

CELLULAR CONSTITUENTS OF THE PINEAL PARENCHYMA IN THE ALBINO RAT: EFFECT OF DIFFERENT PHOTOPERIODS

By

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INTRODUCTION

The pineal gland is an endocrine organ synthesizing and secreting substances of well-defined hormonal nature. The pinealocytes represent the major cellular component of its parenchyma (**Vollrath, 1981**). The term pinealocytes is used as an inclusive term i.e. all pineal cells excluding neurons and glial elements. Several attempts have been made to classify the pinealocytes into structurally and functionally distinct groups of cells. **Blumfield and Tapp (1970)** studying the rat pineal have distinguished three types of pinealocytes according to the nuclear size and suggested a correlation with pinealocyte function. Differences in mitochondria have also been recorded in hamsters where the pinealocytes were classified into P1 pinealocytes with small cristiform mitochondria and P2 pinealocytes with mitochondria exhibiting a plexiform array of crests in a dense matrix (**Clabough, 1971**). **Pevet and Collin (1976)** studying the mole pineals have introduced another classification according to the number and form of pinealocyte processes. Using the terminology employed to nerve cells, they have distinguished three types of pinealocytes. Type I pinealocytes with two processes have been considered bipolar cells, type II pinealocytes with one process have been classified as unipolar elements and type III pinealocytes have been regarded as multipolar cells due to the presence of multiple processes. Light and dark pinealocytes have also been distinguished in many species, e.g. mouse (**Upson et al., 1976**), rat (**Johnson, 1980; Milin et al., 1990; Humbert and Pevet, 1995**), horse (**Cozzi, 1986**), rabbit (**Krakowski and Cieciora 1992**), gerbil (**Redecker, 1993**) and pig (**Przybylska, 1989; Lewczuk et al., 1994**). However, the nature of the two forms of cells was controversial. Some investigators have considered them as two distinct cell populations (**Upson et al., 1976; Milin et al., 1990**). Others have regarded them as different functional states of one and the same cell type (**Przybylska, 1989; Redecker, 1993**).

Pineal cells containing pigment material have been identified in several species, e.g. noctule bat (**Pevet et al., 1977**), dog (**Calvo et al., 1988**), mongolian ground squirrel (**Song and Huang 1990**), cat (**Calvo et al., 1992**), Japanese quail (**Ohashima and Hiramatsu, 1993**), brown bat (**Bhatnagar and Hilton, 1994**) and ovine (**Regodon et al., 1998**). These

cells have been considered by **Pevet et al. (1977)** as special type of pinealocytes. Other investigators regarded them as pigment cells rather than pinealocytes (**Song and Huang, 1990; Ohashima and Hiramatsu, 1993; Bhatnagar and Hilton, 1994; Regodon et al., 1998**). Although the pigment material has been identified as melanin (**Calvo et al., 1988; Song and Huang, 1990; Calvo et al., 1992; Bhatnagar and Hilton, 1994**), its exact functional significance in the pineal gland has not yet been clearly defined. However, a melanin-based photopigment and other different photopigments were recently identified in the pineal glands of several mammalian and non-mammalian species pointing to a photoreceptor function (**Mano et al., 1999; Moutsaki et al., 2000; Foster et al., 2003-a**).

Although the pineal gland contains and releases different substances of hormonal and non-hormonal nature, there is insufficient evidence as yet for the subdivision of the pineal cells into structurally and functionally distinct groups of cells. The aim of the present study was to define whether or not different types of pinealocytes could be distinguished on a morphological ground. Morphological assessments were made under different photoperiodic conditions to allow interpretation of the findings satisfactorily in terms of function.

MATERIAL AND METHODS

Fifteen male albino rats were utilized in the present study. They were divided into three groups of five animals each. The first group (control) was kept under a cycle of 10 hours light and 14 hours dark per day. The second group was exposed to continuous illumination while the third group was kept under continuous darkness. The animals were kept under these lightning conditions for four weeks and allowed free water ad libitum. At the end of the experiment, they were sacrificed by lethal dose of ether inhalation. The pineal glands were then rapidly removed and subjected for the following processings:

A. For electron microscopy the specimens were fixed by immersion in a mixture of glutaraldehyde and formaldehyde in phosphate buffer (pH 7.4). The specimens were then post-fixed in osmium tetroxide (in the same phosphate buffer), dehydrated in ethanol/propylene oxide and then embedded in Epon. Simithin sections were cut at 0.75 μm and stained with toluidine blue. Ultrathin sections were stained with uranyl acetate followed by lead citrate.

B. For the demonstration of melanin, parts of the pineal glands from the different experimental groups were fixed in 4% formaldehyde in saline, dehydrated in ascending grades of ethanol, cleared in benzene and embedded in paraffin. Sections were cut at 5 μm and stained with Masson-Fontana silver stain (**Masson, 1914; Fontana, 1925**).

Cell counts were performed on semithin sections using twenty-five randomly chosen high power light microscopic fields (X1000). As the outline of the cells was difficult to distinguish by light microscopy and as pineal cells with more than one nucleus were extremely rare, counts were made of the number of the nuclei in the selected areas.

Measurements were performed on electron microscopic photographs using a scaled magnifying lens.

The obtained data were subjected for statistical analysis. Statistical significance of the differences among the values obtained was tested by the student's "t" test.

RESULTS

The pineal gland was formed of tightly packed cells with very scarce connective tissue and hardly intercellular spaces. In certain areas, however, the intercellular spaces might widen considerably forming intercellular canaliculi (Figs. 1, 2). The parenchymal cells varied in appearance from small cells with little cytoplasm and small darkly stained nuclei to large cells with abundant cytoplasm and large pale nuclei with prominent nucleoli (Fig. 1). Based on certain morphological criteria, two basic populations of pinealocytes were distinguished: pinealocytes of population I (PI) forming 77% of the pineal parenchymal cells and pinealocytes of population II (PII) forming 19% of the cells. In addition, glial cells were found sparsely distributed among the pinealocytes, forming about 4% of the total cell count.

The PI consisted of a cell body and multiple processes of varying size and shape (Figs. 2, 3). The processes were commonly seen extending between adjacent pinealocytes to reach the perivascular spaces or intercellular canaliculi (Fig. 3). The nuclei demonstrated pronounced polymorphism with marked indentations of the nuclear membrane (Figs. 1, 2). They typically demonstrated high electron lucency of their nucleoplasm. The amount of condensed chromatin was usually small. In most cases, the nuclei comprised a conspicuously large prominent nucleolus. The cytoplasm contained relatively large amounts of cell organelles (Figs. 2, 4). Multiple Golgi areas were frequently noticed in the vicinity of the nucleus. Mitochondria measuring $0.45 \pm 0.05 \mu\text{m}$ in diameter (0.33-0.75) were widely distributed in the cytoplasm and extending into the cell processes. The endoplasmic reticulum was generally devoid of ribosomes and typically lacked the tubular profiles. Its major part was formed of vesicles and cisternae of smooth endoplasmic reticulum (Fig. 4). The majority of ribosomes lay free in the cytoplasm either single or groups forming polysomes. Profiles of rough endoplasmic reticulum were found only occasionally. Subsurface cisternae of smooth endoplasmic reticulum closely related to the cell membrane were characteristic feature of this cell type. They were frequently seen confronting similar cisternae of the adjoining cells of PI. Plasma membrane thicken-

ing and fusion might be seen in between. Similar membrane specialization might also be noticed at the margins of omega profiles suggestive of pinocytotic pit. Dense core vesicles, although altogether rare, were detected in almost all cells of this population with a frequency of 1-4 vesicles per cell. Their mean diameter was 267 ± 26.6 nm (range: 197-302 nm). In favorable sections, these vesicles were seen budding out from cisternae of smooth endoplasmic reticulum (Fig. 2-b). Microtubular sheaves, apparently formed of microtubules embedded in a dense matrix, were also noticed in few cases (Fig. 4).

