Egyptian Journal of Anatomy, Jan. 2017; 40(1):54-74

Original Article	**Changes in Pinealocytes type I and II of Adult Albino Rat Exposed to Different Wavelengths of Light or Confined to Constant Darkness: A Morphological and Electron Microscopic Study**

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ABSTRACT

Introduction: the pineal gland is a neuro-endocrine gland that secretes melatonin hormone which regulates the circadian rhythm, psychiatric disorder and age changes resulting from exposure to different light wavelengths or darkness. Intensity, duration and wavelength of light control the activity of pineal gland.

Aim of work: the purpose of this study was to monitor the morphological and electron microscopic changes in the pinealocytes type I and II of albino rats exposed to either constant (24hrs/day) red light (long wavelength) or blue light (short wavelength) as well as confined to constant darkness as compared to control group subjected to normal diurnal conditions.

Material and methods: forty adult male albino rats were divided into four subgroups. Group I (GI): the rats were kept under normal diurnal conditions, group II (GII): the rats were confined to constant darkness, group III (GIII): the rats were exposed to constant red light (long wavelength; 670nm) and group IV (GIV): the rats were subjected to constant blue light (short wavelength, 450nm); for four weeks continuously.

Results: morphological findings of pinealocytes type I (PI) were analyzed statistically and revealed an increase in the nuclear number of PI in GII, decrease in the nuclear number of PI in GIV and non-significant difference in nuclear number of PI in GIII as compared to the control group. In addition, rats of GIV reported a statistically significant increase in the amount of collagen fibers between loosely packed parenchymal cells. Electron microscopic results of the cytoplasm of PI in GII showed abundant cell organelles such as mitochondria, numerous ribosomes and multiple lipid droplets together with well-developed Golgi bodies, scarce dense core vesicles (DCV), few cisternae of smooth endoplasmic reticulum (SER) and rough endoplasmic reticulum (RER). GIII demonstrated similar results to that of GI. On the other hand, GIV showed scarce cytoplasmic organelles such as few mitochondria; mostly distended with disturbed cristae, scattered lipid droplets with abundant DVC and numerous cisternae of SER. Ultrastructurally, PI nuclei of GII exhibited infolded nuclear envelope with heterochromatic karyoplasm and prominent nucleoli while GIV revealed reduction of the nuclear chromatin with rupture of nuclear envelope at certain sites. However, GIII nuclei demonstrated results similar to that of GI. No observable changes were detected by light and electron microscopy of PII in the different experimental groups apart from the presence of statistically significant increase in the nuclear number of PII in GII. Moreover, the PII cytoplasm showed scarce mitochondria in GI, II and RER in GIV. The glial cells demonstrated statistically significant increase in GIV but non-significant differences were noticed in the other groups as compared to GI.

Conclusion: it could be concluded that the morphological and electron microscopic changes of pinealocyte type I indicate decrease in the activity of pinealocytes type I on exposure to constant short wavelength blue light and increase in the activity of PI confined to constant darkness. While the exposure to constant long wavelength red light demonstrates results similar to the control group. There were no changes in the structure of pinealocytes type II in the different experimental groups, exposed to different light wavelengths or confined to darkness.

Key Words: Pineal gland, wavelengths of light; morphological; darkness; electron microscopic study.

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INTRODUCTION

The pineal gland is a pine cone-shaped neuroendocrine gland that regulates diurnal rhythm (*Ross and Pawlina, 2011*). It is located in the midline outside the blood-brain barrier (*Macchi and Bruce, 2004*) and covered by pia mater layer (*Young et al., 2006*). The gland is divided into lobules of different sizes by the penetrating meningeal septa (*Gartner and Hiatt, 2007*).

The pineal gland receives information about light and dark from the retina via the retino-hypothalamic pathway which reaches the gland via sympathetic nerve fibers by being converted into electrical impulses in the photoreceptors of retina (*Ganguly et al., 2002*). Through this pathway, light regulates the activity of pineal gland and secretion of its main hormone, melatonin which controls the circadian rhythm; broad spectrum light reduces melatonin production while darkness stimulates its production (*Korf et al., 2003*).

The frequency of visible light (interval not color), determines the color sensation. The daylight contains all the wavelengths of the color spectrum which known for albino animal species as photopic (black/white) and scotopic (colored vision) electro-retinograms (*Szel and Rohlich, 1992*). Intensity, duration and wavelength of light control the activity of pineal gland (*Ross and Pawlina, 2011*).

The main cell types of the mammalian pineal gland are the melatonin synthesizing pinealocytes, interstitial cells located between the pinealocytes and few perivascular phagocytes (*Møller et al., 2002*).

Some studies demonstrated increase in the metabolic and synthetic activity of pinealocytes in some species as reported by electron microscopic changes of these cells (*Dominguez et al., 1987; Bhatnagar, 1992*). Although it is known that constant darkness exposure mostly enhances the morphology of pineal gland and its melatonin production (*Kus et al., 2004*), little knowledge is available regarding the morphological and ultrastructural changes in the pinealocytes on exposure to different light wavelengths; long (red), short (blue) or confined to constant darkness.

The aim of this study was to monitor the effect of constant exposure to red illumination (long wavelength, 670nm) and blue illumination (short wavelength, 450nm) as well as constant darkness, on the morphology and ultrastructure of pinealocytes in adult rat as compared to rats exposed to normal diurnal rhythm.

AIM OF THE WORK

A- Experimental animals:

Forty adult male albino rats (Sprague Dawely) weighing 200±30g were obtained from animal house of Faculty of Medicine, Kasr Al-Ainy, Cairo University, Egypt. The rats were housed in well ventilated cages (5 rats per cage), each cage measured 40 cmx 25cm x 20 cm.

B- Experimental design:

The animals were randomly segregated into four equal groups (10 rats each); group I (control): the rats were kept under standard diurnal conditions (10h of light, 14h of darkness), group II: the rats were confined to constant darkness, group III: the rats were constantly exposed to red illumination (long wavelength, 670nm) and group IV: the rats were constantly exposed to blue illumination (short wavelength, 450nm) (Aral et al., 2006) for four weeks continuously. The different light wavelengths were obtained by a light bulb (40 Watt and 220 Volt).

In the present work, all animals were allowed free access to food and water. All aspects of the research were complied with protocols approved by Local Ethical Committee of Faculty of Medicine, Kasr Al-Ainy, Egypt.

For constant darkness, the animals were kept in a dark room and the animal cage was covered with thick black cloth allowing fresh air to circulate. While the different lights were applied by putting constant corresponding coloured light bulb above the animal cage at a distance of 4 inches with a heat filter in between.

C- Methods:

- Histological study

After four experimental weeks, all rats were sacrificed between 11am to 12 noon by deep anesthesia with i.p. injection of pentobarbital sodium (60 mg/kg body weight) (*Gaertner et al., 2008*), followed by cervical dislocation. In five animals from each group, the pineal gland was excised In Toto and then immediately fixed in 4% buffered formalin solution overnight at room temperature. Tissue samples were dehydrated in ascending grades of ethyl alcohol (50%, 70%, 90%) for 24hrs, cleared in xylol and embedded in paraffin blocks; first on hot plate oven for 24hrs at 60◦C to assure penetration of wax between particles then for 2-3hrs in refrigerator. Sections of 5µm thickness were sliced, mounted on glass slide then stained with hematoxylin and eosin to study the architectural pattern of the pineal gland and Masson's trichrome stain was done to study the distribution of collagen bundles and their thickness within the gland.

- Electron microscopic study

Fresh microspecimens of the pineal glands were excised and immediately fixed in 2.5% gluteraldhyde at 4°C for 2hrs for electron microscopy. The specimens were post-fixed in 2% osmium tetraoxide, dehydrated in ascending grades of ethanol/propylene oxide and then embedded in Epon. Semithin sections were cut at 0.5µm and stained with toluidine blue to localize the site of lesion for electron microscopy monitoring. Ultrathin sections (70-80nm) were mounted on copper grids and double stained with uranyl acetate followed by lead citrate then examined by Zeiss 100S transmission electron microscopy.

- Image analysis

The mean area percent of collagen bundles in Masson's trichrome-stained sections as well as the nuclear number of pinealocytes type I, pinealocytes type II and glial cells in semithin sections were measured in 10 non-overlapping high power fields. The measurements were taken using Leica Qwin 500 LTD image analyzer computer system (software Qwin 500, Cambridge, UK).

- Statistical analysis

The obtained data from image analyzer were presented as mean (± SD). Paired sample t-test was used for comparison of means of different groups, using SPSS version 20 (SPSS Inc., Chicago, IL). The significance between variables was considered when p value < 0.05.

RESULTS

A- Light microscopic examination:

- Group I (GI-control)

The pineal gland of GI consisted of cell plates within parenchymal lobules, incompletely separated from each other by fine connective tissue septae (Figs. 1A-C). The pinealocytes form most of cells of pineal gland which appeared tightly packed (Figs. 1A,B). Pinealocytes type I (PI) constituted the major proportion of other cells e.g. pinealocytes type II and glial cells (Fig. 1A) and demonstrated pale cytoplasm surrounding large, euchromatic nuclei with prominent nucleoli (Fig. 1B). Pinealocytes type II (PII) located near the perivascular spaces and containing a very thin rim of cytoplasm with small darkly-stained nuclei (Fig. 1B). Scattered glial cells were seen among pinealocytes, distinguished by their scanty cytoplasm surrounding small deeply-stained elongated nuclei (Figs. 1A,B). Blood vessels (Figs. 1A,B) surrounded by perivascular spaces were distributed in the gland within the septa between the lobules, while blood capillaries were seen between the parenchymal cells inside the pineal lobules (Fig. 1A). Masson's trichrome-stained sections revealed fine collagen fibers between plates of the parenchymal cells as well as in the interlobular septae (Fig. 1C).

