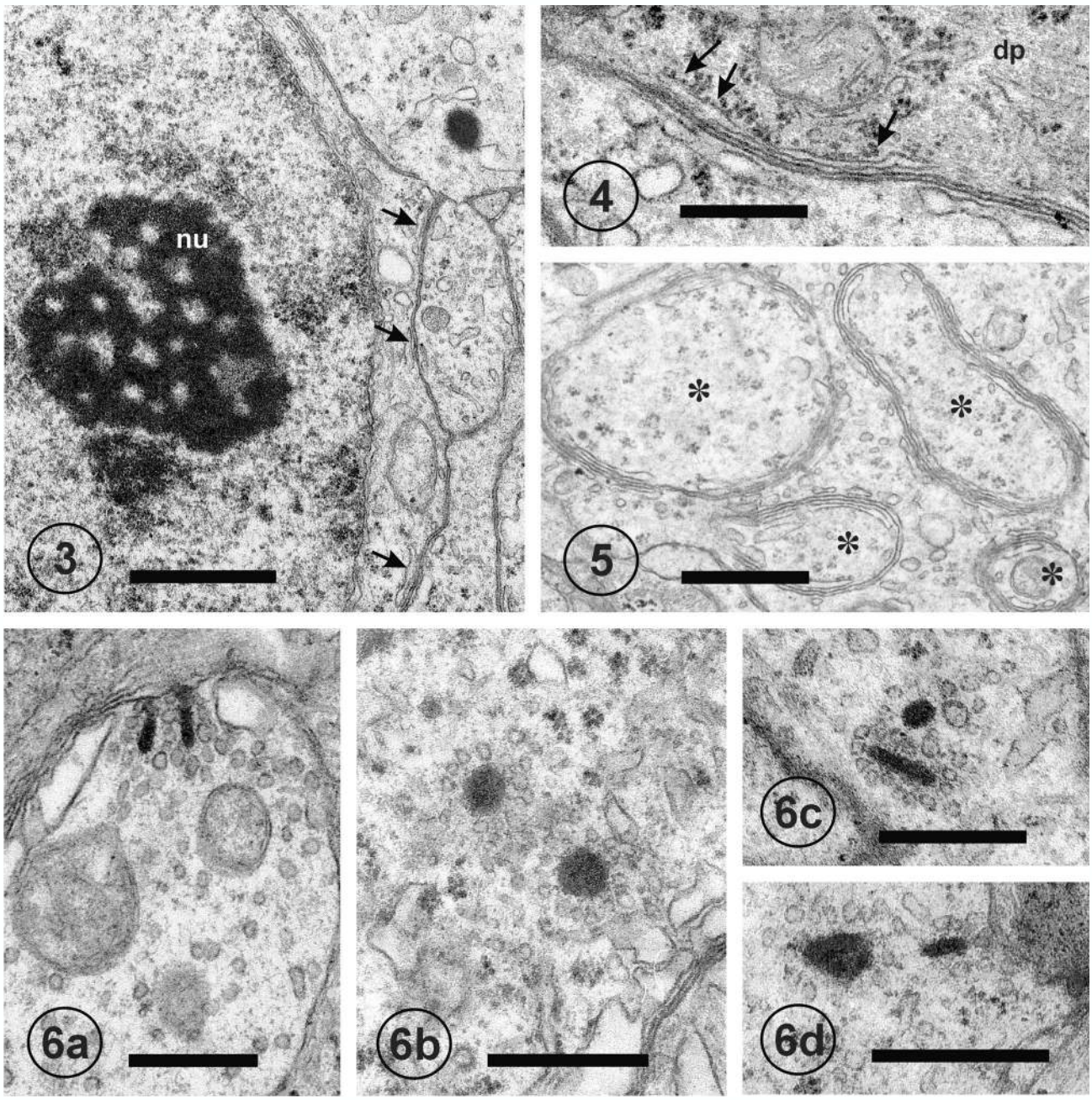


Fig. 3. A pinealocyte nucleus shows a prominent nucleolus (white arrow). Most of them possess a fibrillar part exhibiting a reticular morphology. Subsurface cisterns (black arrows) attached to the plasma membrane can also be observed in this micrograph of a sample taken in winter. x 25000. Calibration bar: 1 μm.

Fig. 4. Occasionally, the subsurface cisterns have ribosomes (arrows) attached on the cytoplasmic side. The image corresponds to a winter sample. dp: dark pinealocyte. x 38000. Calibration bar: 0.3 μm.

