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# Ultrastructure of the pineal gland in the adult rat

J. CALVO AND J. BOYA

*Department of Histology and General Embryology, Faculty of Medicine, University Complutense, Madrid, Spain*

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## INTRODUCTION

The ultrastructural features of the adult rat pineal gland have been studied by various authors. Wolfe (1965) and Arstila (1967) describe pineal cell types in detail. Other components, mostly those related to the principal pinealocyte, such as synaptic ribbons (Krstic, 1976; King & Dougherty, 1980) and nucleoli (Lew, Payer & Quay, 1982), have also been described. However, the ultrastructure of the second pineal cell type or Type II pinealocyte (Pevet, 1977; Calvo & Boya, 1983) and the components of pineal connective tissue spaces, are much less well known. Furthermore, all the investigations referred to have been carried out on adult rats of a fixed age. Until now, the modifications in rat pineal gland ultrastructure during adult age have not been described.

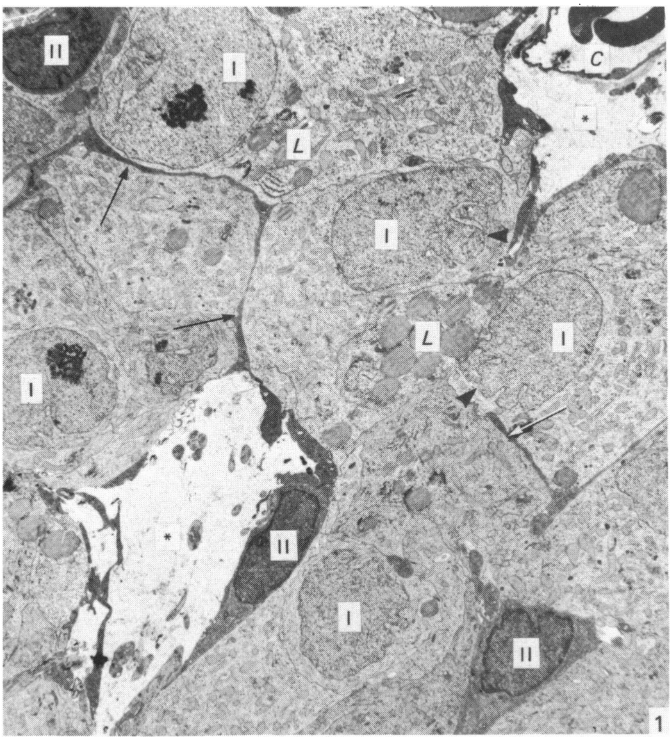
The ultrastructural differentiation of pineal cell types during the postnatal period until 60 days of age has recently been described (Calvo & Boya, 1983). In the present study the ultrastructure of the pineal gland in the adult rat is described, especially its lesser known aspects, as well as those features which show a clearer evolution with increasing age during the adult period.

## MATERIALS AND METHODS

A total of 36 albino Wistar rats of both sexes was used, kept under standard conditions of light and feeding. The animals were anaesthetised with ether and killed by decapitation at 75 days and at intervals of 30 days from 3 to 10 months of age. For each age interval, four pineal glands (2 from each sex) were taken and fixed by immersion in 0·1 M phosphate-buffered 2% glutaraldehyde—2% paraformaldehyde, pH 7·4. After washing them through the same buffer, they were post-fixed in phosphate-buffered 1% osmium tetroxide and embedded in Vestopal. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in an EM Philips 201.