- Group II (GII-constant darkness)

The pineal gland of GII revealed plates of abundant tightly packed pinealocytes surrounding PII within the parenchymal lobules (Figs. 2A,B). PI demonstrated cytoplasm that surround heterochromatic nuclei with nucleoli (Figs. 2A,B). PII contained small dark nuclei (Figs. 2A,B). Glial cells were infrequently encountered between pinealocytes (Figs. 2A,B), characteristically showed very small, darkly-stained nuclei (Fig. 2B). Blood vessels were

seen within interlobular septa (Figs. 2A,B) while blood capillaries were observed between cells (Fig. 2A). There was moderate amount of collagen fibers separating plates of closely packed cells within the parenchyma and in septa between the lobules (Fig. 2C).

-Group III (GIII-constant long wavelength red illumination)

Sections from GIII demonstrated plates of almost packed pinealocytes (PI, PII) within the parenchymal lobules (Figs. 3A,B). PI demonstrated large euchromatic nuclei with prominent nucleoli, while PII enclosed small dark nuclei (Fig. 3B). Few glial cells (Fig. 3A) appeared between parenchymal cells. Blood vessels (Figs. 3A,B) were encountered between the lobules surrounded by perivascular spaces (Fig. 3B), while blood capillaries (Fig. 3A) were noticed between the parenchymal cells. There were few amounts of collagen fibers between plates of packed parenchymal cells as well as in interlobular septa (Fig. 3C).

-Group IV (GIV-constant short wavelength blue illumination)

GIV showed plates of loosely packed of both types of pinealocytes within the parenchymal lobules (Figs. 4A,B). PI demonstrated nuclei with reduced chromatin; some having prominent nucleoli, while PII revealed small dark nuclei (Fig. 4B). A number of glial cells appeared between parenchymal cells (Fig. 4A). Blood vessels (Figs. 4A,B) between the lobules as well as numerous blood capillaries (Fig. 4A) between the parenchymal cells were also observed. There were abundant bundles of collagen (Fig. 4C) between loosely packed parenchymal cells.

B- Electron microscopic examination:

-Group I

Ultrastructurally, the pineal gland of GI demonstrated PI with irregular outline with cytoplasmic processes extending between the cells as far as the perivascular spaces (Figs. 5A,B). The cytoplasm of PI showed multiple cell organelles including intact cristal pattern of mitochondria (Figs. 5A-D) and free ribosomes (Fig. 5D). Scattered rough endoplasmic reticulum (RER) (Figs. 5A-D) and cisternae of smooth endoplasmic reticulum (SER) (Fig. 5D) were also encountered. Dense core vesicles (DCV) appeared in contact with the cell membrane (Fig. 5B). The PI nuclei revealed infoldings of the nuclear envelope, euchromatic karyoplasm and prominent nucleoli (Figs. 5A,B); some exhibited double nucleoli (Fig. 5A).

PII were seen as small regular-outline cells adjacent to the perivascular space (Figs. 5A,B). Their cytoplasm showed mitochondria (Figs. 5C,D), cisternae of RER (Fig. 5C), free ribosomes (Fig. 5D). PII demonstrated oval heterochromatic nuclei (Figs. 5A,C,D) with indented nuclear envelop (Fig. 5A).

-Group II

Pinealocytes type I showed multiple cytoplasmic processes (Figs. 6A-C). Their cytoplasm contained abundant cell organelles; numerous mitochondria appeared either well-defined (Figs. 6A-E) or distended with disturbed cristae (Figs. 6B,C). Abundant free ribosomes (Figs. 6B,E), RER (Fig. 6E), well developed GB (Figs. C,E), scattered cisternae of SER (Figs. 6C-E) were also observed. Multiple lipid droplets (Fig. 6D) and few dense core vesicles (DCV) (Figs. 6B,D) were also seen. The nuclei exhibited heterochromatic karyoplasm (Figs. 6A,B,E), prominent nucleoli (Figs. 6A,B) and sometimes double (Fig. 6B) and deep infoldings of nuclear envelope (Figs. 6B,D). PII were encountered adjacent to blood vessels and perivascular spaces (Figs. 6A,C). Their cytoplasm showed mitochondria (Fig. 6C). Their nuclei appeared indented (Fig. 6A) and exhibited heterochromatic karyoplasm (Figs. 6A,C).

-Group III

Pinealocytes type I showed cytoplasmic processes (Figs. 7A,D) and their cytoplasm contained multiple well-defined mitochondria (Figs. 7B-E). Free ribosomes, RER (Fig. 7C) and cisternae of SER (Figs. 7A,D,E) were also observed in the cytoplasm. DCV (Figs. 7A,C,E) and brain sand (Fig. 7D) were also encountered. The nuclei revealed infoldings of nuclear envelope (Figs. 7A,C), prominent nucleoli (Fig.7A) and euchromatic karyoplasm (Figs. 7A,C,D,E). PII were identified by their

heterochromatic nuclei (Figs.7A,B,D) located in contact with perivascular spaces (Fig. 7B).

-Group IV

Pinealocytes type I showed cytoplasmic cell processes (Figs. 8A,B) extending to widened perivascular spaces (Figs. 8B,C). Their scanty cytoplasm contained scarce organelles (Figs. 8B-E). Few number of mitochondria appeared either intact (Fig. 8D) or distended with disturbed cristae (Fig. 8E). Abundant cisternae of SER (Fig. 8B) and numerous DCV (Figs. 8A,B,E) were also encountered free in the cytoplasm. Scattered lipid droplets (Figs. 8B,C,E) were seen. The nuclei demonstrated reduced amount of chromatin (Figs. 8A-E). The nuclear envelope ruptured causing leakage of chromatin into the cytoplasm (Figs. 8B,C,E). Some nuclei exhibited prominent nucleoli (Figs. 8B,E). PII appeared closely related to widened perivascular spaces (Figs. 8A,B). Their cytoplasm showed mitochondria (Fig. 8C), RER and lipid droplet (Fig. 8D).Their nuclei exhibited heterochromatic karyoplasm (Fig. 8A,C,D).

C- Statistical results:

The mean area percent of collagen fibers revealed a statistically significant increase (p<0.05) in group IV (exposed to constant blue illumination) as compared to control group. On the other hand, there was statistically non-significant difference (p>0.05) neither in group GII (confined to constant darkness) nor GIII (exposed to constant red illumination) as compared to control group (Table 1).

In group II (confined to constant darkness), there was a statistically significant (p<0.05) increase in the nuclear number of pinealocytes type I as compared to control group. Additionally, group IV (exposed to constant blue short wavelength) showed a statistically significant decrease in nuclear number of pinealocytes type I as compared to control group. However, group III (exposed to constant red long wavelength) revealed statistically non-significant difference (p>0.05) in the number of nuclei of pinealocytes type I as compared to the control group (Table 2).

As shown in (Table 3), there was a statistically significant (p<0.05) increase in the nuclear number of PII in GII (confined to constant darkness) as compared to control group. However, there was statistically non-significant difference (p>0.05) in the nuclear number of PII neither in group III (exposed to constant red illumination) nor in group IV (exposed to constant blue illumination) as compared to the control group.

(Table 4) revealed a statistically significant (p<0.05) increase in the nuclear number of glial cells in in group IV (exposed to constant blue illumination) as compared to the control group. However, there was statistically non-significant difference (p>0.05) in the nuclear number of glial cells neither in group II (confined to constant darkness) nor in group III (exposed to constant red illumination) as compared to the control group.

Table 1: The mean area percent of collagen fibers in all experimental groups

Groups		Mean±SD	SEM	Mean difference	*p* value
Pair 1	Control	4.38±0.679	.226	.40	0.843
	Darkness	3.97±1.057	.352		
Pair 2	Control	4.38±0.679	.226	-.17	0.984
	Red	4.55±.821	.273		
Pair 3	Control	4.38±0.679	.226	-11.04	0.000*
	Blue	15.42±4.986	.479		

SD: standard deviation; SEM: standard error of mean; *: statistically significant (p< 0.05)

Table 2: Nuclear number of pinealocytes type I in all groups

Groups		Mean±SD	SEM	Mean difference	*p* value
Pair 1	Control PI	20.20±9.283	2.855	-3.2	0.000*
	Darkness PI	23.40±9.008	2.848		
Pair 2	Control PI	20.20±9.283	2.855	4.9	0.197
	Red PI	15.30±8.3937	2.654		
Pair 3	Control PI	20.20±9.283	2.855	9.4	0.020*
	Blue PI	10.80±3.457	1.093		

SD: standard deviation; SEM: standard error of mean; *: statistically significant ($p< 0.05$); PI: pinealocytes type I.

Table 3: Nuclear number of pinealocytes type II in all experimental groups

Groups		Mean±SD	SEM	Mean difference	*p* value
Pair 1	Control PII	6.80±1.932	.611	-1.50	0.034*
	Dark PII	8.30±1.494	.472		
Pair 2	Control PII	6.80±1.932	.611	2.1	0.142
	Red PII	4.70±3.400	1.075		
Pair 3	Control PII	6.80±1.932	.611	1.1	0.399
	Blue PII	5.70±2.710	.857		

SD: standard deviation; SEM: standard error of mean; PII: Pinealocytes type II; *: statistically significant ($p< 0.05$).