Fig. 5. The subsurface cisterns seem to be more developed in the summer. Several pinealocytic processes (asterisks) penetrate into a neighbouring pinealocyte and form false images of triads of cisterns. Note that the membranes of the central cistern at the triad are actually the plasmalemmas of the two adjacent pinealocytes. x 30000. Calibration bar: 0.5 μm.

Fig. 6. Different types of synaptic-like structures are found in the winter samples. **a**. A synapse-like formation between two pinealocytes is observed. Twin dense ribbons attached to the plasmalemma are associated with vesicles of light content in a pinealocytic process. x 40000. **b**. Two large dense spherules associated with vesicles can be observed. x 42000. **c**. A synaptic-like mixed group showing a ribbon and a spherule is present in a pinealocyte process. x 40000. **d**. A ribbon (right) appears to be in close relation with a large synaptic-like structure (left). Both of them are associated with vesicles of a clear content. x 45000. Calibration bars: 0.5 μm.

cells that showed a cytoplasm of light or dark electron-density, with a great variety of organelles. These cells displayed a round or oval clear nuclei (Fig. 2) with prominent nucleoli (Figs. 2, 3). Their cytoplasm possessed numerous mitochondria, some of which had lipid deposits in their matrix, some cisterns of smooth endoplasmic reticulum (sER), well developed Golgi apparatus and rough endoplasmic reticulum (rER), abundant polyribosomes and also coated pits and vesicles. Occasionally, a single cilium with its basal body could be observed. Some of these cilia showed a widened ending. Centrioles were also present either in association with ciliated structures or as isolated elements. Microtubules and microfilaments could be seen widely spread throughout the cytoplasm. Very occasionally, some laminated structures in continuity with rER cisterns, as well as sER-like cisterns with a geometric arrangement were observed (not shown). No qualitative differences were detected regarding the mentioned pinelocytic organellae between either season.

All of the pinealocytes presented subsurface cisterns (Fig. 3), which sometimes seemed to be in continuity with cisterns of rER, showing ribosomes at the cytoplasmic side (Fig. 4). The contour of these cells was found to be extremely tortuous and numerous processes with a round/oval shape could be distinguished contacting intimately with other cells. The summer samples showed many examples of these bulbs, which, due to the presence of abundant subsurface cisterns, gave false images of triads of cisterns that closely resembled dictyosomes (Fig. 5).

In many cases these pinealocytic processes appeared full of vesicles of clear content and showed synapses and related structures that will be described below. Some adherent junctions were present between pinealocytes and were also present between pinealocytes and type II cells.

Synaptic-like structures in Type I cells

Although rather scarce, small-sized ribbons, 45 nm width and 200-250 nm length (Fig. 6a), and spherules, 130-170 nm in diameter (Fig. 6b), either alone or in groups of two or more, could be visualized in different locations in the pinealocyte cytoplasm in the winter samples. Both of them were associated with clear vesicles, 45-65 nm in diameter. Groups of ribbons (Fig. 6a), spherules (Fig. 6b) or a mixture of them (Fig. 6c) were observed. Some of the ribbons appeared attached perpendicularly to the plasma membrane and some others in a cytoplasmic site distant from the membrane. A third type of dense "synaptic" formation of large size, 150 nm width and 220 nm length, and irregular shape was found (Fig. 6d). Most of the synaptic-like contacts occurred in the cellular processes, but some of them occurred in the perikaryon in the proximity of the nucleus. This type of structure was found both in light and dark pinealocytes. Synaptic-like contacts were usually seen between pinealocytes, but also some of these contacts were sporadically seen between pinealocytes and type II cells. Synaptic-like formations were almost absent in the samples collected in the summer, which showed only very occasional and isolated ribbons.

Ultrastructural features of type II cells

Basically, type II cells could be distinguished from neighbouring pinealocytes (type I cells) by the morphology of their nuclei. These nuclei were irregular in shape and possessed more heterochromatin than those of pinealocytes (Figs. 2, 7, 9). Their cytoplasm was also reduced in volume. In addition, dense glycogen-like particles were spread throughout the cytoplasm. This was a distinctive feature for cell type II (Figs. 7-10). Similarly to pinealocytes, these cells showed many cellular processes. Nevertheless, neither subsurface cisterns nor synaptic-like structures were seen in type II cells. A characteristic structure with numerous concentric cisterns surrounding a dense core was always observed associated to this cell type (Figs. 7, 8). These myelin-like structures were often localized in terminal bulbs in the vicinity of the perivascular spaces. In some cases, these cisterns were not concentric and were continuous with rER. Some adherent junctions between these cells could be seen. Lysosomes of variable electron density were also abundant. Cisterns of rER and sER, numerous mitochondria and centrioles surrounded by dictyosomes were also found. Single cilia were occasionally observed. Cellular processes containing bundles of microfilaments, which could correspond either to these cells or to glial (type III) cells, were occasionally observed among the pinealocytes (not shown). However, such bundles could not be detected in the cytoplasm surrounding the nucleus of type II cells. In the summer, in addition to the mentioned features, type II cells presented large round bodies of variable size (0.5-10 μm in diameter), with a heterogeneous content (Figs 9, 10), which corresponded to those observed in semithin sections (Fig. 1a). These bodies were surrounded by flattened cisterns and round vesicles and, in some cases, located in the proximity of the Golgi apparatus (Fig. 10).