The PII were distinguished by their general ultrastructural features and localization within the parenchyma. They were widely dispersed within the pineal parenchyma with preferential location near blood vessels and intercellular canaliculi (Figs. 1, 5). Their outline was very regular and, in most cases, showed a single basal process. The nuclei were regularly shaped and contained relatively larger amounts of condensed heterochromatin. The cytoplasm displayed somewhat more electron density than that in PI. It contained more tubular profiles of rough endoplasmic reticulum typically lacking or rare in PI. Additionally, the presence of irregular bodies comprising electron dense flocculent material and the absence of dense core vesicles were common features characterizing this cell type. Microfilaments and fibrils were not detected excluding their glial nature.

Glial cells, probably oligodendrocytes, stood out clear among the surrounding cells by their characteristic electron density (Fig. 6). Their outline was very irregular with multiple processes extending between adjacent cells and seemed to follow their contour. They have small, irregular deeply stained nuclei with clearly demarcated heterochromatin. The cell body was characterized by very little cytoplasm with sparse cell organelles. Dense core vesicles and dense bodies identified in PI and PII were typically lacking in glial cells.

Effect of continuous illumination and continuous darkness

Exposure to continuous illumination resulted in a general increase in the PI nuclear packing density (table 1). This was always associated with marked widening of the perivascular spaces and intercellular canaliculi (Fig. 7). Ultrastructurally, PI demonstrated very little cytoplasm containing fewer cell organelles accompanied by larger distribution of lipid droplets (Fig. 8-a). PII, on the other hand, showed darker nuclei comprising larger amounts of heterochromatin. Dense bodies characteristic of this cell type were hardly detected and instead, membrane-bound materials of light to medium electron density were frequently noticed. Most of these membrane-bound materials were found closely related to the cell membrane (Fig. 8-b).

By continuous darkness, on the other hand, there was a decrease in PI nuclear packing density with concomitant narrowing of the perivascular spaces and intercellular canaliculi (Fig. 9; table 1). At the ultrastructural level, large PI comprising abundant cell organelles were observed. An increase in the mitochondrial diameter was particularly noticed (Fig. 10-a; table 2). This increase was found to be statistically very highly significant ($P < 0.0005$). Dense core vesicles, by contrast, showed no significant changes when compared with the control cases. On the other hand, PII demonstrated well-developed Golgi apparatus mostly located in the juxtannuclear cytoplasm. Profiles showing undulations of the nuclear membrane towards the Golgi zone were noticed. In most cases, a material of medium electron density was detected in the perinuclear space at the site of the undulation (Fig. 10-b). In such cases, an abundance of membrane-bound dense bodies was found in the perinuclear cytoplasm. The polymorphism of these dense bodies was impressive. They ranged from small granules up to extremely large membrane-bound floccules. Coincidence of autophagic vacuoles and other lysosomal structures was a common feature. In favorable sections, extremely large dense bodies confronting corresponding bodies of the adjacent cells were observed. Their enclosed materials were apparently continuous across intercellular membrane gaps.

In contrast to PI and PII, it was observed that glial cells did not possess any unusual morphological features under either photoperiods.

Using Masson-Fontana stain for the demonstration of melanin, photoperiod-induced changes were observed in the amount and distribution of melanin pigment within the pineal parenchyma. In control animals, the pigment granules appeared finely distributed through out the parenchyma (Fig. 11-a). No specific localization of such granules was found in any of the glands belonging to this group. Exposure to continuous illumination resulted in marked reduction in melanin content of the pineal parenchyma (Fig. 11-b). Conversely, constant darkness resulted in intensification of the stain where the pigment granules formed clumps in certain specific cells (Fig. 11-c).

Table (1): The effect of different photoperiods on the nuclear packing density of pinealocytes of population I.

Experimental group	Mean \pm SD (Range)	"t" test
a) Control	42.67 \pm 2.9 (38 – 46)	Between a & b S (P<0.01)
b) Continuous illumination	51.4 \pm 11.4 (42 – 59)	
c) Continuous darkness	38.61 \pm 6.2 (30 – 43)	Between a & c S (P<0.05)

Mean = Arithmetic mean of the number of nuclei per high power field.
 SD = Standard deviation.
 S = Significant.

Table (2): The mitochondrial diameter in pinealocytes of population I under different photoperiods.

Experimental group	Mean \pm SD (Range)	"t" test
a) Control	0.45 $\mu\text{m} \pm$ 0.05 (0.33 μm – 0.75 μm)	Between a & b VHS (P<0.0005)
b) Continuous illumination	0.39 $\mu\text{m} \pm$ 0.09 (0.37 μm – 0.44 μm)	
c) Continuous darkness	0.74 $\mu\text{m} \pm$ 0.19 (0.65 μm – 0.98 μm)	Between a & c VHS (P<0.0005)

Mean = Arithmetic mean.
 SD = Standard deviation.
 VHS = Very highly significant.

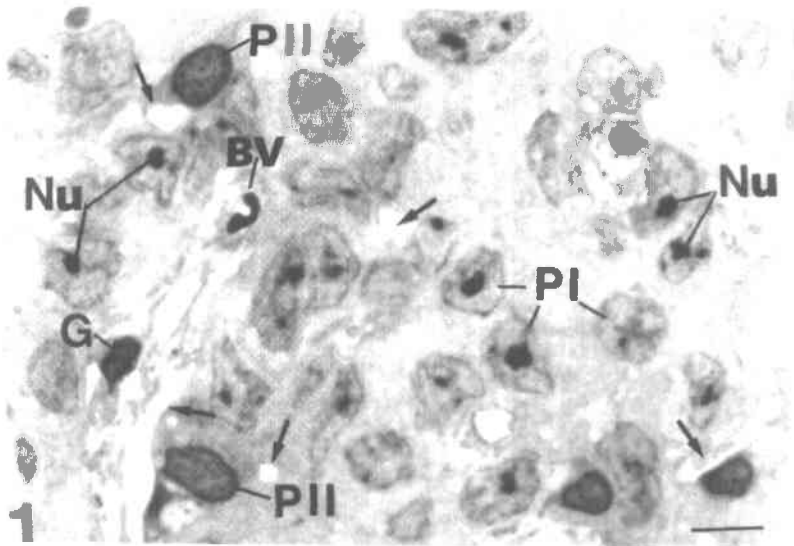


Fig. (1): A photograph of the pineal gland of a control rat showing tightly packed cells with nuclei ranging in appearance from small darkly stained to large pale with prominent nucleoli. In certain areas, the intercellular spaces widen considerably forming intercellular canaliculi (arrows). Two populations of pinealocytes can be distinguished: pinealocytes of population I (PI) forming the major portion of the parenchyma, and pinealocytes of population II (PII) preferentially located near the intercellular canaliculi. Some of these canaliculi are shown connected with the perivascular space surrounding a blood vessel (BV). The cells of population I demonstrate large pale lobulated nuclei with conspicuously large prominent nucleoli (Nu). The cells of population II are distinguished by their regularly shaped darkly stained oval nuclei. The nuclei of the glial cells (G) appear smaller and more intensely stained than the other two cell types.