Table 4: Nuclear number of glial cells in all experimental groups

Groups		Mean±SD	SEM	Mean difference	*p* value
Pair 1	Control G	2.80±1.175	.553	0.3	0.591
	Dark G	2.50±1.178	.686		
Pair 2	Control G	2.80±1.175	.553	0.4	0.599
	Red G	2.40±1.429	.512		
Pair 3	Control G	2.80±1.175	.553	-2.7	0.004*
	Blue G	5.50±2.121	.670		

SD: standard deviation; SEM: standard error of mean; G: glial cells; *: statistically significant ($p< 0.05$)

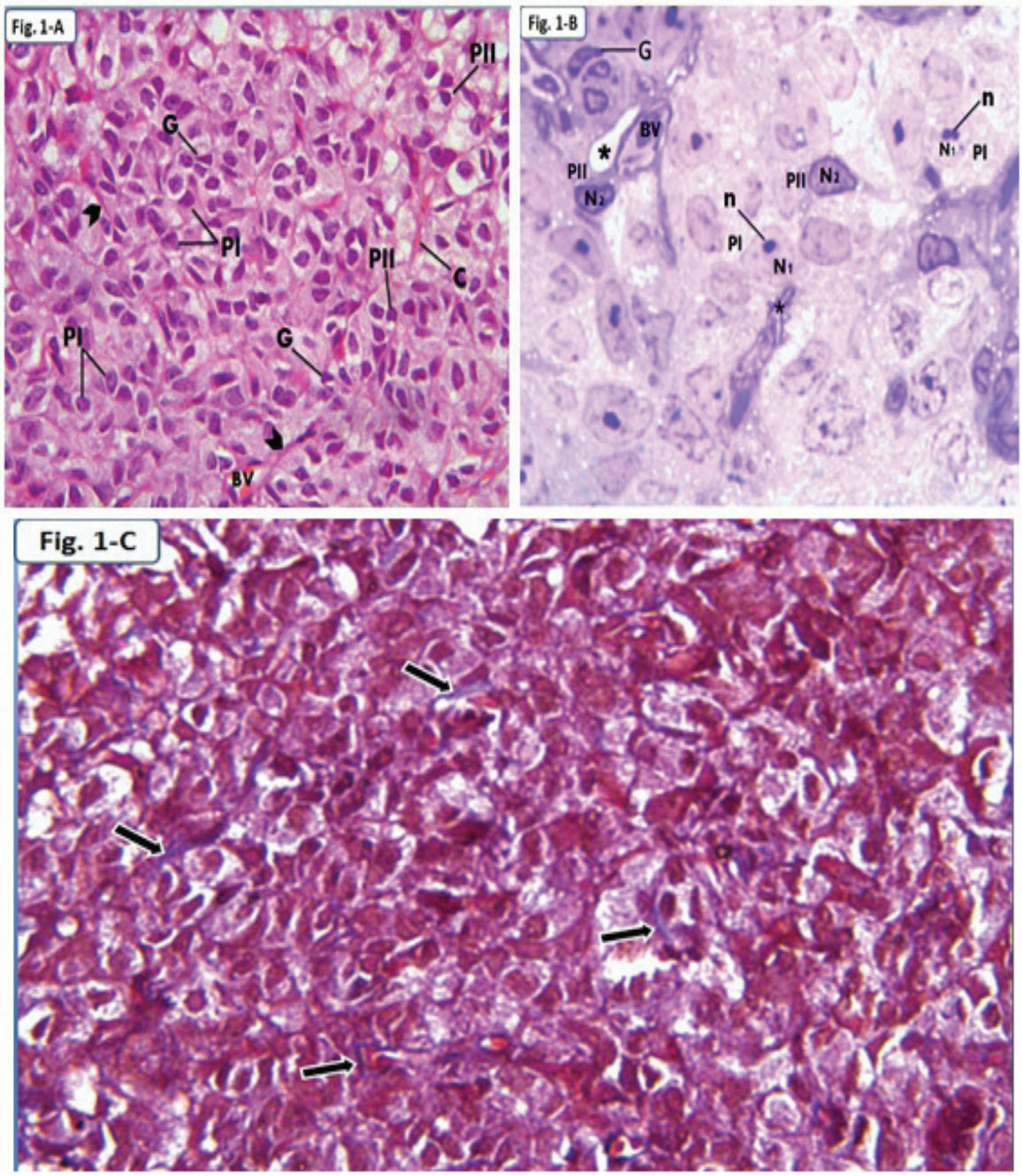

Fig. 1: Photomicrographs of pineal gland from GI showing: A. plates of closely packed pinealocytes. Pinealocytes type I (PI) and pinealocytes type II (PII) appear within parenchymal lobules (circle), separated by fibrous septae (arrowheads) containing blood vessels (BV). Glial cells (G) scattered among pinealocytes whereas the blood capillaries (C) are situated among parenchymal cells. (Hx & E x 400) B. tightly packed pinealocytes within the parenchymal lobules in semithin section. Pinealocytes type I (PI); pale cytoplasm surrounding large euchromatic nuclei (N1) with prominent nucleoli (n). Pinealocytes type II (PII) demonstrate darkly-stained nuclei (N2) and appear near the perivascular space (*) which surrounds the blood vessels (BV) seen between the parenchymal lobules. Glial cells (G), deeply-stained nuclei. (Toluidine blue x 1000) C. fine distribution of collagen fibers (arrows) between lobules and between tightly packed parenchymal cells. (Masson's trichrome x 400)

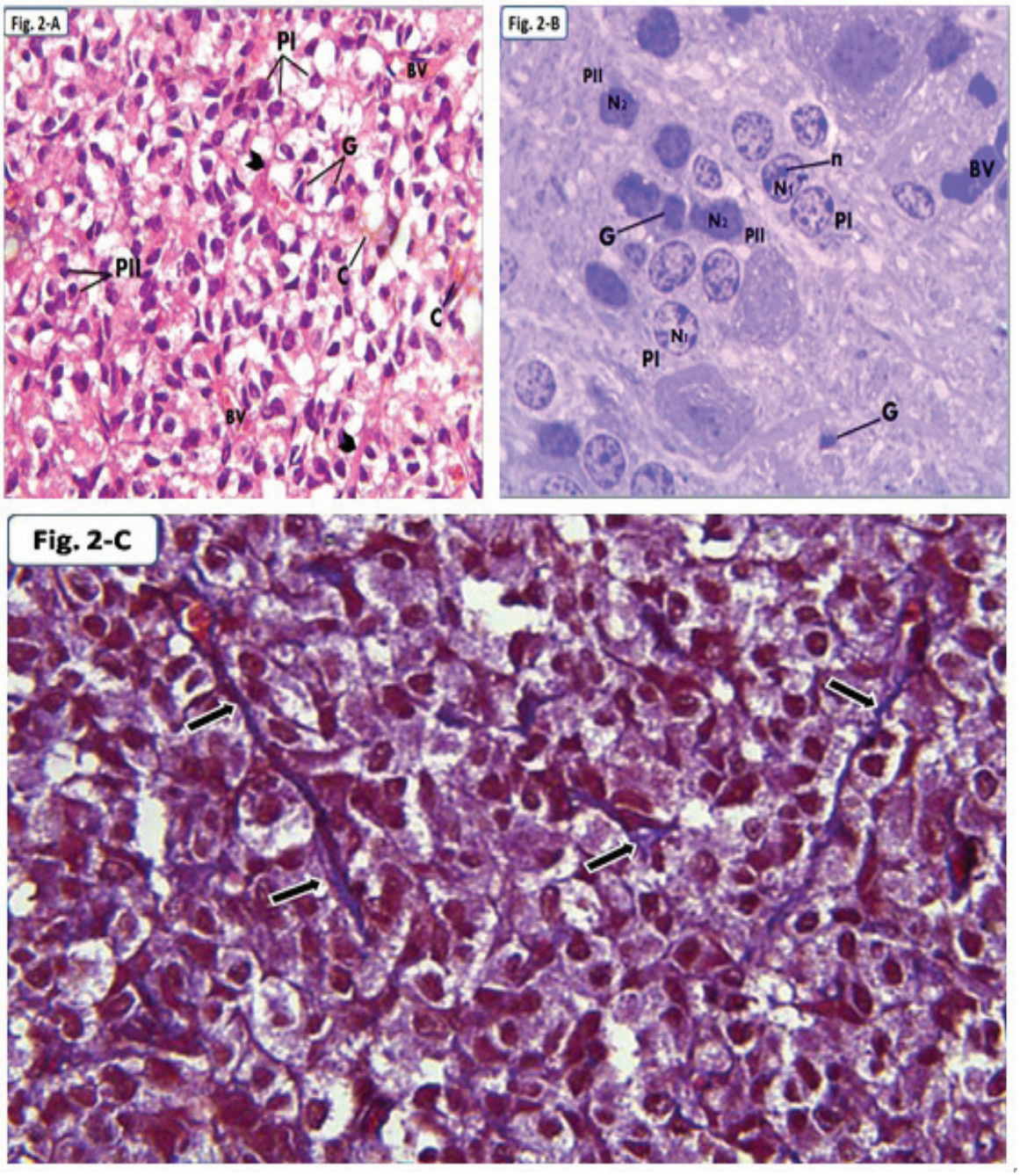

Fig. 2: Photomicrographs of pineal gland from GII demonstrating: A. abundant closely packed pinealocytes in semithin section; pinealocyte type I (PI) mainly and pinealocyte type II (PII) within the parenchymal lobules demarcated by fibrous septa (arrow heads). Scattered glial cells (G) are seen between the pinealocytes. Blood vessels (BV) between the lobules and blood capillaries (C) between the parenchymal cells are also observed. (Hx & E x 400) B. numerous tightly packed pinealocytes type I (PI) with pale cytoplasm surrounding heterochromatic nuclei (N1) and prominent nucleoli (n). Pinealocytes type II (PII) are also seen with small dark nuclei (N2). Glial cells (G) appear between the pinealocytes and reveal very small, darkly-stained nuclei (N3). Blood vessels (BV) are also observed between lobules. (Toluidine blue x 1000) C. moderate amount of collagen fibers (arrows) between plates of closely packed cells. (Masson's trichrome x 400)