Nerves, capillaries and perivascular spaces

Numerous unmyelinated nerve fibers (Figs. 2, 11) and vessels appeared surrounded by a thick basal membrane showing dense fibers of collagen. These structures constituted septa that penetrated from a connective capsule and distributed profusely within the pineal parenchyma (Fig. 2). Two types of vesicles, one with a clear content, 40-55 nm in diameter, and another with a dense content, 110-140 nm in diameter (Figs. 12, 13), could be distinguished in the nerve endings. These nerves were sometimes found outside the perivascular spaces (Fig. 12) in the vicinity of pinealocytes and type II cells. Some isolated nerve endings showed only

vesicles with dense content (Fig. 13). However, no synapses could be observed between nerves and pinealocytes. Regarding these nerve constituents, no qualitative differences were detected between the winter and the summer samples.

Some scarce processes of glial (type III) cells characterized by a dense cytoplasm and by the presence of abundant filaments (Fig. 13) could be found mainly surrounding the nerve/vascular spaces and sometimes also spread throughout the pineal parenchyma. Dense granules similar to those described in Type II cells as glycogen-like particles were also present in the cytoplasm of these cells. Large dense bodies, with a variable diameter (0.5-5 μm), were observed at these glial cells in the summer samples.

Although very occasionally, some pinealocyte processes full of vesicles crossed the basal membrane of the septa and seemed to end in the perivascular spaces. Other pinealocyte processes, which could not be distinguished clearly from nerve endings, were found in the proximity of the blood vessels.

Around the vessels, which were of non-fenestrated endothelium (Fig. 14), some pericytes were seen. Furthermore, some other cells with numerous dense round bodies of heterogeneous content (Fig. 14), these being much larger in the summer samples, were located in these perivascular spaces. Dilated rER cisterns could be observed in these cells.

Discussion

The ultrastructure of this gland shows many parallelisms to other mammalian pineals. We have made an attempt to define in detail the different cell populations of this gland, taking into account exclusively ultrastructural criteria, which could be useful in future studies on other mammals. The presence of several cell types in the pineal parenchyma has been reported in several studies (Vollrath, 1981). However, there is no agreement in the classification of these cell types. Some authors consider two types of pinealocytes, depending on the electron-density of their cytoplasm. Although we have found dark and light pinealocytes, they shared similar ultrastructural features. We therefore think it is appropriate to consider them as different functional states of the same cell type, as other authors also suggested (Pévet and Racey, 1981). The widened endings seen on some of the pinealocyte cilia resemble the club-shaped terminals observed in the cilia of pinealocytes in the tropical bat (Chang et al., 1987). These terminals have been compared to the outer segments of rudimentary photoreceptor cells of non-mammalian vertebrates reported by Oksche (1984); however, it is widely accepted that the mammalian pineal has lost the photoreceptive role present in the lower vertebrates.

Laminated structures in association with the rER were reported previously in the pinealocytes of the mole rat (*Spalax ehrenbergi*) by Pévet et al. (1976), who considered them as a kind of smooth endoplasmic reticulum (sER) specialization. In addition, the canaliculate lamellar bodies described by Lin (1967) and McNeill (1977) in rat pinealocytes were also identified as unusual configurations of the sER. No function has been identified for these lamellar bodies.

Welsh and Reiter (1978) and others have reported the presence of subsurface cisterns. The cistern relationship with rER has also been seen in the rat (González and Álvarez-Uría, 1984) and the degu (Uría et al., 1992). These cisterns may thus be considered as part of the sER. Regarding their function, Tutter et al. (1991) demonstrated the storage of calcium ions and suggested a functional role in cell stimulation. The abundance of such structures in the summer is thus unclear, since it is

Fig. 7. Typical appearance of a type II cell (asterisk) in a micrograph of a winter sample: a nucleus of irregular contour which can be distinguished from those (round) of neighbouring pinealocytes, and a concentric lamellar structure (arrow) surrounding a dense core. x 8000. Bar: 1 μm.