(Toluidine blue; x 1000; Bar = 10 μ m)

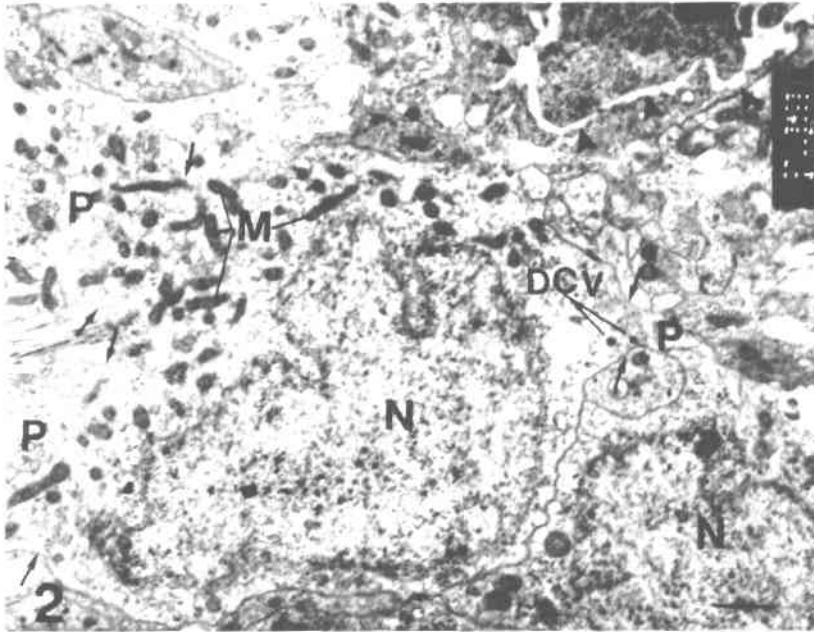


Fig. (2): Electron photomicrograph of the pineal gland of a control rat demonstrating the ultrastructural appearance of a pinealocyte of population I. It consists of a cell body and a number of processes (P) of variable dimensions. The arrows point to the initial segments of these processes. The nucleus (N) shows marked indentation of the nuclear membrane. It displays a typical euchromatin pattern with highly dispersed chromatin. An abundance of mitochondria (M) and few dense core vesicles (DCV) are shown in the cell body and extending into the processes. The intercellular space in the upper part of the picture is apparently widened over a long distance forming an intercellular canaliculus (arrowheads).

(x 6000; Bar = 2 μ m)

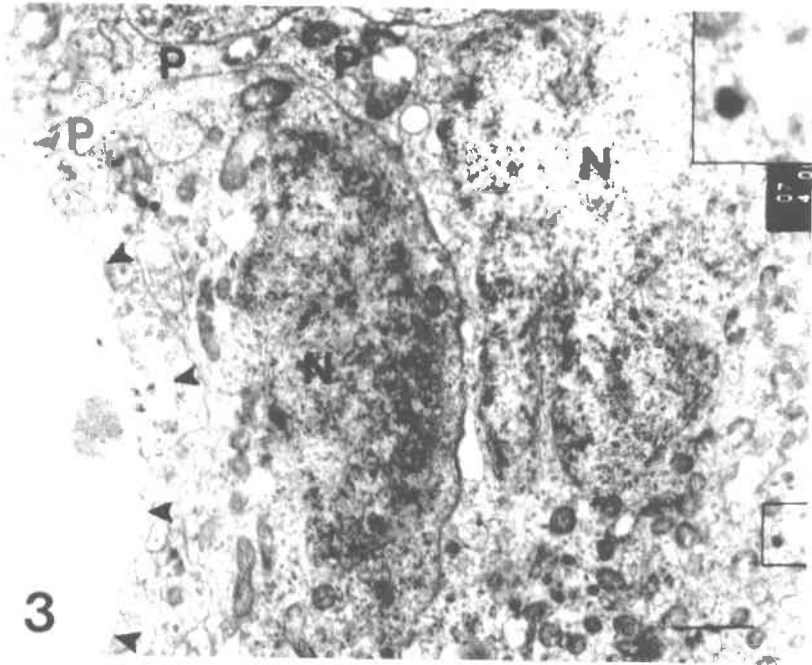


Fig. (3): Electron photomicrograph of the pineal gland of a control rat illustrating a pinealocyte of population I with a characteristic large indented nucleus (N). Its process (P) is shown extending between adjacent cells to reach the perivascular space (arrowheads). A higher magnification of the area enclosed by rectangle (inset) shows a dense core vesicle budding out from a dilated cistern of smooth endoplasmic reticulum.

(x 8000; Bar = 2 μ m)

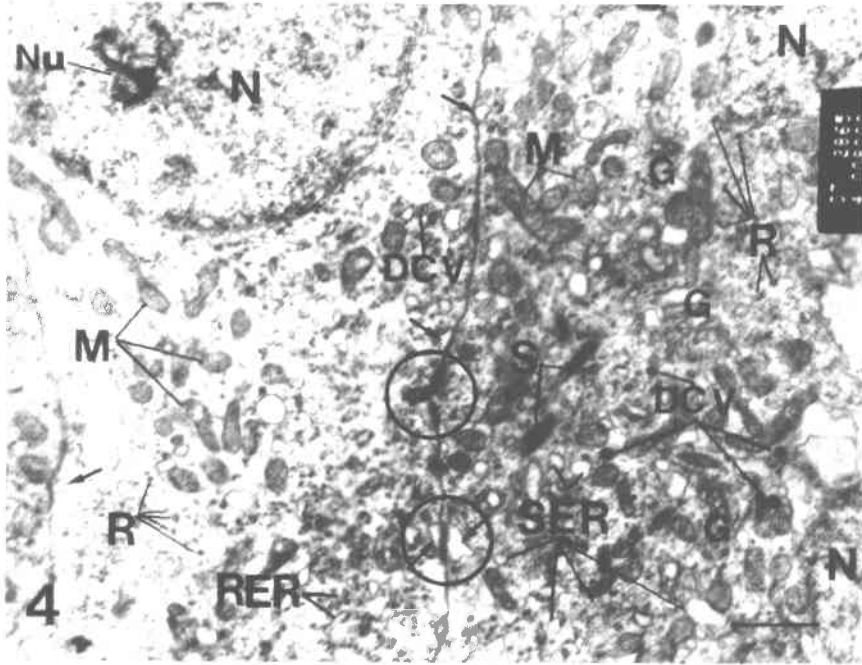


Fig. (4): Electronphotomicrograph of the pineal gland of a control rat showing adjacent pinealocytes of population I. Their cytoplasm comprise large amounts of mitochondria (M), endoplasmic reticulum, and ribosomes (R). The endoplasmic reticulum appears generally devoid of ribosomes. Most of the ribosomes lie free in the cytoplasm either single or groups forming polysomes. The major part of the endoplasmic reticulum is formed of vesicles and cisternae of smooth endoplasmic reticulum (SER). Tubular profiles of rough endoplasmic reticulum (RER) are very scarce. Subsurface cisternae of smooth endoplasmic reticulum (arrows) are shown closely related to the cell membrane. The encircled areas demonstrate an intercellular membrane thickening and fusion between two confronted subsurface cisternae, and a similar membrane specialization at the margins of an omega profile of a pino- or exocytotic pit. Multiple Golgi zones (G) and a number of dense core vesicles (DCV) are also demonstrated. Two microtubular sheaves (S), apparently formed of microtubules embedded in electron dense matrix, can also be seen in this area. Note the euchromatic nucleus (N) and the prominent nucleolus (Nu).