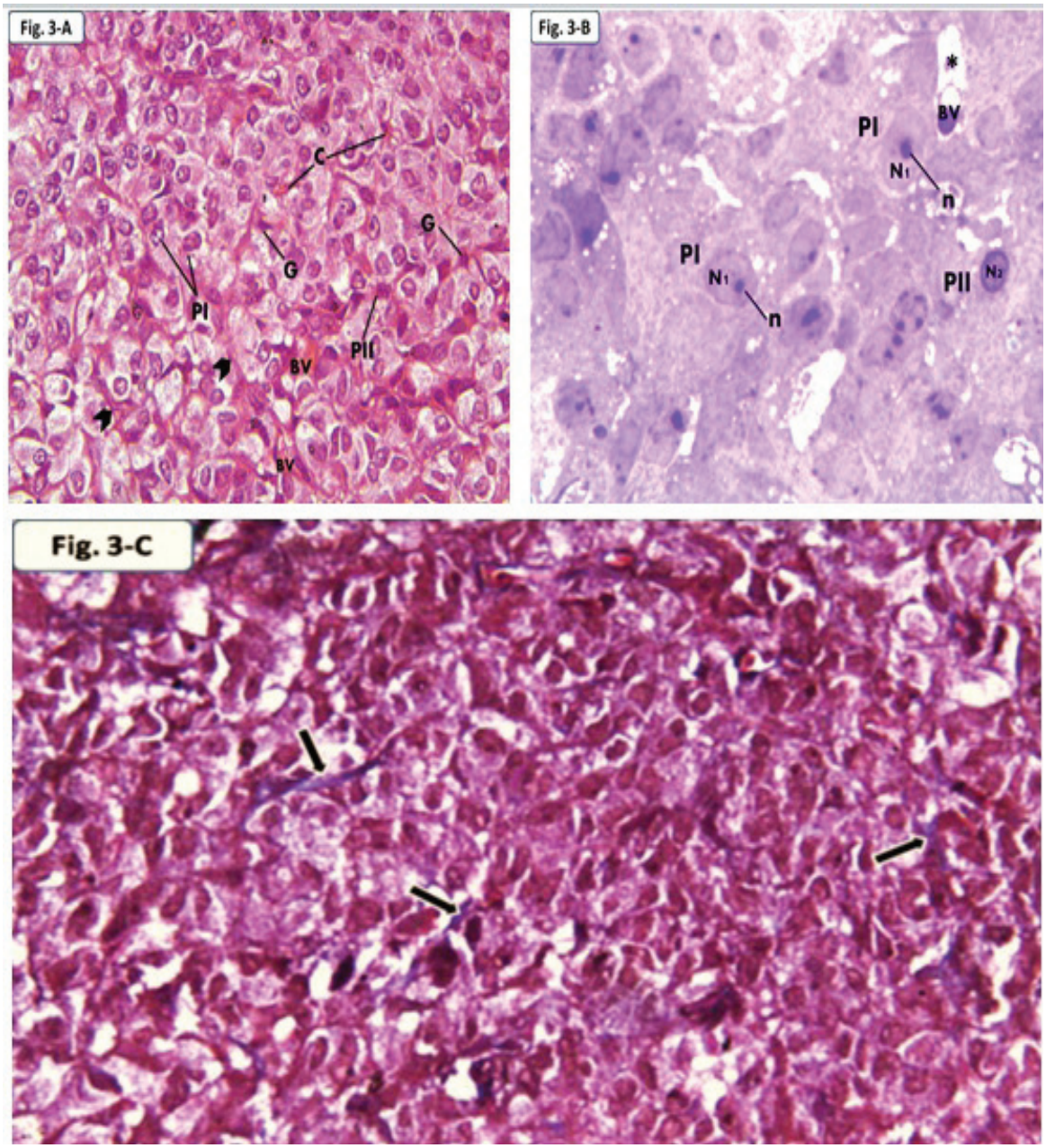

Fig. 3: Photomicrographs of pineal gland from GIII displaying: A. plates of almost packed pinealocytes; pinealocytes type I (PI) and pinealocytes type II (PII) within the parenchymal lobules. Few glial cells (G) appear between the parenchymal cells. Blood vessels (BV) in the fibrous tissue septa between the parenchymal lobules and blood capillaries (C) between the parenchymal cells are also seen. (Hx & E x 400)
Fig. 3B: almost packed pinealocytes within the parenchymal lobules in semithin section. Pinealocytes type I (P1) demonstrate large euchromatic nuclei (N1) with prominent nucleoli (n). Pinealocyte type II (PII) features a small dark nucleus (N2). Perivascular space (*) surrounding blood vessels (BV) are also seen. (Toluidine blue x 1000) C. few amount of collagen fibers (arrows) between plates of packed parenchymal cells as well as in the interlobular septa. (Masson's trichrome x 400)

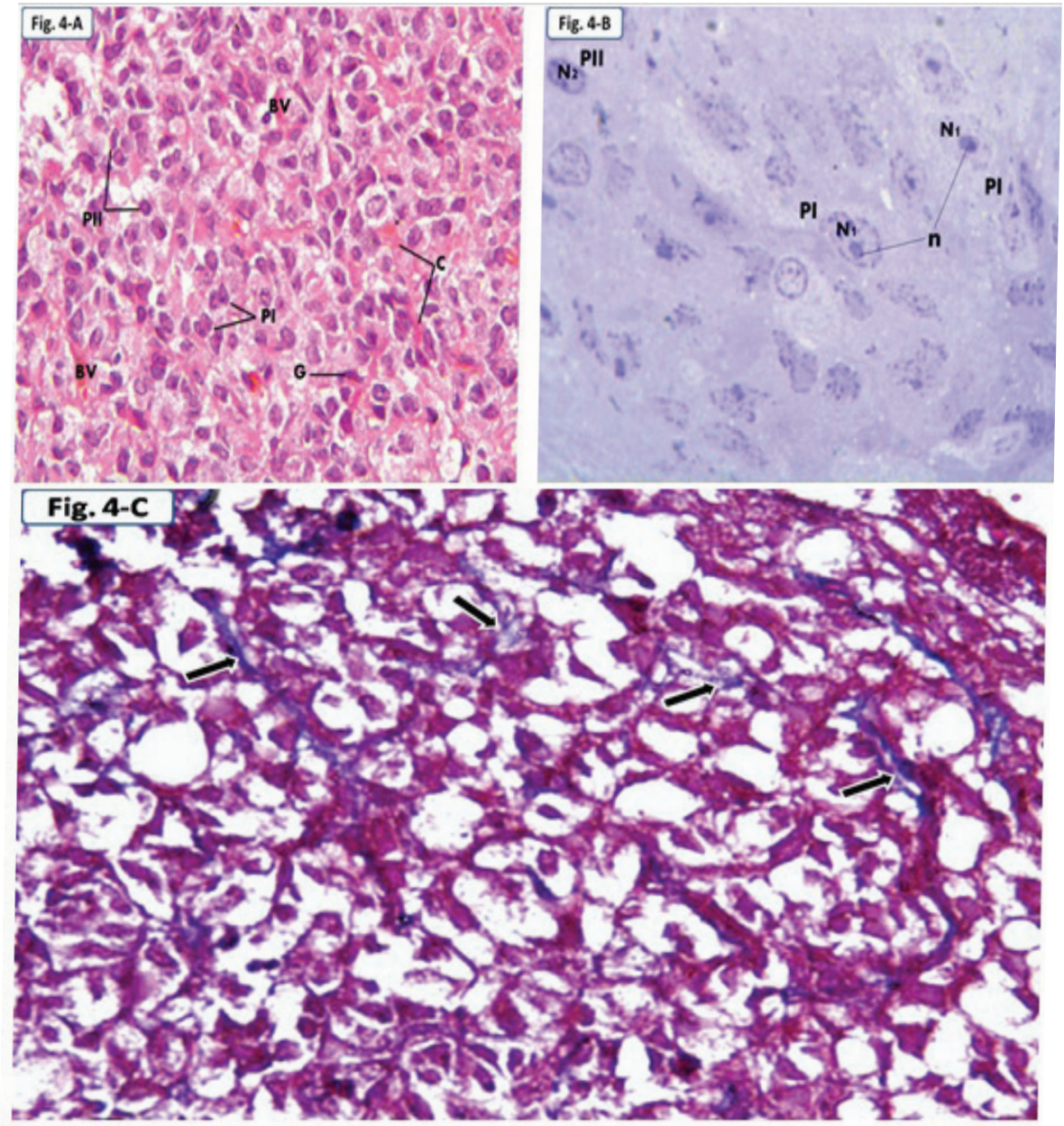

Fig. 4: Photomicrographs of pineal gland from GIV revealing: A. plates of loosely packed pinealocytes (PI,PII) within the parenchyma. Glial cells (A) are also observed between pinealocytes. Blood vessels (BV) between the lobules and numerous congested blood capillaries (C) between the cells are also seen. (Hx & E x 400) B. loosely packed pinealocytes within the parenchymal lobules in semithin section. Pinealocytes type I (PI) have nuclei (N1) and exhibit reduced amount of chromatin, some show prominent nucleoli (n). Few pinealocyte type II (PII) reveals small dark nucleus (N2). (Toluidine blue x 1000) C. large amount of collagen bundles (arrows) between plates of loosely packed cells. (Masson's trichrome x 400)