Fig. 8. A concentric lamellar structure surrounding a dense probably lipidic core (asterisk) can be observed in a type II cell process close to a perivascular region. Arrows point to some of the many glycogen-like particles present. x 15500. Bar: 1 μm.

Fig. 9. In the summer, type II cells (asterisk) show large, round structures with amorphous content. Arrows point to glycogen-like particles. x 8000. Bar: 1 μm.

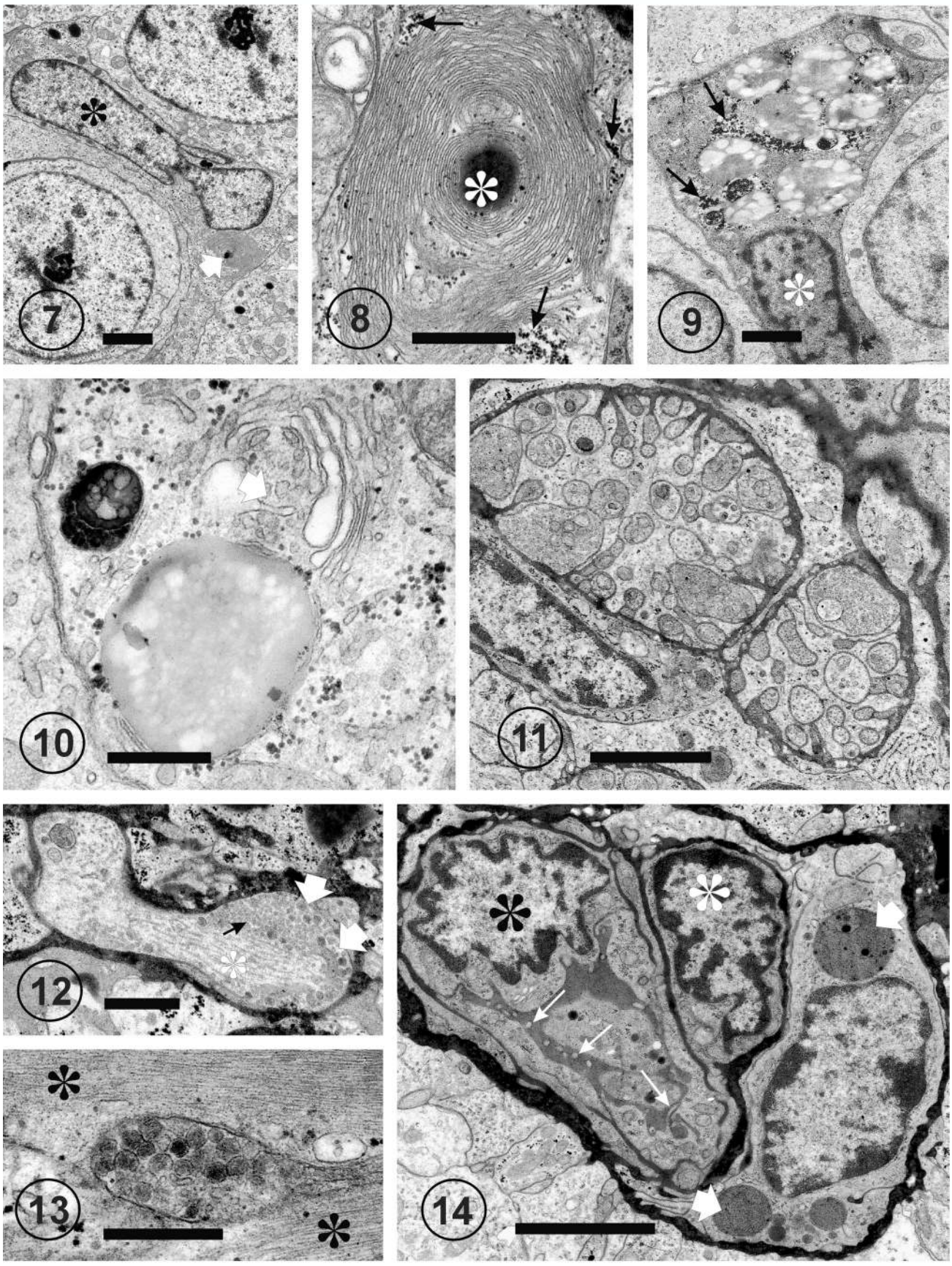
Fig. 10. The mentioned structures are often surrounded by cisterns and vesicles and are sometimes located in the proximity of Golgi apparatus (arrow). x 30000. Calibration bar: 0.5 μm.