## RESULTS

The nucleus of Type I pinealocytes displayed numerous infoldings in its envelope. These infoldings had a tendency to be grouped in one area of the nuclear contour (Fig. 1). Mitochondria varying greatly in size and shape occupied a large part of the cytoplasm. Granular endoplasmic reticulum formed short cisterns, either isolated or in stacks. Cells in young adult glands frequently displayed large clusters of smooth endoplasmic reticulum located in the periphery near the cell membrane (Fig. 2). Among elements of the smooth endoplasmic reticulum, numerous free ribosomes were observed. Clusters of smooth endoplasmic reticulum were mostly found in the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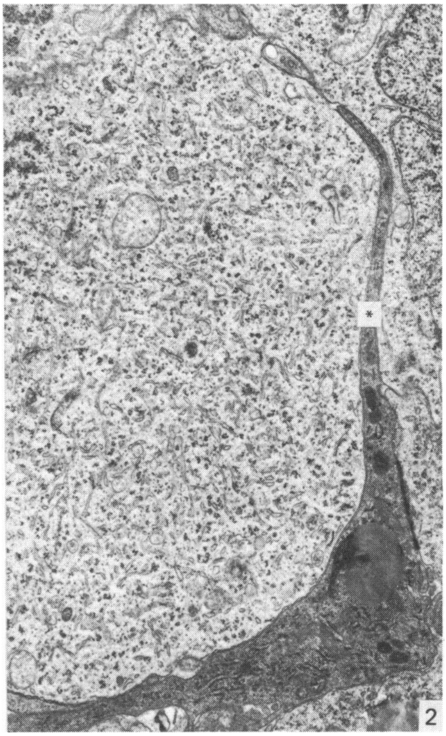
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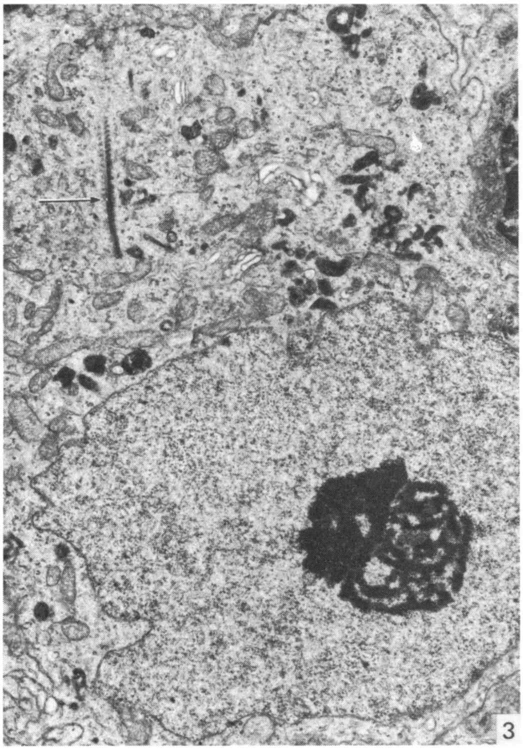
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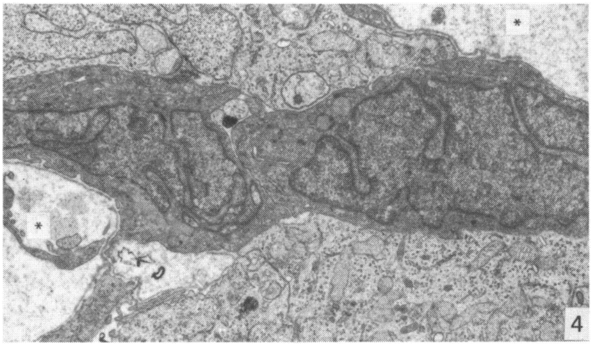
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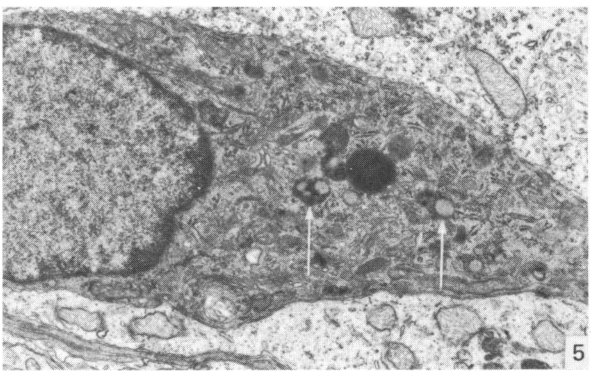
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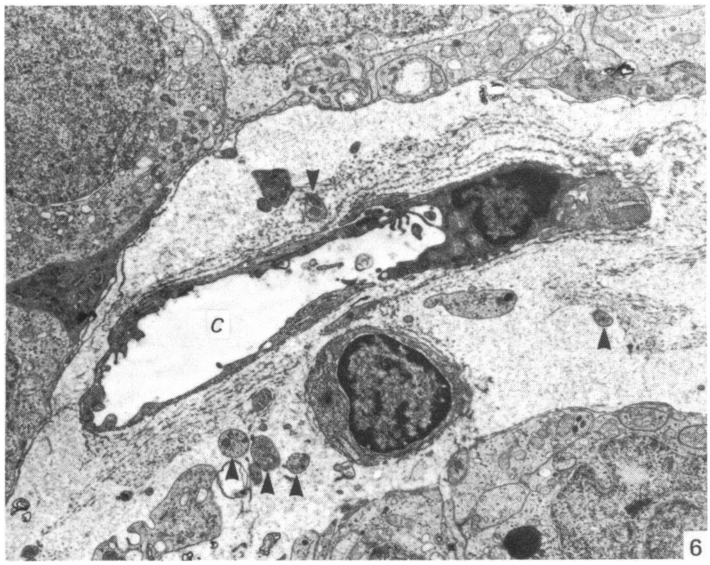