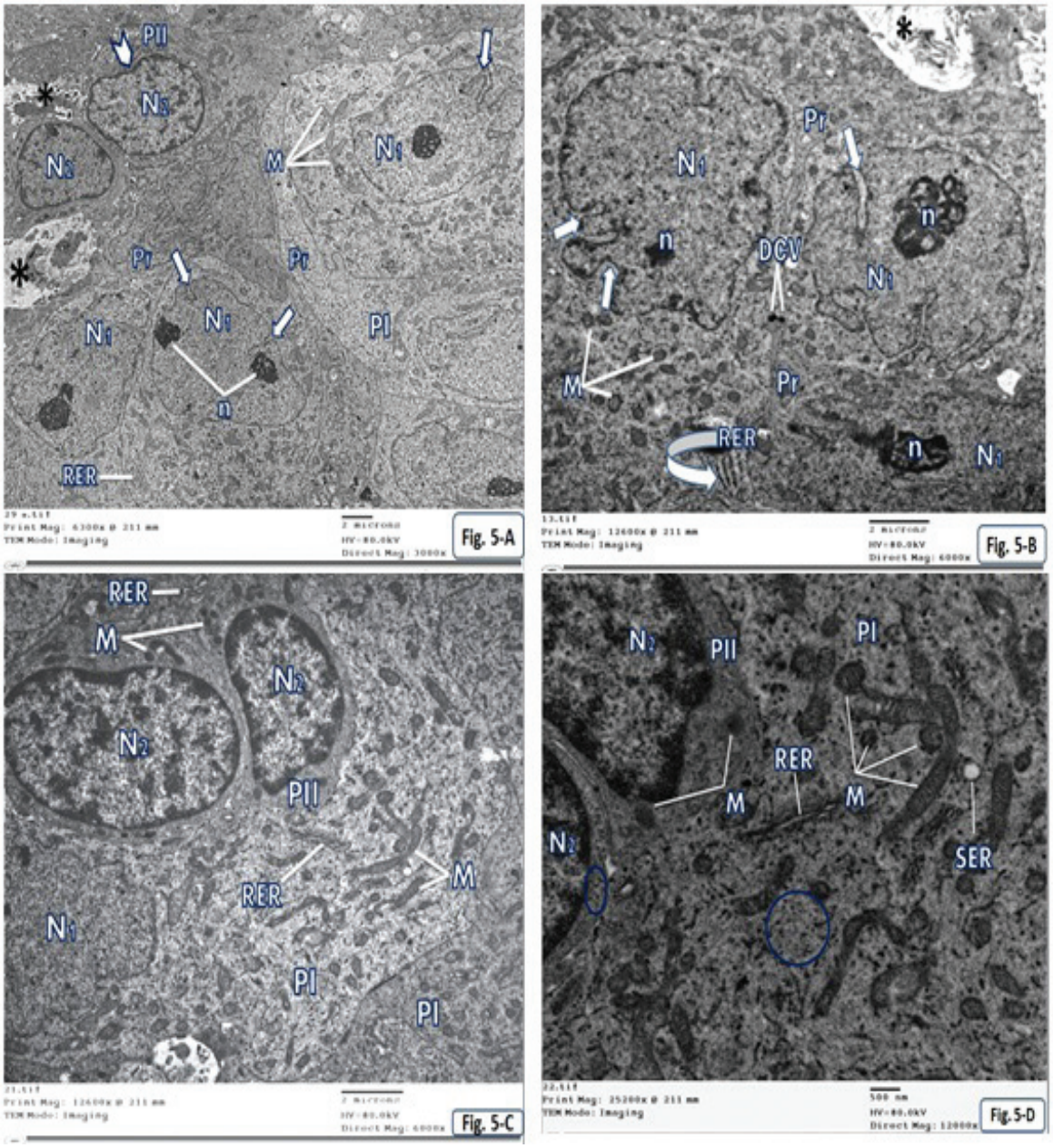

Fig. 5: Electron photomicrographs of pineal gland from GI demonstrating: A. pinealocytes type I (PI) with irregular outline and cytoplasmic processes (Pr) extending between the cells to the perivascular space (*) as well as pinealocytes type II (PII). The cytoplasm of PI contains numerous mitochondria (M) and rough endoplasmic reticulum (RER). The nuclei (N1) show infoldings (arrows) of the nuclear envelope and euchromatic karyoplasm. Some exhibit double nucleoli (n). PII shows heterochromatic nuclei (N2) with indented nuclear envelope (arrow head). (EM x 3000)
B. two adjacent pinealocytes type I (PI) with multiple cytoplasmic processes (Pr), extending between the cells to the perivascular space (*). Their cytoplasm contains intact mitochondria (M) and rough endoplasmic reticulum (RER). Dense core vesicles (DCV) are also seen. The nuclei (N1) illustrate infolding of the nuclear envelope (arrows), prominent nucleoli (n) and euchromatic karyoplasm. (EM x 6000) C. two adjacent pinealocytes type I (PI). Their cytoplasm demonstrates intact mitochondria (M) and rough endoplasmic reticulum (RER). Pinealocytes type II (PII) show mitochondria (M) and rough endoplasmic reticulum (RER) in their cytoplasm. Their nuclei (N2) demonstrate heterochromatic karyoplasm (N2). (EM x 6000) D. intact mitochondria (M), rough endoplasmic reticulum (RER), scattered smooth endoplasmic reticulum (SER) and free ribosomes (large circle) in the cytoplasm of pinealocytes type I (PI). The cytoplasm of pinealocytes type II (PII) contains numerous ribosomes (small circle) and mitochondria (M). Their nuclei (N2) demonstrate heterochromatin pattern. (EM x 12000)