Fig. 11. Two unmyelinated nerve fibers can be seen in a perivascular region. x 10000. Bar: 2 μm.

Fig. 12. A nerve ending, which seems to cross the thick basal collagenous membrane around the perivascular region, shows both types of vesicles: of clear content (small black arrow) and of dark content (large white arrows). Asterisk, microtubules. x 21000. Bar: 1 μm.

Fig. 13. Some nerve endings are full of vesicles of dark content. Asterisks: glial cell processes. x 50000. Calibration bar: 0.5 μm.

Fig. 14. Around the vessels, which show a non-fenestrated endothelium (black asterisk) with numerous luminal microvilli (small black arrows), some pericytes (large white arrow) can be seen. Furthermore, cells with numerous round dense bodies of heterogeneous staining (white asterisks) can also be found in the perivascular spaces. This sample was collected in the summer. x 10000. Bar: 2 μm.


7
8
9
10
11
12
13
14

during this season when the pineal synthetic activity is expected to be the lowest.

Synaptic-like structures such as ribbons and spherules were frequently seen in mammalian pineal glands (Vollrath, 1981). Our observations confirm their presence in the viscacha, although these structures have not been reported previously in this species (Domínguez et al., 1987), due to their low frequency. In the guinea pig (Vollrath, 1986; Khaledpour and Vollrath, 1987) and in the Syrian hamster (Díaz et al., 1990) ribbons and spherules did not appear to lie within the same pinealocyte region, in contrast to some of our observations. In the Guinea pig, an inverse day versus night rhythm was observed for these structures (Khaledpour and Vollrath, 1987): spherules reach a peak around midday and ribbons at about 4:00 a.m. Díaz et al. (1990) also observed a marked peak for ribbons during the night in the Syrian hamster. As our samples were taken at night, the presence of more ribbons than spherules is in agreement with these findings. Large synaptic-like dense structures described here may be compared to transitional forms between ribbons and spherules, as reported in the Guinea pig by Khaledpour and Vollrath (1987). Although the precise function of these structures is unknown, the existence of intercellular communication suggested by many authors is widely accepted. No relation was found between the ribbon and spherule structures and nerve endings, such as González and Alvarez-Uría (1984) and also Ichimura et al. (1986) described in other mammals. The low frequency of these synaptic-like formations in the summer samples fully supports the idea of low activity of the gland during the summer. In fact, pineal activity is known to be inhibited by the long period of light (Reiter, 1981; Pévet, 2000) and low levels of synaptic-like structures in the summer were reported in other mammals (Martínez-Soriano et al., 1999, 2002).

We have classified type II cells as interstitial cells due to their dissimilar characteristics to pinealocytes. Interstitial cells were classically reported to be different from pinealocytes by Arstila and Hopsu (1964) and others. However, cells with features similar to those described in the present paper have been considered as a type of pinealocyte by Pévet et al. (1976), Karasek and Hansen (1982), and Chang et al. (1987). Similar concentric lamellar structures have been reported as rare constituents in pinealocytes of the tropical bat (Chang et al., 1987), but numerous in pinealocytes of the mole-rat (Pévet et al., 1976). The function of these structures is unknown; nonetheless, Bucana et al. (1973) suggested that they were due to degenerative activity prior to the formation of a new membrane system. The presence of glycogen-like particles and diverse dense bodies were reported in type II pinealocytes of the fox by Karasek and Hansen (1982). Thus, the identity and function of type II cells is controversial.

Vollrath (1981) considered the interstitial cells as a type of astrocyte since immunohistochemical studies using antibodies against glial markers were positive (Møller et al., 1978). Boya and Calvo (1993) also reported a notable population of astrocytes in the cat and the dog pineals. Cells with similar ultrastructural appearance were also classified as astrocytes by Sakai et al. (1996) in a study on the cotton rat pineal. In our samples, some features of the type II cells, such as the presence of glycogen-like particles and dense bodies, were also found in glial (type III) cell processes. Møller and Baeres (2002) reported that interstitial cells contain a high number of microfilaments in most species, thus their absence in the main regions of the type II cells found in our samples is difficult to explain. As we have mentioned in our results, some sparse processes containing microfilaments could correspond either to type II cells or to glial (type III) cells. In such a case, these two cell types in the viscacha pineal would also share this cytological feature as it occurs in other species. The lack of synaptic-like structures and subsurface cisterns in these cells seems to favour a functional difference with active pinealocytes. The number of type II cells did not significantly change between summer and winter, though their relative volume increased slightly in summer. This increase in volume was paralleled by the accumulation of large dense bodies and suggests seasonal variation, although the role of such bodies is unclear.