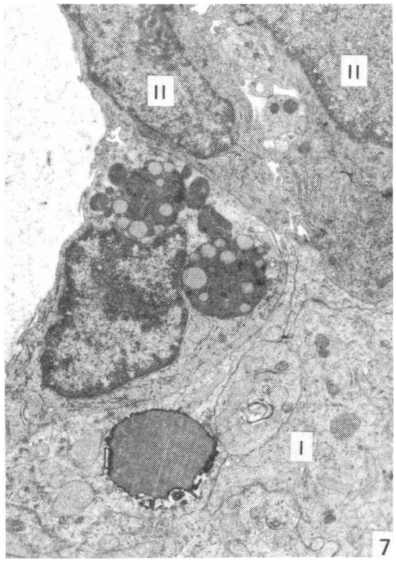


Fig. 6. Female, 3 months. Wide connective tissue space containing a capillary (*C*) and doublings of the capillary basement membrane. Arrowheads; nerve fibres. × 5800.

Fig. 7. Female, 8 months. Cell, located inside the parenchymal basement membrane, with a structure different to Type I (I) and Type II (II) pinealocytes. The cell contains large lipofuscin granules. × 6700.

clear cell variety of the Type I pinealocyte. Over 6 months of age, they were rarely found.

The Golgi complex occupied a large area near the nucleus. In the middle of the Golgi area there were remnants of centrioles in the form of several long clusters of microtubules, surrounded by very dense and sometimes striated material (Fig. 3). No centrioles or cilia were found in adult rats.

Dense bodies or lysosomes within the Type I pinealocyte obviously increased in number with age (Fig. 3). They frequently formed groups related to autophagic vacuoles, multivesicular bodies, and sometimes to lipid droplets. The constancy and frequency of autophagic vacuoles in the cytoplasm, described in prepubertal rats (Calvo & Boya, 1983), was also a characteristic of the adult Type I pinealocyte. Lipid droplets whose size and density differed from one cell to another were also

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Fig. 1. Female, 75 days. The appearance of the pineal gland at low magnification. Parenchymal cell cords are separated by connective tissue spaces (asterisks). There are numerous large Type I pinealocytes (I). Denser Type II pinealocytes are located in the margin of the parenchyma (II) with thin processes (arrows) placed among Type I pinealocytes. *L*, lipid droplets; *C*, capillary; arrowheads, infoldings of the nuclear envelope. × 2900.

Fig. 2. Male, 75 days. Type I pinealocyte containing a cluster of endoplasmic reticulum and free ribosomes in its peripheral cytoplasm. A lamellar process of a Type II pinealocyte is located between Type I cells (asterisk). × 10000.

Fig. 3. Female, 9 months. Type I pinealocyte containing a well developed nucleolus with an attached chromatin granule. In the cytoplasm, there are groups of small dense bodies and centriole remnants in relation to the Golgi area (arrow). × 8900.

Fig. 4. Female, 8 months. Two Type II pinealocytes with very irregular nuclei in contact with two connective tissue spaces (asterisks). × 7100.

Fig. 5. Female, 5 months. Type II pinealocyte containing lipofuscin-like dense bodies (arrows). × 10800.

found in the cytoplasm of this cell type (Fig. 1). Larger lipid droplets were found in older animals.

Type II pinealocytes were characterised by their great nuclear and cytoplasmic density as well as by their cytoplasmic processes (Figs. 1, 4, 5). The cell body was usually located in the margin of the parenchyma, either occurring singly or forming groups of two or three cells (Figs. 1, 4). The processes followed the contour of pineal cell cords but did not form a continuous layer (Fig. 1). Other very thin lamellar processes projected into the cell cords separating Type I pinealocytes (Figs. 1, 2). Contact surfaces between two cell bodies or between a cell body and a process showed gap-like junctions.