(x 8000; Bar = 2 μ m)

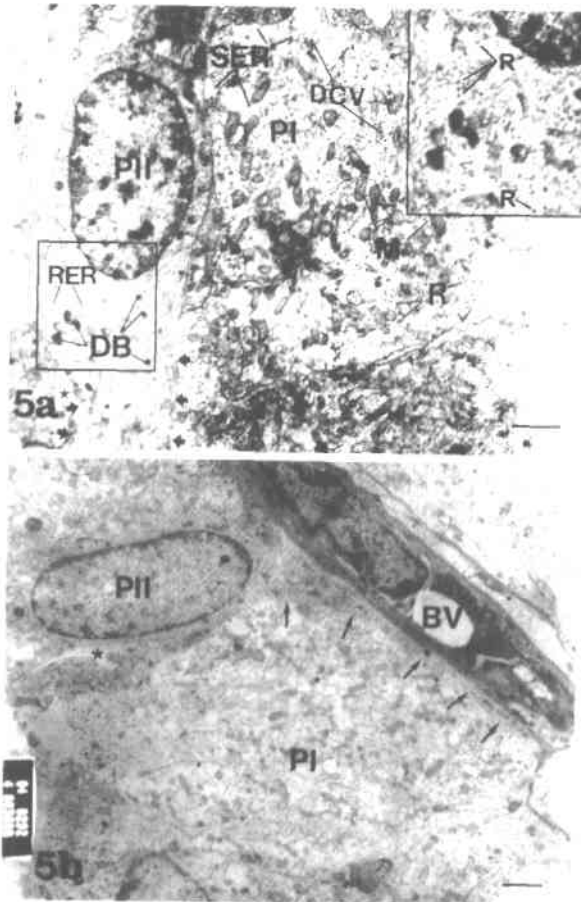


Fig. (5-a, b): Electron photomicrographs of pineal glands of control rats demonstrating the characteristics of pinealocytes of population II (PII). These cells appear closely applied to a blood vessel (BV) or intercellular canaliculi (*). They consist of a regularly shaped cell body and a single process (pointed to by arrows). They contain regularly shaped oval nuclei with relatively larger amounts of heterochromatin. Their cytoplasm shows somewhat more electron density than the surrounding profiles of pinealocytes of population I (PI). Dense bodies (DB) containing electron dense flocculent material, and tubular profiles of rough endoplasmic reticulum (RER) are characteristic features of their cytoplasm. A higher magnification of the area enclosed by rectangle (inset) demonstrates frequent ribosomes (R) and tubular profiles of rough endoplasmic reticulum in the vicinity of the dense bodies. Note the dense core vesicles (DCV), the mitochondria (M) and the smooth endoplasmic reticulum (SER) in the pinealocytes of population I.

(x 4600 in a, 3600 in b; Bar = 3 μ m)

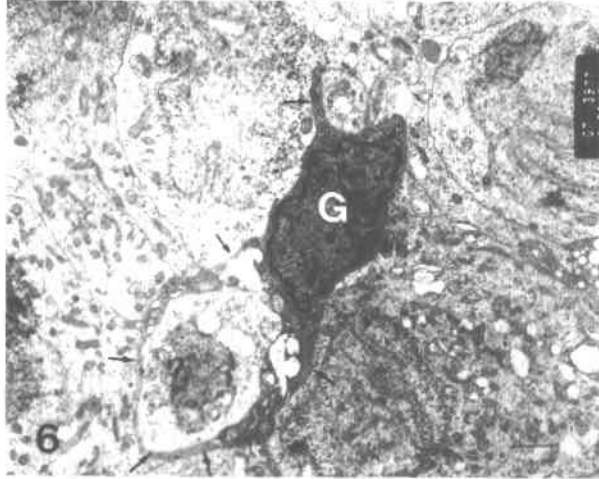


Fig. (6): Electron photomicrograph of the pineal parenchyma of a control rat showing a glial cell (G) standing out clear among the other parenchymal cells. It displays a characteristic electron density contrasting with the surrounding pinealocytes. It has irregular cell body and multiple processes (arrows) extending between and surrounding adjacent pinealocytes. Very little cytoplasm, comprising scarce cell organelles characterizes it. Its irregular deeply stained nucleus demonstrates well-demarcated condensed heterochromatin.

(x 4600; Bar = 3 μ m)

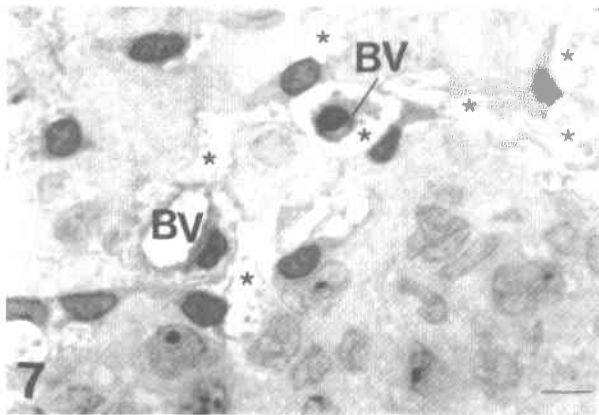


Fig. (7): This photomicrograph demonstrates the appearance of the pineal gland after exposure to continuous illumination. Extreme widening of the perivascular spaces surrounding the blood vessels (BV) and the intercellular canaliculi (*) is characteristic for this experimental group.

(Toluidine blue; x1000; Bar = 10 μ m)

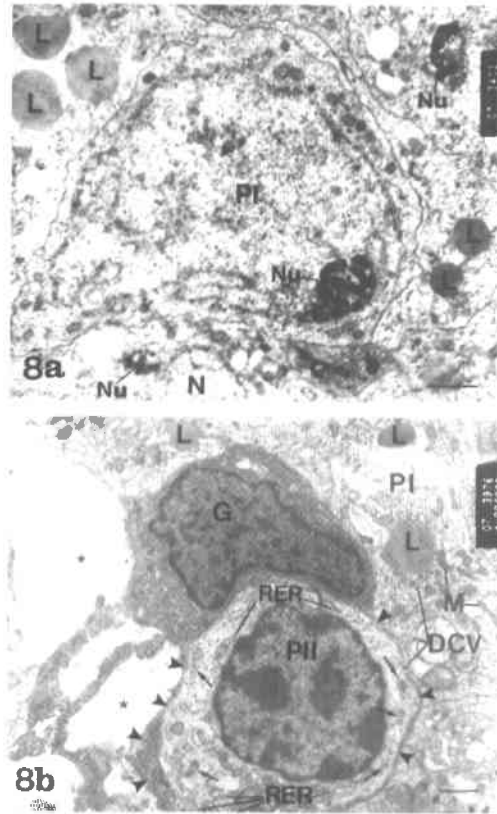


Fig. (8): These electronphotomicrographs demonstrate the ultrastructural appearance of the pineal parenchymal cells after exposure to continuous illumination.