Our observations of glial cell processes are in agreement with many other papers. Their location near the perivascular spaces and the presence of numerous microfilaments in the cytoplasm has confirmed their classification as fibrous astrocytes (Vollrath, 1981; Karasek and Hansen, 1982; Boya et al., 1995).

The presence of two types of vesicles in the sympathetic nerve endings, which are typical of adrenergic innervation, confirm previous observations on other mammals (Vollrath, 1981). Nevertheless, large dense-cored vesicles, such as those described here, are not very usual in other mammals. Their size and content are more similar to the large vesicles found in the sympathetic endings of the rat (Duffy and Markesbery, 1970; Vollrath, 1981). The ultrastructure of such large dense vesicles seems, nevertheless, to be closer to the typical peptidergic ones. In fact, diverse peptidic neurotransmitters were reported in the pineal fibers, either corresponding to the sympathetic (Cozzi et al. 1992; Mikkelsen and Møller, 1999) or the parasympathetic innervation (Møller and Mikkelsen, 1989) of the pineal. On the other hand, neuropeptides were also described in the pineal innervation originating in the trigeminal ganglion (Reuss, 1999; Møller et al., 1999), and recently, a new peptide, orexin, was discovered in some pineal fibers corresponding to the central innervation of the gland (Mikkelsen et al., 2001). Therefore, some of the nerve endings shown here could be peptidergic. Although some nerve endings close to pinealocyte processes were seen, no defined synapses could be observed. This is in accordance with other mammalian species investigated, in which the majority of the nerve fibers, specially the sympathetic ones, have

a perivascular location (Vollrath, 1981). A more detailed study focussed on the innervation of the pineal of this rodent, using markers for diverse neurotransmitters, is needed to elucidate the nature of the nerve endings and their relationship with the pineal cells.

Although exceptions like the mouse and the rat can be cited (Møller et al., 1978; Wartenberg, 1968), blood capillaries of non-fenestrated endothelium were reported in most species. Luminal cytoplasmic processes were found in rat, but are rare in other rodents such as the mouse. Wartenberg (1968) reported that the pineal parenchyma was separated by an incomplete basal lamina, and some pinealocyte processes penetrate the perivascular spaces. This is in agreement with our findings. The structure of the perivascular spaces of the viscacha pineal can be compared to those of the cat and the rhesus monkey, which are wide, with a thick collagenous basal lamina and contain large numbers of nerve fibers (Wartenberg, 1968). Cells with large dense bodies similar to those described here are not usual. Although its precise function is unknown, the appearance of such heterogeneous content in these structures suggests a role of phagocytosis for these cells. In fact, phagocytic cells located in the perivascular spaces have been described before (Møller and Bares, 2002). Such cells, which might be microglial-like cells, were also reported to be antigen-presenting cells since they showed immunoreactivity for the class II major histocompatibility system (Sato et al., 1996).

The pineal of the viscacha shows, in conclusion, many similarities to other mammalian pineals, as well as some morphological evidence of circannual rhythmicity. The changes observed in this species, which is predominantly nocturnal, such as the low number of synaptic-like structures in the summer, might be related to the gradual exposure to twilight during the long daylight period of this season. This correlates well with published data about the development of the gonads of this species occurring in the summer (Weir, 1971; Fuentes et al., 1991, 1993). The viscachas in east-central Argentina have a single breeding season, and mating takes place in March-April (late summer/early autumn) (Branch, 1993). A seasonal rhythm was detected for several gonadal constituents (Muñoz et al., 1997, 1998, 2001). Although other factors could also be involved in this process, it is unquestionable that the pineal gland and its principal hormone, melatonin, is mainly responsible for the synchronisation of the gonadal activity with the annual rhythm.

In addition to the morphological changes described, in the present paper we have characterized in detail the different cell populations of this organ at the electron microscopic level. This is undoubtedly of interest for future multidisciplinary studies, either on this species or on other mammals, which need to be done for a better understanding of the physiology of this still very enigmatic gland.

Acknowledgements. CONICET and the Universidad Nacional de San Luis (Argentina) supported this research. We are grateful to Teresa Fogal and Juan Arroyuelo for their collaboration.

References

Arstila A.U. and Hopsu V.K. (1964). Studies in the rat pineal gland. I. Ultrastructure. Ann. Acad. Sci. fenn. A II 113, 3-21.

Bhatnagar K.P. (1992) The ultrastructure of mammalian pinealocytes: a systematic investigation. Microsc. Res. Tech. 21, 85-115.

Boya J. and Calvo J.L. (1993). Immunohistochemical study of the pineal astrocytes in the postnatal development of the cat and dog pineal gland. J. Pineal Res. 15, 13-20.