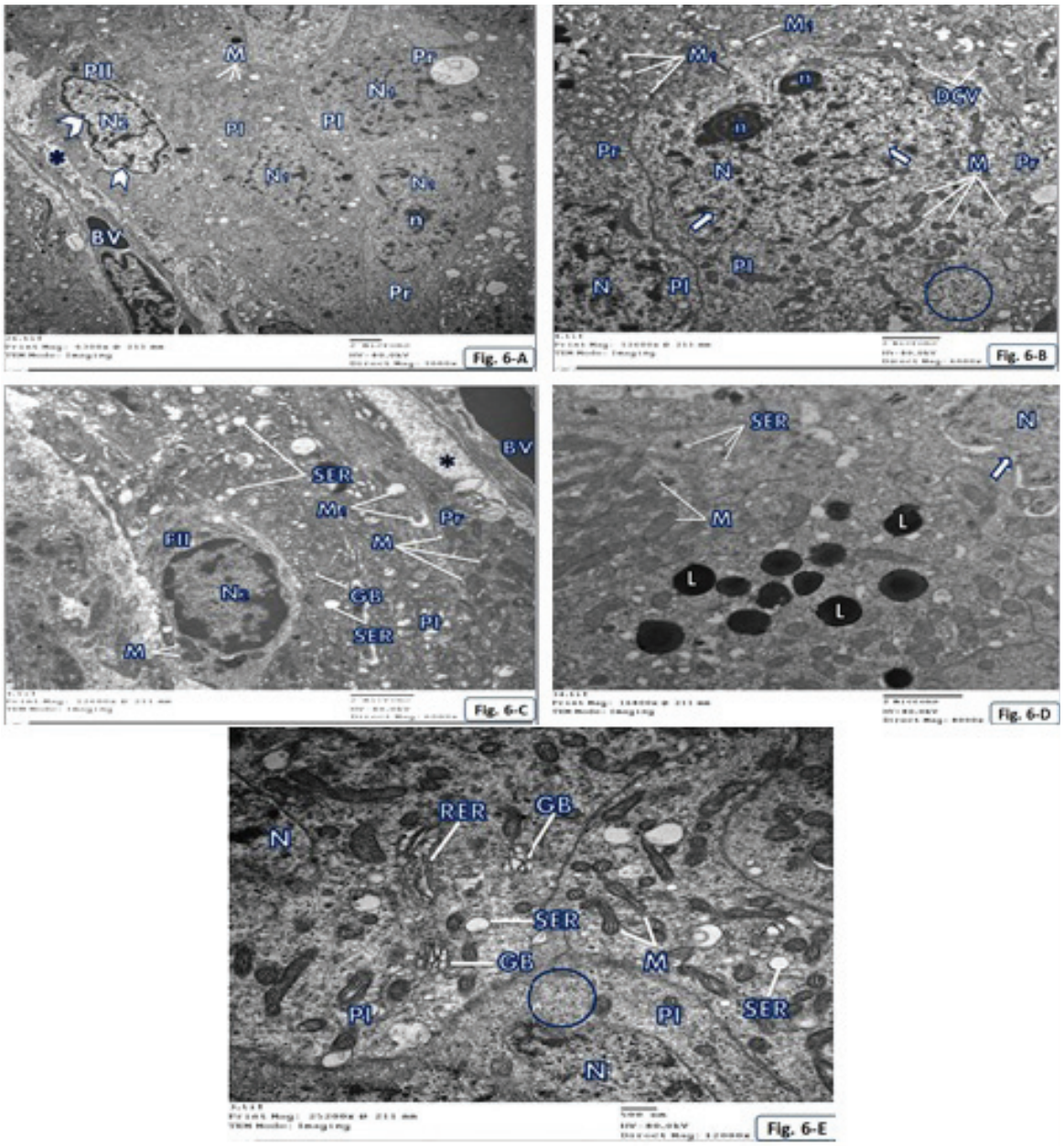

Fig. 6: Electron photomicrographs of pineal gland from GII displaying: A. pinealocytes type I (PI) with multiple cytoplasmic processes (Pr). Their cytoplasm show abundant mitochondria and the nuclei (N1) demonstrate prominent nucleoli (n) with heterochromatic karyoplasm. Pinealocytes type II (PII) appear adjacent to perivascular space (*) surrounding the blood vessels (BV). Their nuclei show indentation (arrowheads) of the nuclear envelope and heterochromatin pattern. (EM x 3000) B. two adjacent pinealocytes type I (PI) with multiple cytoplasmic processes (Pr). Their cytoplasm shows numerous free ribosomes (circle), abundant mitochondria (M) and few dense core vesicles (DCV). Some mitochondria appear distended with disturbed cristae (M1). The nuclei (N) demonstrate deeply infolded nuclear envelope (arrows), double prominent nucleoli (n) and heterochromatic karyoplasm. (EM x 6000)
Fig. 6C: pinealocyte type II (PII) adjacent to the blood vessels (BV) and perivascular space (*). Mitochondria (M) are seen in the cytoplasm of PII. Its nucleus (N2) located at the center of the cell exhibiting heterochromatin pattern. Numerous mitochondria (M), cisternae of smooth endoplasmic reticulum (SER) and Golgi bodies (GB) are also observed in the cytoplasm of a process (Pr) of pinealocyte type I (PI). Distended mitochondria with disturbed cristae are also seen (M1). (EM x 6000) D. pinealocytes type I demonstrating mitochondria (M), cisternae of smooth endoplasmic reticulum (SER) and multiple lipid droplets (L). The nucleus (N) demonstrates infolding of the nuclear envelope (arrow). (EM x 8000)
Fig. 6E: numerous mitochondria (M), free ribosomes (circle), rough endoplasmic reticulum (RER), cisternae of smooth endoplasmic reticulum (SER) and prominent Golgi bodies (GB) in the cytoplasm of adjacent pinealocytes type I (PI). Their nuclei (N) show heterochromatic pattern. (EM x 12000)

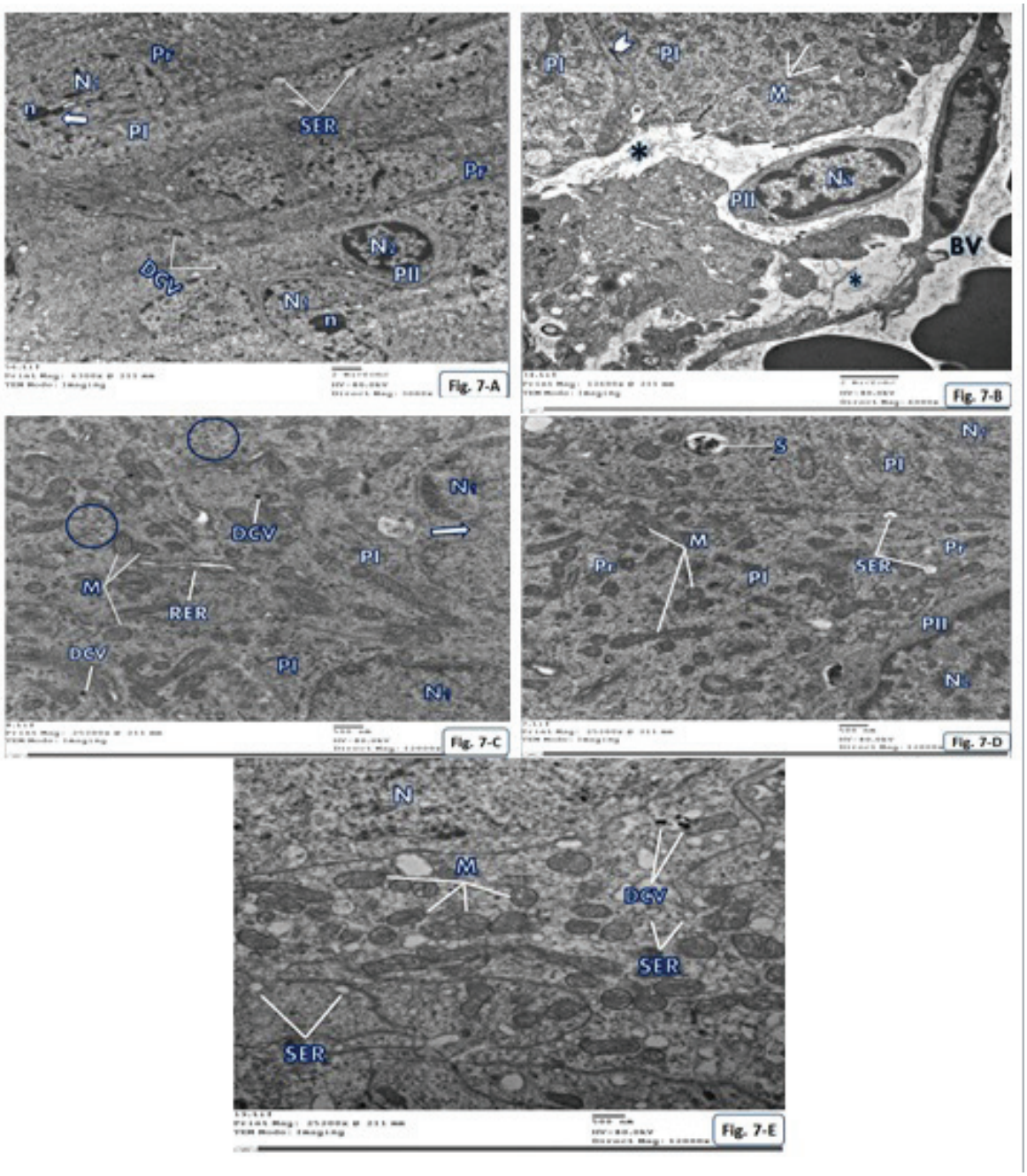

Fig. 7: Electron photomicrographs of pineal gland from GIII revealing: A. pinealocytes type I (PI) with multiple cytoplasmic processes (Pr). Cisternae of smooth endoplasmic reticulum (SER) and dense core vesicles (DCV) are also seen in their cytoplasm. Their nuclei (N1) show infoldings (arrows) of nuclear envelope with euchromatic karyolplasm and prominent nucleoli (n). Pinealocytes type II (PII) exhibit heterochromatic nucleus (N2). (EM x 3000)
Fig. 7B: pinealocyte type II (PII) in contact with the perivascular space (*) surrounding blood vessels (BV). Its nucleus (N2) exhibit heterochromatic karyoplasm. The cytoplasm of pinealocytes type I (PI) demonstrates intact mitochondria (M). (EM x 6000) C: the cytoplasm of pinealocytes type I (PI) with numerous mitochondria (M), rough endoplasmic reticulum (RER) and free ribosomes (circles). Dense core vesicles (DCV) are also seen. Their nuclei (N1) show infoldings (arrow) of the nuclear envelope and demonstrate euchromatic karyoplasm. (EM x 12000) D. numerous mitochondria (M) in the cytoplasm of a process (Pr) of pinealocytes type I (PI). Brain sand and cisternae of smooth endoplasmic reticulum (SER) are observed. Their nuclei (N1) demonstrate euchromatic karyoplasm. Pinealocyte type II (PII) exhibits heterochromatic pattern of its nucleus (N2). (EM x 12000) E. pinealocyte type I (PI). The cytoplasm shows numerous well-defined mitochondria (M), cisternae of smooth endoplasmic reticulum (SER) and dense core vesicles (DCV). The nucleus (N) illustrates euchromatic karyoplasm. (EM x 12000)