The nucleus of the Type II pinealocyte was generally ovoid (Fig. 1). Infoldings in the nuclear envelope, sometimes deep and complex, were frequently found in older rats (Fig. 4). The nucleolus was very small and generally peripherally placed.

The dense cytoplasm displayed cisterns of granular endoplasmic reticulum containing flocculent material and were very rich in ribosomes. In younger rats, there were a few dense bodies in the cytoplasm of the soma. With increasing age, small lipofuscin granules appeared (Fig. 5). Centrioles and even cilia were relatively frequent in Type II pinealocytes. Some large round lipid droplets were also observed, mostly in younger adults. Vacuoles surrounded by a double membrane characteristic of this cell type (Calvo & Boya, 1983) were also found. Filaments were sometimes observed, mostly in the processes.

With increasing age, the connective tissue spaces contained progressively more connective tissue fibres, as well as duplications of capillary basement membrane (Fig. 6) and material with a dense fibrillar-like appearance. Migrant cells, probably coming from the blood, were an almost constant component in the connective tissue spaces of the adult gland. Occasionally, lymphoid nodules were found. Migrant cells sometimes invaded the parenchyma. There was also a cell type characterised by a spherical nucleus with dense granules of peripheral chromatin and a cytoplasm containing long cisterns of granular endoplasmic reticulum. In rats older than 6 months, these cells showed spherical lipofuscin inclusions much larger than those observed in Type II pinealocytes (Fig. 7).

## DISCUSSION

In general, the structure of Type I pinealocytes in the adult rat described in this paper agrees with the results of other authors (Wolfe, 1965; Arstila, 1967; see revision in Vollrath, 1981). However, some of the findings require comment. The ultrastructure of Type I pinealocytes in the adult rat represents the final stage in a process of development which begins in the early postnatal period (Calvo & Boya, 1983). The differences observed between the prepubertal period and the adult stage are not very important and are mostly quantitative. Also, with the light microscope, there seems to be only a slight increase in pinealocyte size between these two stages (Calvo & Boya, 1984). Moreover, the results show some evolution in the ultrastructure of the Type I pinealocyte during adult life.

The ultrastructure of the adult Type I pinealocyte seems to indicate a high level of cell activity. The dispersed chromatin, well developed nucleoli, infoldings in the nuclear envelope, and numerous mitochondria in the cytoplasm are characteristics of active cells. The endoplasmic reticulum has been considered to be a poorly developed organelle in the Type I pinealocyte. According to Krstic (1977) it occupies only 5·3 % of cell volume. However, the present results show that at least in some cells the smooth endoplasmic reticulum forms important clusters with a typical peripheral location which occupy a considerable proportion of the cell volume. It is important to point out that these clusters are first observed near puberty (Calvo & Boya, 1983), and persist in young adult rats, stages at which pineal activity must be high. Dense bodies or lysosomes are considered rare elements in mammalian pinealocytes (Vollrath, 1981). However, in the adult rat, groups of dense bodies are a constant finding in Type I pinealocytes, and the number of lysosomes definitely increases with age. Related to the groups of dense bodies there are multivesicular bodies and autophagic vacuoles. The latter findings, which are almost constant in the Type I pinealocyte, could suggest that autophagy is an important process in the physiology of these cells.

Type II pinealocytes display a similar ultrastructural aspect to that observed in the pubertal stage (Calvo & Boya, 1983). The ultrastructure of Type II pinealocytes also evolves during the adult stage. Most of these changes concern the shape of the nucleus and the appearance of small granules of lipofuscin in their cytoplasm.

## SUMMARY

The ultrastructure of the rat pineal gland was studied from 75 days until 10 months of age. Type I pinealocytes of young adults showed nuclei with dispersed chromatin, numerous infoldings of the nuclear envelope and well developed nucleoli. The cytoplasm displayed many mitochondria and clusters of smooth endoplasmic reticulum. With increasing age, there was a clear increase in the number of dense bodies or lysosomes in the Type I pinealocyte. The changes in the Type II pinealocytes with age were mainly in nuclear shape and in the appearance of lipofuscin granules.

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