(a) The pinealocytes of population I (PI) demonstrate little cytoplasm containing scarce cell organelles and large lipid droplets (L).

(b) A pinealocyte of population II (PII) and a glial cell (G) are located in the vicinity of an extremely dilated intercellular canaliculus (*). The pinealocyte of population II has a rounded nucleus with larger amounts of condensed heterochromatin. No electron dense bodies can be seen in its cytoplasm. Instead, a membrane-bound material of medium electron density (arrows) is shown closely related to the cell membrane. The glial cell appears more electron dense with irregular outline and multiple processes (arrowheads) embracing the adjoining pinealocyte. No obvious changes can be noticed in its structural appearance when compared with the control (Fig. 5).

Note the nucleus (N), the nucleolus (Nu), the mitochondria (M) and the dense core vesicles (DCV) in pinealocytes of population I, and the rough endoplasmic reticulum (RER) in the pinealocyte of population II.

(x 8000 in a, 6000 in b; Bar = 2 μ m)

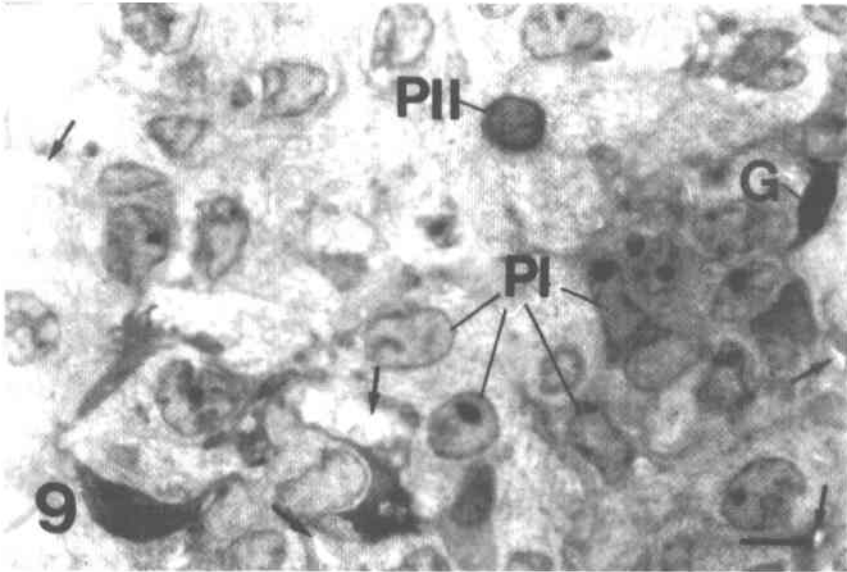


Fig. (9): A photomicrograph of the pineal gland after exposure to constant darkness showing tightly-packed pinealocytes with extreme narrowing of the intercellular canaliculi (arrows). Note the pinealocytes of population I (PI), the pinealocytes of population II (PII) and the glial cells (G).

(Toluidine blue; x 1000; Bar = 10 μ m)

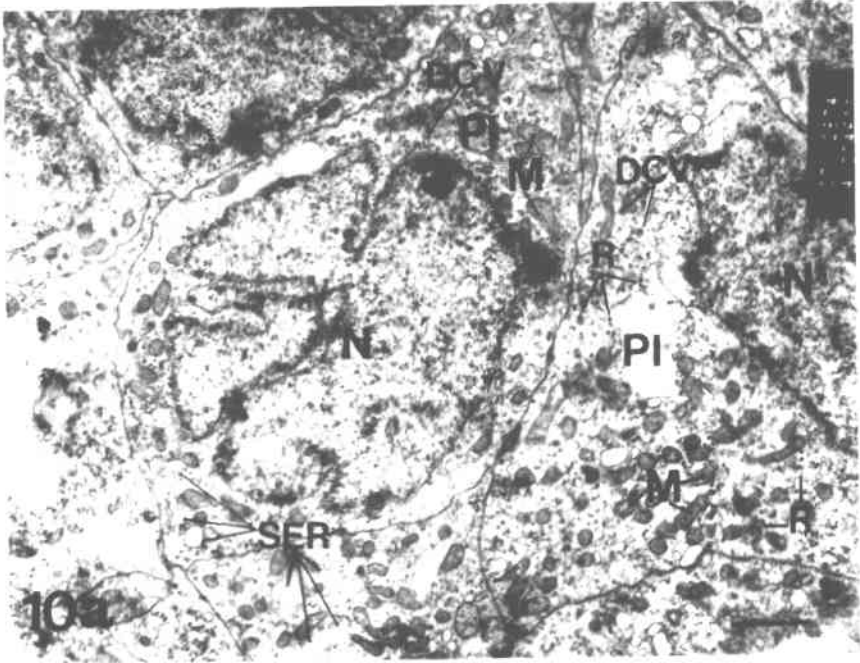
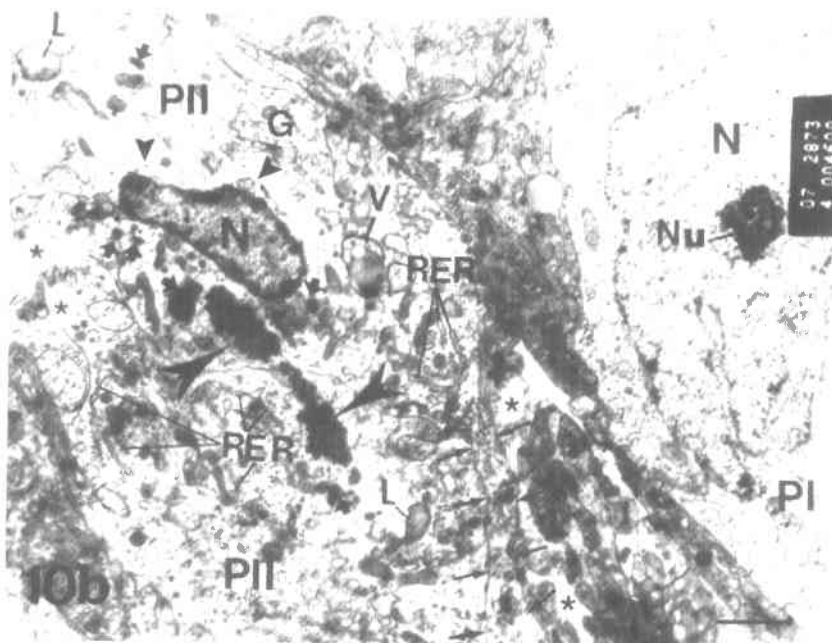


Fig. (10): Electron photomicrographs demonstrating the ultrastructural appearance of pinealocytes of population I and pinealocytes of population II after a period of constant darkness.