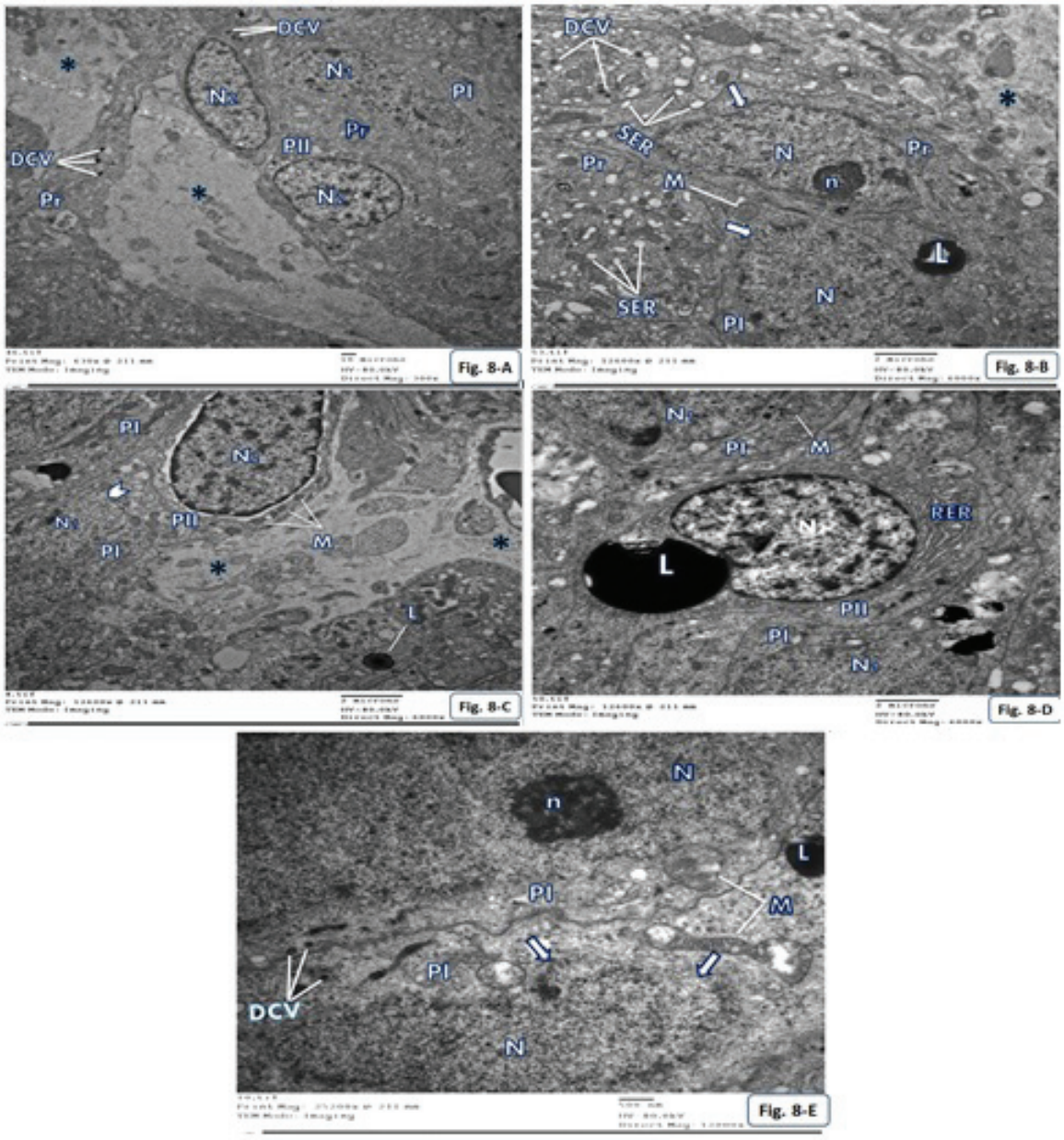

Fig. 8: Electron photomicrographs of pineal gland from GIV illustrating: A. pinealocytes type I (PI) with cytoplasmic processes (Pr) that show multiple dense core vesicles (DCV). Their nuclei (N1) demonstrate small amount of chromatin. Pinealocytes type II (PII) are located adjacent to widened perivascular space (*). Their nuclei (N2) reveal heterochromatin pattern. (EM x 3000) B. pinealocytes type I (PI) with cytoplasmic processes (Pr) extending to widened perivascular space (*). Their cytoplasm shows scarce mitochondria (M) and abundant cisternae of smooth endoplasmic reticulum (SER). Numerous dense core vesicles (DCV) and one lipid droplet (L) are also appear. The nuclei (N) demonstrate reduction in the amount of chromatin, some show prominent nucleoli (n). At certain areas, the nuclear envelope is ruptured (arrows). (EM x 6000) C. pinealocytes type II (PII) located near to perivascular space (*). Their cytoplasm demonstrates mitochondria (M). Their nuclei (N2) exhibit heterochromatic karyoplasm. The cytoplasm of pinealocytes type I (PI) shows lipid droplet (L). Their nuclei (N1) demonstrate reduction in the nuclear chromatin and rupture in the nuclear envelope (arrowhead). (EM x 6000) D. numerous cisternae of rough endoplasmic reticulum (RER) and large lipid droplet (L) within the cytoplasm of pinealocytes type II (PII). Their nuclei (N2) exhibit heterochromatic karyoplasm. Pinealocytes types I (PI) demonstrate scarce cell organelles including mitochondria (M). Reduction of the amount of its nuclear chromatin (N1) of PI is also visible. (EM x 6000) E. two adjacent pinealocytes type I (PI). Their cytoplasm appears little with scarce cellular organelles apart from few mitochondria with disturbed cristae (M). Scattered dense core vesicles (DCV) and lipid droplet (L) are also seen. The nuclei (N) containing prominent nucleoli (n) and ruptured nuclear envelope (arrows). (EM x 12000)

DISCUSSION

In the present work, parenchymal cells were distinguished into: pinealocytes and glial cells. Identical observation was noticed by (*Kus et al. 2004*). In the present study, two types of pinealocytes were identified in the control group; pinealocyte type I (PI), constitutes the major part of parenchymal cells and pinealocytes type II (PII) which were observed fewer in number and located adjacent to the perivascular spaces. Corresponding findings were encountered by (*Borhamy 2004*) who reported that PI forms about 77% of parenchymal cells and PII represents 19% of parenchymal cells. Cernuda- (*Cernuda et al. 2003*) considered the light cells (PI) and dark cells (PII) as successive stage of pinealocytes due to similar electron microscopic characteristics. In addition to the result of the present work, (*Ekstrom and Meissl 2003*) demonstrated neuroendocrine photoreceptor cells in the pineal gland of non-mammalian vertebrates.

In the present work, ultrastructural examination of pineal gland from different groups revealed PI with irregular outlines and multiple cytoplasmic processes. These processes extend between adjacent cells to reach the perivascular spaces. Similar result was reported by (*Borhamy 2004*) who suggested that these processes most probably transmit their active products to the blood stream.

In the present study, electron microscopic examination of PI in GII confined to constant darkness revealed abundant cytoplasmic cell organelles such as mitochondria, numerous free ribosomes and multiple lipid droplets. Earlier, the study of (*Vollrath 1981*) considered these lipid droplets as secretory material of pinealocytes or stored sites for melatonin secretion. Studies on rats and hamsters by (*Karasek et al. 1990*) and (*Swieto¬slawski and Karasek 1993*), respectively found an increase in number of cytoplasmic organelles of the pinealocytes exposed to darkness. The latter authors attributed these observations to the increase in the pinealocyte activity with darkness.

In the present work, electron microscopic study of GIII exposed to constant long wavelength red illumination, demonstrated results similar to that of the control group such as numerous mitochondria with intact cristal pattern and free ribosomes. In addition, scattered cisterna of SER and infrequent DCV were also noticed. While, after exposure to blue short wavelength light in GIV of the present study, little cytoplasm containing scarce cell organelles were observed including few mitochondria, ribosomes and scattered lipid droplets. Nearly similar results were demonstrated by (*Karasek et al. 1990*) and Kus et al. (2004). The former author added that there was reduction in the synthesis of melatonin as a result of constant broad band white light exposure.

In the present work, PI in GII demonstrated numerous mitochondria either normal or distended with disturbed cristal pattern of some of them. Similar result was observed by (*Srivastava 2003*) who emphasized that this finding is an evident of increased metabolic activity of the pineal gland. Earlier, (*Cross and Mercer 1999*) attributed the mitochondrial size increase to their crucial role in for hormone synthesis in endocrine glands by reactions occurred in the mitochondria and endoplasmic reticulum with shuttling back and forth between these two organelles; constant darkness resulting in some physiological changes in these organelles leading to their increase in size. Additionally in the present study, the mitochondria of GIII were similar to that of the control group, while in GIV, there was scattered mitochondria were observed and mostly dilated with disturbed cristae. In contrary with the observation of the present study, (*Aral et al. 2006*) found numerous mitochondria on exposure to blue light. The authors pointed out that the serum level of melatonin was higher in darkness than short wavelength illumination exposure and added that the pineal gland reaction was highly sensitive to blue light short wavelengths of l max = 450nm.

Scattered dense core vesicles (DCV) were observed in GII of the present work, while a large number of DCV were encountered in group IV exposed to constant blue light and no changes in DCV in group III as compared to the control group. Similar results were demonstrated by (*Karasek et al. 1990*) who reported an increase in the number of DCV during the day, with decrease melatonin levels in the blood and decrease in the number of DCV during the night

when melatonin levels in the blood increase; these data indicate that DCV are considered as storage of melatonin. (*Lewczuk and Przybylska 2000*) added that the number of DCV in the pinealocytes depends on light conditions and controlled by norepinephrine. Moreover, the results of (*Redins et al. 2001*) in vivo found that melatonin decreases the number and volume of DCV, indicating their role in the pinealocytes secretory activity, while increases in the count and volume of lipid droplets and Golgi apparatus. Similarly, the prominent Golgi bodies were encountered in GII (confined to constant darkness) of the present study.

Brain sand, an identifying feature of pineal gland also observed in present study in GIII. It has been described by (*Ross and Pawlina 2011*) as calcified concretions derived from precipitation of calcium phosphates and carbonates on carrier proteins released into the cytoplasm when the pineal secretions are exocytosed.

In the present work, few cisternae of smooth endoplasmic reticulum (SER) were demonstrated in PI of GII, while abundant cisternae of SER were reported in PI of GIV. However, the findings of SER in GIII were similar to that of the control group. There is lack in the literature that support the observation of the present work regarding SER apart from earlier explanation by (*Tutter et al. 1991*) who demonstrated calcium ions in the cisterns of SER and emphasized their functional role in cell stimulation.

The prominent Golgi bodies were encountered in GII (confined to constant darkness) of the present study. (*Cernuda-Cernuda et al. 2003*) reported well developed Golgi bodies in pinealocytes of control rats subjected to normal day/night rhythm.

In the present study, in GII confined to constant darkness, there was increase in the nuclear number of PI which confirmed statistically, a feature of increased metabolic activity of the gland. In partial agreement to the data of (*Redondo et al. 2000*) who found an increase in the pinealocytes volume of sheep sacrificed at night more than in those sacrificed by day.

In the present study, electron microscopic study of the nuclei of PI in GI appeared with irregular outlines and prominent nucleoli; occasionally double nucleoli and the nuclear envelope showed many infoldings. Similar observations were reported by (*Cernuda-Cernuda et al. 2003*). In the present study, nuclei of PI in GII, exhibited heterochromatic karyoplasm with infolded nuclear envelope and prominent nucleoli, occasionally doubled. Furthermore, the nuclei of PI of GIII were similar to those of control group. In contrast, in GIV, the nuclei showed a reduction in chromatin and ruptured nuclear envelope at certain sites. In partial agreement with (*Kus et al. 2004*) who attributed this finding to decrease in pinealocytes activity in rates exposed to constant broad band white light as indicated by nuclear microscopic changes.