Boya J., Calvo J. L. and Rancaño D. (1995). Structure of the pineal gland in the adult cat. J. Pineal Res. 18, 112-118.

Branch L.C. (1993). Social organization and mating system of the plains viscacha. J. Zool. 229, 473-491.

Bucana C.D., Nadakabukaren M.J. and Frehn J.L. (1973). Ultrastructural features of the pineal gland from cold-exposed golden hamsters. J. Neurocytol. 2, 237-247.

Chang N., Bhatnagar K.P., Tseng M.T. and Karim K.B. (1987). Ultrastructure of the pineal gland of the tropical bat Rousettus leschenaulti. Acta Anat. 128, 194-203.

Cozzi B., Mikkelsen J.D., Ravault J.-P. and Møller M. (1992). Neuropeptide Y (NPY) and C-flanking peptide of NPY in the pineal gland of normal and ganglionectomized sheep. J. Comp. Neurol. 316, 238-250.

Díaz C., Álvarez-Uría M., Tolivia J. and López J.M. (1990). Circadian changes in synaptic ribbons and spherules in pinealocytes of the Syrian hamster (*Mesocricetus auratus*). Cell Tissue Res. 262, 165-169

Domínguez S., Piezzi R.S., Scardapane L. and Guzmán J.A. (1987). A light and electron microscopic study of the pineal gland of the viscacha (*Lagostomus maximus* maximus). J. Pineal Res. 4, 211-219.

Duffy P.E. and Markesbery W.R. (1970). Granulated vesicles in sympathetic nerve endings in the pineal gland: observations on the effects of pharmacologic agents by electron microscopy. Am. J. Anat. 128, 97-115.

Fuentes L., Calvo J.C., Charreau E. and Guzmán J. (1993). Seasonal variations in testicular LH, FSH, and Prl receptors; in vitro testosterone production; and serum testosterone concentration in adult male viscacha (*Lagostomus maximus* maximus). Gen. Comp. Endocrinol. 90, 133–141.

Fuentes L., Caravaca N., Pelzer L., Scardapane L., Piezzi R.S. and Guzmán J. (1991). Seasonal variations in the testis and epididymis of viscacha (*Lagostomus maximus* maximus). Biol. Reprod. 45, 493-497.

González G. and Álvarez-Uría M. (1984). La glándula pineal de los mamíferos. Univ. Oviedo, Servicio de publicaciones. Oviedo. p 210.

Ichimura T., Arikuni T. and Hashimoto P.H. (1986). Fine-structural study of the pineal body of the monkey (*Macaca fuscata*) with special reference to synaptic formations. Cell. Tissue Res. 244, 569-576.

Karasek M. and Hansen J.T. (1982). Ultrastructure of the pineal gland of the fox. Am. J. Anat. 163, 257-267.

Khaledpour C. and Vollrath L. (1987). Evidence for the presence of two

24-h rhythms 180° out of phase in the pineal gland of male Pirbright-White guinea pigs as monitored by counting "synaptic" ribbons and spherules. Exp. Brain. Res. 66, 185-190.

Lin H.S. (1967). A peculiar configuration of agranular reticulum (lamellar body) in the rat pinealocyte. J. Cell Biol. 33, 15-25.

Martínez-Soriano F., Hernández-Gil de Tejada T., López Bigorra M., Ballester Carmona S. and Vollrath L. (1999). Circadian and seasonal changes of synaptic bodies in different parts of the rabbit pineal gland. Histol. Histopathol. 14,1079-1091.

Martínez-Soriano F., Ruiz-Torner A., Armananzas E. and Valverde-Navarro A.A. (2002). Influence of light/dark, seasonal and lunar cycles on serum melatonin levels and synaptic bodies number of the pineal gland of the rat. Histol. Histopathol. 17, 213-22.

McNeill M.E. (1977). An unusual organelle in the pineal gland of the rat. Cell Tissue Res. 184, 133-137.

Mikkelsen J.D. and Møller M. (1999). Neuropeptide Y in the mammalian pineal gland. Microsc. Res. Tech. 46, 239-256.

Mikkelsen J.D., Hauser F., deLecea L., Sutcliffe J.G., Kilduff T.S., Calgari C., Pévet P. and Simonneaux V. (2001). Hypocretin (orexin) in the rat pineal gland: a central transmitter with effects on noradrenaline-induced release of melatonin. Eur. J. Neurosci. 14, 419–425.

Møller M. and Baeres F.M.M. (2002). The anatomy and innervation of the mammalian pineal gland. Cell Tissue Res. 309, 139-150.