(a) The pinealocytes of population I (PI) appear large as compared with the control (Fig. 5). Their deeply indented nuclei (N) demonstrate typical euchromatin pattern with finely dispersed chromatin. The cytoplasm contains abundant ribosomes (R), smooth endoplasmic reticulum (SER), large mitochondria (M) and few dense core vesicles (DCV).

(x 4600; Bar = 3 μ m)



(b) A pinealocyte of population II (PII) is closely related to the intercellular canaliculi (*) and typically demonstrates a cell body with a single slender process (pointed to by thin arrows). Its cytoplasm contains multiple Golgi zones (G) in the vicinity of the nucleus. They are formed of layers of membranous sacs that are distended in most parts. The nuclear membrane shows undulations (small arrowheads) towards the Golgi zones with a material accumulated in the perinuclear space at the sites of the undulation. An abundance of electron dense bodies (thick arrows) are also found in the juxtannuclear cytoplasm. They range from singly lying granules to large membrane-bound irregular floccules. The electron dense material contained in one of these floccules (large arrowhead) is apparently continuous with the material in a corresponding floccule of adjacent cell of the population II. An autophagic vacuole (V) and other lysosomal structures (L) are seen in association with these dense bodies. Note the tubular profiles of rough endoplasmic reticulum (RER) in pinealocytes of population II, and the large prominent nucleolus (Nu) in pinealocyte of population I.

(x 4600; Bar = 3 μ m)

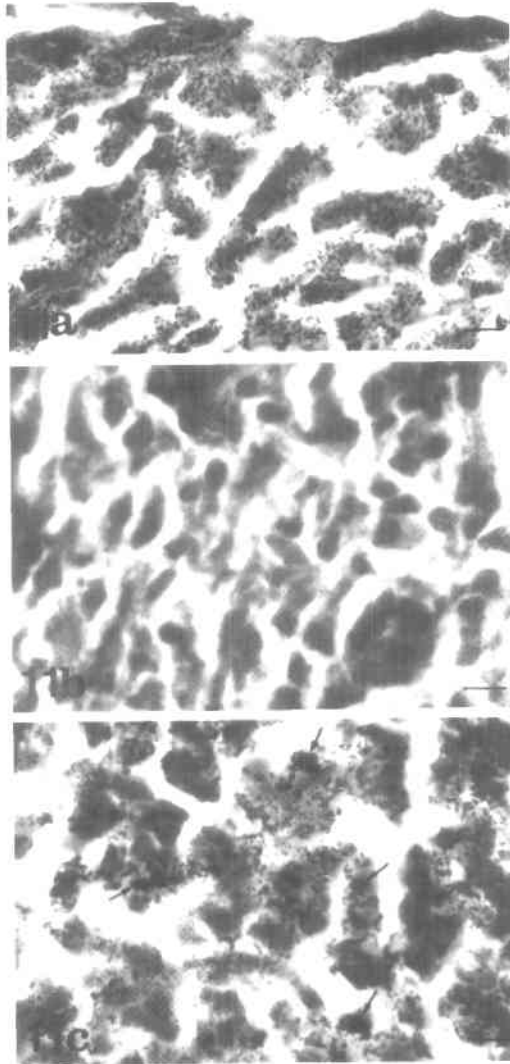


Fig. (11): Photomicrographs showing the amount and distribution of melanin pigment within the pineal parenchyma under different photoperiods.

(a) In control animals, the pigment granules appear finely distributed all-over the parenchyma.

(b) By continuous illumination there is considerable reduction in the staining intensity.

(c) By constant darkness, the stain is intensified and the pigment granules form clumps in certain specific areas (arrows).

(Masson-Fontana; x 1000; Bar = 10 μ m)

DISCUSSION

The pineal gland is an endocrine organ exhibiting circadian changes in biochemical and physiological processes. Its active substances are synthesized and released in a rhythmic fashion during the dark phase of the day-night cycle (**Vollrath, 1981**). In view of the concept that the mammalian pineal gland has a photoreceptor function, besides being an endocrine organ (**Lolly et al., 1992; Blackshaw and Snyder, 1997; Macchi and Bruce, 2004**), it was of particular interest as to whether or not different types of pinealocytes could be distinguished. Based on certain morphological criteria, the present study characterized two basic populations of pinealocytes, namely pinealocytes of population I (PI) and pinealocytes of population II (PII).

The PI formed the major part of the pineal parenchyma amounting to 77% of the parenchymal cells. They were distinguished by their large irregular cell body with multiple processes and large deeply enfolded nuclei. These cells demonstrated certain ultrastructural features suggesting their endocrine nature. Their nuclei displayed the morphological characteristics of metabolically highly active cells. They were typically euchromatic with highly dispersed chromatin and comprised, in most cases, a large prominent nucleolus. The cytoplasm contained abundant cell organelles such as Golgi apparatus, free ribosomes, mitochondria, endoplasmic reticulum, and dense core vesicles. The structural appearance of the endoplasmic reticulum was peculiar for this cell type. It lacked the tubular profiles and was generally devoid of ribosomes. Its major part was formed of vesicles and cisternae of smooth endoplasmic reticulum; a morphological feature known to be characteristic for hormone secreting cells (**Cross and Mercer, 1999**). Profiles showing budding out of dense core vesicles from these cisternae were noticed. This gave indirect evidence for the participation of the smooth endoplasmic reticulum in the formation of dense core vesicles that were considered a morphological correlate of secretory products in the pineal gland (**Vollrath, 1981**). In addition to the above-mentioned organelles, microtubular sheaves were occasionally seen in PI. Their functional significance was, however, obscure. But according to **Vollrath (1981)**, they might play a role in the secretory process of the pinealocytes.

The PII formed a smaller portion of the pineal parenchyma amounting to 19% of the cells counted. The shape, cytoplasmic content, and localization within the parenchyma were sufficiently obvious to be used as criteria for these cells. They have very regular outline and possessed regularly shaped, round to oval nuclei with somewhat larger amounts of condensed heterochromatin. In most cases, a single, relatively narrow process was noticed at one side of the cell body. The cytoplasm of PII lacked dense core vesicles present in PI and instead contained large, irregular bodies comprising electron dense flocculent material. Also, PII demonstrated more

tubular profiles of rough endoplasmic reticulum which were typically lacking or scarce in PI.

The PII were predominantly located near blood vessels and intercellular canaliculi. Previously, pineal cells showing such specific localization have been regarded as astrocytes (Cozzi, 1986; Franco et al., 1997). Actually, these cells differed in many respects from astrocytes. They demonstrated very regular outline and usually possessed a single basal process when compared to the astrocytes which are known to be star-shaped cells with multiple processes (Young and Heath, 2000). In addition, PII contained frequent ribosomes and rough endoplasmic reticulum known to be very scarce in astrocytes. Also, microfilaments and fibrils characteristic of astrocytes (Boya et al., 1995; Young and Heath, 2000) were not detected in PII. Furthermore, in rats, immunohistochemical techniques for the demonstration of glial fibrillary acidic protein specific for astrocytes, revealed that astrocytes were restricted to the pineal stalk (Lopez-Munoz et al., 1992; Pedersen et al., 1993). However, PII described here were found widely dispersed all over the pineal gland.