In the present work, the nuclear number of PI in GIII showed statistically non-significant difference when compared to the control group. On the other hand, rats of GIV revealed statistically significant decrease in PI nuclear number together with a statistically significant increase in the amount of collagen fibers between loosely packed parenchymal cells. Nearly similar results were reported by (*Kus et al. 2004*) but on exposure to white broad band light.

In the present study, no detectable changes were found by light microscopy in PII of the different experimental groups exposed to the different light wavelengths apart from the presence of statistically significant increase in the nuclear number of PII in GII confined to constant darkness. However, there was no literature support the finding of the present study. In the present work, a characteristic feature of PII in all experimental groups, its location adjacent to the blood vessels and perivascular spaces. Similar observation was reported by (*Borhamy 2004*) who attributed this result to the participation of PII in the transmission of secretory products of the pineal gland to blood stream.

In addition, electron microscopic examination of PII revealed no remarkable changes in all groups in response to different light wavelengths or constant darkness. PII demonstrated regular cellular outline with well-defined nuclear envelope apart from indentation in GII, with

condensed chromatin material. Moreover, the PII cytoplasm lacked cell organelles apart from scattered mitochondria in GI, II and RER in GIV which were scarce in PI of all experimental groups. Identical findings were observed by (*Borhamy 2004*).

In the present study, glial cells were sparsely distributed in the interstitial tissue among the pinealocytes. Additionally, glial cells in the current work showed statistically significant increase in GIV exposed to constant blue short wavelength illumination but non-significant differences were noticed in the other groups as compared to the control group. In partial agreement with (*Kus et al. 2004*) who demonstrated glial cells in rats exposed to constant broad band light. Earlier, (*Vollrath 1981*) found positive glial markers and considered interstitial cells as a type of astrocyte which was reported later in cat and dog by (*Boya and Calvo 1993*) and in cotton rat by (*Sakai et al. 1996*). Moreover, (*Srivastava 2003*) stated that the functional role of microglial cells is phagocytosis of necrotic cells or phagocytotic activity of the outer segments of photoreceptor cells.

(*Brainard et al. 2001*) pointed out that light with wavelength between 430 and 500 nm (short wavelength) was more effective in suppressing nocturnal melatonin than longer wavelengths. The authors added that blue sensitive pigment is also fully effective in controlling the circadian biological rhythm humans.

(*Freedman et al. 1999*) stated that the photoreceptors; rods and cones; are responsible for the image-produced vision i.e. visual perception, whereas retinal ganglion cells are considered intrinsically photosensitive receptors (ipRGCs). Moreover, (*Reppert and Weaver 2002*) pointed out that there were rhythmic changes of several physiological functions in the human body within a 24h period produced by the hypothalamic supra-chiasmatic nucleus (SCN); the circadian pacemaker. Furthermore, (*Hatori et al. 2008*) described melanopsin as a subtype of retinal ganglion cells (RGCs) that mediate through the retino-hypothalamic tract the photo-entrainment i.e. the adjustment of the circadian rhythms phase by the light–dark cycle. There is evidence that mRGCs project to many nonvisual areas; serving many non-visual forming functions, such as melatonin secretion and its suppression by light (*Tsai et al., 2009*).

(*Mathes et al. 2007*) and (*Zaidi et al. 2007*) emphasized that the stimulation of melanopsin levels (at least in the rat) is dependent on light exposure and the length of photoperiod which in turn regulates the release of melatonin from the pineal gland. In addition, (*Mure et al. 2007*) reported that melanopsin retinal ganglion cells (mRGCs) are the most sensitive to blue light short wavelength (between 459 and 483 nm). Moreover, (*Roecklein et al. 2009*) found that blue light (440–480 nm) is highly effective in phase shifting the circadian clock, can increase alertness.

CONCLUSION

In the present study, it could be concluded that there was reduction in the metabolic activity of pinealocytes type I of rat on exposure to constant short wavelength blue illumination more than the exposure to constant long wavelength red illumination which gave results nearer to the control group (normal diurnal rhythm) as reported by the morphological and electron microscopic study. However, there was stimulation of the synthetic activity and the discharge of secreted products of the pinealocytes type I of rats confined to constant darkness. Additionally, there were no remarkable changes in pinealocytes type II neither on exposure to different light wavelengths nor confined to constant darkness.

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تغيرات النوع الأول والثاني لخلايا الغدة الصنوبرية للفأر الأبيض الناتجة عن تعرضه المستمر للظلام أو لأطوال موجية مختلفة للضوء :دراسة مورفولوجية ومجهرية فائقة الدقة

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ملخص البحث

المقدمة: تعتبر الغدة الصنوبرية واحدة من الغدد العصبية الصماء والتي تفرز هرمون الميلاتونين الذي ينظم الساعة البيولوجية، ويعالج الاضطرابات النفسية وأعراض الشيخوخة وفقا للتعرض للضوء و الظلام. وقد وجد أن العوامل التي تتحكم في عمل الغدة الصنوبرية هي كثافة والطول الموجي للضوء وكذلك مده التعرض له .

الهدف: لقد صممت هذه الدراسة لتقييم التغيرات المورفولوجية والتركيبية فائقة الدقة في الخلايا الأولي والثانية للغدة الصنوبرية في الفئران البيضاء البالغة والتي تتعرض باستمرار(لمدة 24 ساعة /اليوم) للضوء الأحمر أو الأزرق أويتم وضعها في الظلام الدائم ومقارنة هذه النتائج بالمجموعة الضابطة التي تتعرض للضوء النهاري العادي.

مواد وطرق البحث: تم تقسيم الفئران البيضاء الذكور البالغين وعددهم أربعون إلى أربع مجموعات متساوية. المجموعة الأولى (الضابطة) والتي تعرضت للضوء النهاري العادي، المجموعة الثانية: والتي تم وضعها في الظلام الدائم، المجموعة الثالثة: والتي تتعرض باستمرار للضوء الأحمر طويل الموجة أما المجموعة الرابعة فهي تتعرض باستمرار للضوء الأزرق قصير الموجه. لمدة أربعة أسابيع مستمرة.

النتائج: لقد أظهر التحليل الاحصائي للنتائج المورفولوجية وجود زيادة في عدد أنوية الخلايا الأولي للغدة الصنوبرية في المجموعة الثانية ونقص في عدد الأنوية في المجموعة الرابعة ولا يوجد أي دلالة إحصائية ملحوظة في المجموعة الثالثة وذلك مقارنة بالمجموعة الضابطة. بالإضافة الي ذلك فقد وجد زيادة في كمية ألياف الكولاجين بين خلايا المجموعة الرابعة. وقد أظهرت النتائج المجهرية فائقة الدقة وجود عضيات وفيرة في سيتوبلازم الخلايا الصنوبرية الأولي في المجموعة الثانية مثل الميتوكوندريا، وعديد الريبوسومات وقطرات الدهون المتعددة جنبا إلى جنب مع وجود أجسام جولجي، أما الحويصلات ذات المراكز الكثيفة وكذلك الشبكة الإندوبلازمية الملساء والخشنة فقد وجدت بأعداد قليلة. وكانت نتائج المجموعة الثالثة مماثلة للمجموعة الضابطة. من ناحية أخرى، أوضحت المجموعة الرابعة ندرة في العضيات السيتوبلازمية مثل عدد قليل من الميتوكوندريا والتي وجدت منتفخة وقطرات الدهون مع وجود عدد وفير من الحويصلات ذات المراكز الكثيفة وكذلك الشبكة الإندوبلازمية الملساء. بالإضافة الي وجود انحناءات بالغلاف النووي للمجموعة الثانية مع وجود تكتلات كروماتين ونويات بارزة في حين كشفت المجموعة الرابعة عن وجود تمزق بالغلاف النووي ونقص في كمية الكروماتين. أما أنوية المجموعة الثالثة فقد أظهرت نتائج مماثلة للمجموعة الضابطة. لم يتم ملاحظة أي تغيرات بواسطة المجهر الضوئي أو الإلكتروني في الخلايا الصنوبرية الثانية في المجموعات المختلفة للتجربة إلا فقط وجود زيادة ذات دلالة إحصائية في العدد النووي للخلايا الصنوبرية الثانية في المجموعة الثانية فقط. وعلاوة على ذلك، فقد أظهر سيتوبلازم الخلايا الصنوبرية الثانية عدد قليل من الميتوكوندريا

فى المجموعات الأولي والثانية وكذلك عدد قليل من الشبكة الإندوبلازمية الخشنة في المجموعة الرابعة. وقد أوضحت الخلايا الدبقية زيادة ذات دلالة إحصائية في المجموعة الرابعة ولكن لا توجد أي تغيرات ملحوظة في الخلايا الدبقية في المجموعات الأخرى مقارنة بالمجموعة الضابطة.

الخلاصة: يمكن أن تستخلص من هذا البحث إلى أن التغيرات المورفولوجية والتركيبية فائقة الدقة لخلايا الغدة الصنوبرية يشير إلى انخفاض في نشاط الخلية الصنوبرية الأولي والتي تتعرض باستمرار للضوء الأزرق ذو الطول الموجي القصير وزيادة في نشاط الخلية الصنوبرية الأولي عندما تتعرض للظلام المستمر. في حين أن التعرض الدائم للضوء الأحمر ذو الطول الموجي الطويل يوضح نتائج مماثلة للمجموعة الضابطة. لم تكن هناك تغييرات في بنية الخلية الصنوبرية الثانية في المجموعات التجريبية المختلفة.