Møller M., Ingild A. and Bock E. (1978). Immunohistochemical demonstration of S-100 protein and GFA protein in interstitial cells of rat pineal gland. Brain Res. 140, 1-15.

Møller M., Fahrenkrug J. and Hannibal J. (1999). Innervation of the rat pineal gland by pituitary adenylate cyclase-activating polypeptide (PACAP)-immunoreactive nerve fibres. Cell Tissue Res. 296, 247-257.

Muñoz E., Fogal T., Domínguez S., Scardapane L., Guzmán J. and Piezzi R.S. (1997). Seasonal changes of the Leydig cells of viscacha (*Lagostomus maximus* maximus). A light and electron microscopy study. Tissue Cell 29, 119-128.

Muñoz E., Fogal T., Domínguez S., Scardapane L., Guzmán J., Cavicchia J.C. and Piezzi RS. (1998). Stages of the cycle of the seminiferous epithelium of the viscacha (*Lagostomus maximus* maximus). Anat Rec 252, 8-16.

Muñoz E., Fogal T., Domínguez S., Scardapane L. and Piezzi R.S. (2001). Ultrastructural and morphometric study of the Sertoli Cell of the viscacha (*Lagostomus maximus* maximus) during the annual reproductive cycle. Anat. Rec. 262, 176-185.

Nowak R.M. (1999). Walker's Mammals of the World. 6th Edition. Johns Hopkins Univ. Pr., Baltimore.

Oksche A. (1984). Evolution of the pineal complex: correlation of structure and function. Ophthalmic Res. 16, 88-95.

Pévet P. (2000). Melatonin and biological rhythms. Biol. Signals Recept. 9, 203-212

Pévet P. and Racey P. A. (1981). The pineal gland of nocturnal mammals. II. The ultrastructure of the pineal gland in the pipistrelle bat (*Pipistrellus pipistrellus* L): presence of two populations of pinealocytes. Cell Tissue Res. 216, 253-271.

Pévet P., Kappers J.A. and Nevo E. (1976) The pineal gland of the mole-rat (*Spalax ehrenbergi*, Nehring). I. The Fine Structure of Pinealocytes. Cell Tissue Res. 174, 1-24.

Reiter R.J. (1981). The mammalian pineal gland: structure and function. Am. J. Anat. 162, 187-313.

Reuss S. (1999). Trigeminal innervation of the mammalian pineal gland. Microsc. Res. Tech. 46, 305-309.

Sakai Y., Hira Y. and Matsushima S. (1996). Regional differences in the pineal gland of the cotton rat, Sigmodon hispidus: Light microscopic, electron microscopic, and immunohistochemical observations. J. Pineal Res. 20,125-137.

Sato T., Kaneko M., Hama A., Kusakari T. and Fujieda H. (1996) Expression of class II MHC molecules in the rat pineal gland during development and effects of treatment with carbon tetrachloride. Cell Tissue Res. 284, 65-76.

Tutter Y., Heinzeller T. and Seitz-Tutter D. (1991). Pinealocyte subsurface cisterns III: storage of calcium ions and their probable role in cell stimulation. J. Pineal Res. 10, 91-99.

Uría H., Antolín I., Tolivia D., Rodríguez-Colunga M. J. and Menéndez-Peláez A. (1992). The pineal gland of the trumpet-tailed rat (*Octodon degus*). J. Pineal Res. 13, 174-183.

Vollrath L. (1981). The pineal organ. In: Handbuch der mikroskopischen anatomie des menschen. Vol VI. part 7. Vollrath L. and Oksche A. (eds). Springer Verlag. Berlin-Heidelberg-New York. pp 1-665.

Vollrath L. (1986). Inverse behaviour of "synaptic" ribbons and spherule numbers in the pineal gland of male guinea-pigs exposed to continuous illumination. Anat. Embryol. 173, 349-354.

Wartenberg H. (1968). The mammalian pineal organ: Electron microscopic studies on the fine structure of pinealocytes, glial cells and on the perivascular compartment. Z. Zellforsch. 86, 74-97.

Weibel E.R. (1979) Practical methods for biological morphometry. In: Stereological methods. Vol. I. Weibel E.R. (ed). Academic Press. London. pp 1-145.

Weir B.J. (1971). The reproductive physiology of the plains viscacha, *Lagostomus maximus*. J. Reprod. Fertil. 25, 355-363.

Welsh M.G. and Reiter R.J. (1978). The pineal gland of the gerbil, Meriones unguiculatus. I. An ultrastructural study. Cell. Tissue Res. 193, 323-336.

Accepted March 28, 2003