Glial elements noticed in the present study were most likely oligodendrocytes. They were sparsely distributed among the pinealocytes, and were easily distinguished by a characteristic electron density contrasting with the surrounding cells. In addition, they demonstrated very little cytoplasm with relatively sparse organelles. An important feature was that the outline of these cells was very irregular. Their multiple processes embraced adjacent pinealocytes with regard to their basic function as supportive cells. In this connection, pinealocyte processes extended between adjacent cells to reach the perivascular spaces and intercellular canaliculi, probably to transmit their active principles to the blood stream.

That PII are not astrocytes was further indicated by their specific response to the different lighting conditions, when compared to the glial cells that showed no response under either photoperiod. In PII, a drastic change in the ultrastructural appearance of the electron dense bodies was impressive. Exposure to constant darkness resulted in considerable enlargement and intensification of these dense bodies. In contrast, after a period of continuous illumination, these bodies were hardly detected in PII and, instead, subsurface cisternae comprising a material of light to medium electron density were frequently noticed. Subsurface cisternae, in particular, have been considered a characteristic feature of pinealocytes (Pevet, 1974; Vollrath, 1981; Heinzeller and Tutter, 1991) and were suggested to be involved in transferring materials between adjacent cells (Vollrath, 1981). This suggestion could be confirmed by the present finding that adjoining subsurface cisternae of two adjacent PI might show a membrane specialization in between. Similar membrane specialization was also noticed at the margins of omega profiles suggestive of pino- or exocytotic vesi-

cles. In this connection, large irregular dense bodies of adjoining PII were also seen *confronting each other*. Their enclosed materials were apparently continuous across intercellular gaps. It was possible that these profiles represented areas of transfer of substances between these cells. The particular location of PII in the immediate vicinity of blood vessels and intercellular canaliculi suggested a possible function of this cell type to participate in transmission of secretory products to the blood stream.

In the present study, PI and PII demonstrated different modes of response to the different photoperiods. This gave supportive evidence for the concept that these two cell types had distinct individuality with a functional heterogeneity. As described above, the main response of PII was reflected by a change in the appearance of their dense bodies. In PI, on the other hand, the cell size and the amount of cell organelles were the main parameters that exhibited changes under different photoperiods. By continuous illumination, the cell size decreased significantly as assessed by increased PI nuclear packing density. Ultrastructurally, these cells showed very little cytoplasm, few cell organelles and greater distribution of lipid droplets, all were conspicuous signs of low metabolic activity. By constant darkness, on the other hand, the cell size increased significantly as indicated by decreased PI nuclear packing density. A concomitant narrowing of the perivascular spaces and intercellular canaliculi was noticed, pointing to an enlargement of PI at the expense of these spaces and canaliculi. At the ultrastructural level, extremely large mitochondria were the most obvious structural feature noticed in this experimental group. This was of particular interest owing to the important role of mitochondria in hormone synthesis in the endocrine cells (**Cross and Mercer, 1999**). These authors stated that hormone synthesis resulted from a series of reactions catalyzed by enzymes in the smooth endoplasmic reticulum and mitochondria with shuttling back and forth between these two organelles. Dense core vesicles, showed, however, no significant changes under different photoperiods contradicting the results of **Lewczuk and Przybylska (2000)** and **Lewczuk et al. (2004)** who reported an increase in the number of these vesicles in pig pineals at the dark. But a rarity of secretory granules or dense core vesicles was a characteristic feature of most, if not all, mammalian pinealocytes despite their endocrine nature (**Vollrath, 1981**). According to these authors, pinealocytes do not store large amounts of its secretion and instead, their active principles are more or less for immediate release.

The present concept that the pineal parenchyma constitutes two distinct populations of pinealocytes could be explained on a phylogenetic basis. It is known that the pineal organ of non-mammalian vertebrates is a *directly photosensory organ* containing neuroendocrine photoreceptor cells (**Pevet and Collin 1976; Collin and Ocksche, 1981; Ekström and Meissl, 2003**). As described by (**Ekström and Meissl, 2003**), some of these photoreceptor cells contain dense core vesicles while others contain

dense bodies simulating respectively PI and PII described in the present study. Furthermore, pinealocytes of mammals have evolved through the vertebrate radiation by a gradual loss of the photoreceptor characters and a gradual increase of the neuroendocrine characters (**Pevet and Collin, 1976 and Klein, 2004**). By analysis of the present findings, in the light of the original data, it appeared that PII described in the present study resembled pineal photoreceptors of lower vertebrates. As illustrated by **Pevet and Collin (1976)**, pineal photoreceptor cells of lower vertebrates had regularly shaped cell body with a single basal process and a regularly shaped oval nucleus. With advancing evolution, these photoreceptor cells have gradually been transformed into irregular cells with multiple processes. Their nuclei have become deeply enfolded and attained large prominent nucleoli. All together, these data suggested that PII described in the present study retained some characteristics of their phylogenetic ancestor, the photoreceptor cell of the lower vertebrate. PI, on the other hand, have likely fully-evolved into the conventional neuroendocrine pinealocytes.

The morphological appearance of the dense bodies of PII described in the current study closely resembled melanin pigment. This was more pronounced after exposure to constant darkness where several types of granules were identified, apparently corresponding to different stages of maturation process of this pigment. Active profiles suggesting formation of the dense bodies by the Golgi apparatus were demonstrated. In such profiles, an abundance of dense bodies was noticed in the vicinity of a well-developed Golgi apparatus located in the juxtannuclear cytoplasm. Undulations of the nuclear membrane facing the Golgi area were apparent denoting formation of cisternae or dictyosomes from the nuclear membrane. Coincidence of these features with different lysosomal structures pointed to an active turnover of the material incorporated in the dense bodies. Histochemically, melanin pigment was identified within the pineal parenchyma using Masson-Fontana stain. But almost a uniform distribution of the pigment was demonstrated among the parenchyma of control animals, indicating that melanin was not restricted to specific cell type. Exposure to constant darkness, however, resulted in striking intensification of the stain, where heavy and intense reaction was localized to certain specific cells. A combination of this finding with the morphological appearance of the dense bodies by constant darkness might be taken as index for melanin formation and / or intensification carried by PII at the dark.

The presence of melanin pigment has previously been identified in the pineal gland of other species as dog (**Calvo et al., 1988**), mongolian ground squirrel (**Song and Huang, 1990**), cat (**Calvo et al., 1992**), Japanese quail (**Ohashima and Hiramatsu, 1993**), brown bat (**Bhatnagar and Hilton, 1994**) and ovine (**Regodon et al., 1998**). In humans, melanotic tumors of the pineal gland has also been reported in the literature (**Hunt and Johnson, 1989; Ogata et al., 1989; Suzuki et al., 2001**). Histological ex-

amination of these tumors revealed atypical cells with scanty melanin pigment, pointing to the presence of melanin containing cells in the human pineal gland as well.

The exact functional significance of melanin pigment in the pineal gland is largely unknown. However, using molecular biological methods, melanopsin, a melanin-based photopigment, has recently been identified in the pineal glands of fish, chicken as well as rodents (**Philp et al., 2000; Bailey and Cassone, 2004**). Its cellular localization, however, has not yet been defined. Melanopsin has been proposed to be a non-rod, non-cone photopigment mediating different responses to light (**Gooley et al., 2003; Foster et al., 2003-b**). Considering that PII represented reminiscent photoreceptor cells containing melanin, it could be suggested that this cell type might play a role in non-visual photoreception that contributes to the circadian rhythm in pineal gland function.

In conclusion, the rat pineal gland has two distinct populations of pinealocytes with a possible functional heterogeneity. Pinealocytes of population I could be regarded as neuroendocrine cells responsible for the formation and secretion of pineal hormones. Pinealocytes of population II were suggested to be an endogenous candidate involved in the non-visual phototransduction mechanism that may play a role in photic regulation of circadian function in this organ.

SUMMARY

The cell types of the pineal parenchyma were investigated to determine whether or not different types of pinealocytes could be distinguished on a morphological ground. Three groups of male albino rats were examined. Group 1 (control) were kept under a cycle of 14 hrs light and 10 hrs dark per day, group 2 were exposed to continuous light, while group 3 were exposed to continuous darkness. The animals were kept under these conditions for four weeks.

Based on certain morphological criteria, three basic cell types of the pineal parenchyma were characterized:

- (1) Pinealocytes of population I were distinguished by their irregular cell bodies with multiple processes, and large deeply enfolded nuclei with large prominent nucleoli. The nuclei demonstrated typical euchromatin pattern with highly dispersed chromatin. The cytoplasm comprised abundant cell organelles. The appearance of the endoplasmic reticulum was characteristic for this cell type. They were largely devoid of ribosomes and typically lacked the tubular profiles. The major part was formed of vesicles and cisternae of smooth endoplasmic reticulum. Dense core vesicles, although not frequent, were peculiar to this cell type.

- (2) Pinealocytes of population II were preferentially located near blood vessels and intercellular canaliculi. They demonstrated regularly outlined cell bodies with a single process and contained regularly shaped nuclei comprising larger amounts of condensed heterochromatin. The presence of irregular electron dense bodies, ranging from small granules to large membrane-bound floccules, and the absence of dense core vesicles were characteristic features of their cytoplasm.
- (3) Glial cells were very irregular cells with multiple processes embracing the surrounding pinealocytes. They were characterized by very little cytoplasm comprising scarce cell organelles. Dense core vesicles and dense bodies identified in pinealocytes were typically lacking in glial cells.

Exposure to different photoperiods yielded different responses among these three cell types suggesting a functional heterogeneity. In pinealocytes of population I, the cell size and mitochondrial diameter were the morphological parameters exhibiting obvious changes under different photoperiods. The response of pinealocytes of population II, on the other hand, was mainly expressed by drastic changes in the amount and intensity of the electron dense bodies. Glial cells, in contrast, showed no response under either photoperiod.

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الملخص العربى

التركيب الخلوي للحمة الغدة الصنوبرية في الفأر الأبيض:

تأثير التعرض للأنظمة الضوئية المختلفة

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يهدف هذا البحث إلى دراسة الخلايا المكونة للحمة الغدة الصنوبرية تحت تأثير فترات مختلفة من الضوء والظلام كوسيلة لمعرفة الدور الوظيفى لكل منها . وقد أجريت هذه الدراسة على ذكور الفئران البيضاء التى تم تقسيمها الى ثلاث مجموعات. المجموعة الأولى استخدمت كمجموعة ضابطة وتم إبقاؤها على دورة منتظمة مكونة من أربعة عشرة ساعة إضاءة وعشر ساعات ظلام يوميا والمجموعة الثانية تم تعريضها إلى إضاءة دائمة أما المجموعة الثالثة فقد تعرضت للظلام الدائم. وقد أقيمت هذه المجموعات الثلاثة تحت تأثير هذه الظروف الضوئية لمدة أربعة أسابيع.

وبالفحص المجهرى الدقيق للحمة الغدة فقد أمكن تمييز نوعين من الخلايا الصنوبرية. النوع الأول تميز بالشكل غير المنتظم مع وجود زوائد خلوية عديدة. وتميزت أنوية هذا النوع من الخلايا بكبر حجمها واحتوائها على الكروماتين الفعال بالإضافة الى وجود نوية كبيرة وكذلك تموجات وتعرجات واضحة فى غشائها النووى. أما السيئوبلازم فقد احتوى على الكثير من العضيات السيتوبلازمية وبالأخص فقد أظهرت الشبكة الإندوبلازمية بعض الصفات المورفولوجية التى ميزتها عن النوع الثانى من الخلايا الصنوبرية حيث كانت هذه الشبكة تفتقد إلى حد كبير الأجسام الريباسية مع ندرة واضحة فى الشكل الأنبوبى لها وبذلك فقد تمثلت فى حويصلات و صهاريج إندوبلازمية ملساء . بالإضافة الى ذلك فقد لوحظ وجود حويصلات داكنة المركز والتي بالرغم من ندرتها فقد كانت من العضيات الأساسية المميزة للنوع الأول من الخلايا الصنوبرية دوناً عن غيرها من الخلايا.

أما النوع الثانى من الخلايا الصنوبرية فقد كان متمركزاً بالقرب من الأوعية الدموية وكذلك الغنبيات البينية للخلايا . وكانت هذه الخلايا منتظمة الشكل مع وجود

زائدة خلوية واحدة فى معظم الأحيان. وكانت أنوية هذه الخلايا منتظمة الشكل مع احتوائها على كم أكبر من الكروماتين المغاير. وكان غياب الحويصلات داكنة المركز ووجود جسيمات داكنة غير منتظمة تراوحت من حبيبات متعددة الأحجام إلى نديفات محاطة بغشاء من الصفات الهامة التى ميزت هذا النوع من الخلايا.

وبالإضافة إلى النوع الأول و النوع الثانى من الخلايا الصنوبرية فقد احتوت لحمة الغدة على الخلايا الدبقية التى أظهرت كثافة الكترونية عالية ميزتها عن الخلايا الصنوبرية المحيطة بها . كما كانت هذه الخلايا الدبقية غير منتظمة الشكل مع وجود زوائد خلوية عديدة امتدت بين الخلايا الصنوبرية المجاورة لها مكونة غلافا حولها فى معظم الأحوال . بالإضافة إلى ذلك فقد أظهرت الخلايا الدبقية قلة ملحوظة فى السيترولازم المحيط بالنواة مع ندرة فى العضيات السيترولازمية. وكان غياب كل من الحويصلات داكنة المركز و الجسيمات الداكنة من الخصائص الهامة لسيترولازم هذا النوع من الخلايا .

وبتعريض الفئران إلى أنظمة ضوئية مختلفة فقد أظهرت هذه الأنواع الثلاثة من الخلايا تباينا ملحوظا فى نمط استجابتها للضوء والظلام مما دل على اختلاف الدور الوظيفى لكل منها. أما النوع الأول من الخلايا الصنوبرية فكان حجم الخلية وكذلك حجم الميتوكوندريا من أهم المعايير التى أظهرت استجابة واضحة. وبالنسبة للنوع الثانى من الخلايا الصنوبرية فقد أظهرت الجسيمات الداكنة تغيرا ملحوظا تحت تأثير التعرض للأنظمة الضوئية المختلفة. أما الخلايا الدبقية فقد كان تركيبها المورفولوجى ثابتا فى المجموعات التجريبية الثلاث مما دل على عدم استجابتها لأنظمة الضوء المختلفة.