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Ultrastructural changes in the rat pineal gland after sympathetic denervation. Quantitative study

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Summary. Ultrastructural changes in the rat pineal gland were studied quantitatively 7 and 60 days after the sympathetic denervation by bilateral excission or decentralization of superior cervical ganglia. The surface occupied by pineal parenchymal cells decreased in rats of experimental groups with respect to the control group. Furthermore, profile areas of the cytoplasm, nucleus and nucleolus of the pinealocytes were also diminished. Cytoplasmic lipid droplets in the pinealocytes were markedly decreased in number and size in experimental rats. As demonstrated by the Kruskal-Wallis H test, statistically significant differences were found between rats of the control and operated groups. Rats treated by superior cervical ganglionectomy or decentralization showed morphological changes indicating a hypofunctional pineal gland, although differences were found between both groups.

Key words: Pinealocyte, Ultrastructure, Rat, Denervation, Morphometry

Introduction

The rat pineal gland is innervated predominantly by sympathetic nerve fibres originating at the superior cervical ganglia (SCG). The integrity of these fibres is necessary for a normal gland function. The sympathetic denervation of the pineal gland by means of superior cervical glanglionectomy leads to pronounced effects on pineal biochemistry. Some of them have been extensively reported; namely the decrease and loss of rhythm of serotonin (Pellegrino de Iraldi et al., 1963; Fiske, 1964), hydroxyindol-o-methyl-transferase activity (Wurtman et al., 1964; Eichler and Moore, 1971; Moore and Rapport, 1971; Nagle et al., 1973; Gallardo and Tramezzani, 1975), and N-acetyl-transferase activity (Klein et al., 1971; Deguchi and Axelrod, 1972).

On the other hand, the morphological effects of pineal denervation have been studied very little. Most reports deal with degeneration of pineal nerve fibres (Vollrath, 1981; Korf and Moller, 1984). Quay (1971) could not find significant changes in pineal volume nor in light microscopic cytology of the pineal parenchyma. Pineal ultrastructural changes following superior cervical ganglionectomy have been described in several species (hamster: Lin et al., 1973, 1975; rabbit: Romijn, 1975; mongolian gerbil: Welsh et al., 1979; cotton rat: Karasek et al., 1983). King and Dougherty (1982) have studied the changes in pineal synaptic ribbons after surgical sympathetic denervation in albino rats.

Two surgical procedures can be used to interfere with sympathetic innervation of the pineal gland: 1) removal of SCG or section of postganglionic fibres (internal carotid nerves or conarii nerves) both leading to degeneration and disappearance of pineal sympathetic nerve fibres, and 2) decentralization of SCG by section of preganglionic fibres located in the cervical sympathetic trunks. This latter procedure leaves pineal nerve fibres undamaged, but prevents the arrival of impulses from the central nervous system to SCG. Although similar biochemical effects have been reported in the pineal gland following removal or decentralization of SCG, these effects might not be necessarily identical. Thus, pineal hydroxyndole-o-methyl-transferase activity is less in decentralized rats than in ganglionectomized ones (Moore and Rapport, 1971). Therefore, SCG should not be considered as a simple relay between the central nervous system and pineal gland but rather as neuroendocrine centres which modify, integrate and modulate incoming signals (Cardinali et al., 1981; Szurszewski, 1982).

The purpose of this study was to perform a quantitative analysis of the ultrastructural changes in the rat pineal gland following superior cervical ganglionectomy and decentralization of SCG.

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Materials and methods

Sixty female albino Wistar rats maintained in identical conditions of temperature (18° C), lighting (14L:10D; lights on at 7 a.m.) and feeding (ad libitum) were used in this study. Animal age was 75 days at the onset of the experiment. The experiment was developed through the months of September and October. Rats of experimental groups were anesthetized with chloral hydrate (300 mg/kg i.p.) and both SCG were removed (GX group). In the decentralized group (DX group), a fragment about 2 mm long of both cervical sympathetic trunks proximal to SCG were removed. The success of the operation was checked by the development of a bilateral ptosis, light microscopic analysis of removed tissue and, in GX group, by the disappearance of sympathetic nerve fibres in pineal connective tissue spaces. Half of the rats of the control group were submitted to the same surgical procedure except for the removal of tissue (sham-operated group).

Ten rats of each experimental group were sacrificed at 11:00 a.m. 7 and 60 days after the operation. In each stage, five intact and five sham-operated rats were also sacrificed to serve as control group. Under ether anesthesia, rats were decapitated and the pineal gland was fixed by immersion in 2% glutaraldehyde-2% paraformaldehyde in 0.1M phosphate buffer, pH 7.4. After fixation, the gland was divided into two halves along its longitudinal axis, washed in 0.1M phosphate buffer, postfixed in 1% osmium tetroxide and embedded in Vestopal. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Phillips EM 201 electron microscope.

Twenty micrographs chosen at random according to Weibel (1979) were taken of each pineal gland at a magnification of × 2,000. Each micrograph covered a field of 7850 μm^2. The surface occupied by connective tissue spaces and both pineal cell types was measured on each micrograph. In addition, we measured the profile area of the following elements of the main pineal cell type, the pinealocytes: cytoplasm; nucleus; nucleolus; and lipid droplets. The number of both nuclei and nucleoli, the nuclear perimeter and the number and diameter of the lipid droplets were also assessed. Morphometric measurements were made using a computerized image analysis system VIDS III. The statistical analysis and the significance of the data were determined using the non-parametric Kruskal-Wallis H test.

Results

The results obtained are shown in Table 1. Significant differences were not found among intact or sham-operated rats of either ages and all the values obtained in these non-denervated rats were pooled as control group (Figs. 1-4). Table 1 shows results of both experimental groups in the two post-operative stages studied. The second pineal cell type, known as the interstitial cell (Vollrath, 1981; Karasek, 1983) or according to our previous reports, the type II pinealocyte (Calvo and Boya, 1983, 1984; Boya and Calvo, 1984), is referred to throughout the present work as glial cell, as suggested by the expression of specific glial cell antigenic markers in this pineal cell type (Schachner et al., 1984; Calvo et al., 1988).

The statistical analysis of the results after the application of the Kruskal-Wallis H test points to the significant differences among the different groups for all the features studied except for the nuclear perimeter of the pinealocytes. The level of signifance ranged from 0.01 to 0.001, as shown in Table 1.

Table 2 shows the relationships between the morphometric data on Table 2. The surface of the different components of the pineal gland is expressed as a percentage of the total area of the micrograph. The quotient parenchyma/stroma expresses the relationship between the total surface occupied by the two types of parenchymal cells and the connective tissue spaces. The mean pinealocyte nuclear and nucleolar area results from dividing the total area by the number of elements counted. Finally, Table 2 shows the relation between the nuclear and cytoplasmic area of the pinealocytes, and the percentage of pinealocyte cytoplasm occupied by lipid droplets.

Discussion

The results of this study showed that sympathetic denervation produces remarkable changes in the rat pineal gland ultrastructure. The choice of the parameters included in this study was made so that measurement of structures could be made at low magnifications of the electron microscope. This permits a more random and homogeneous sampling. Moreover, the results obtained from this study can also be the basis for evaluation of changes in other structures. Thus, for example, any change in number or volume of pinealocyte organelles must take into account the variations in the proportion and amount of cytoplasm of these cells found in this study.

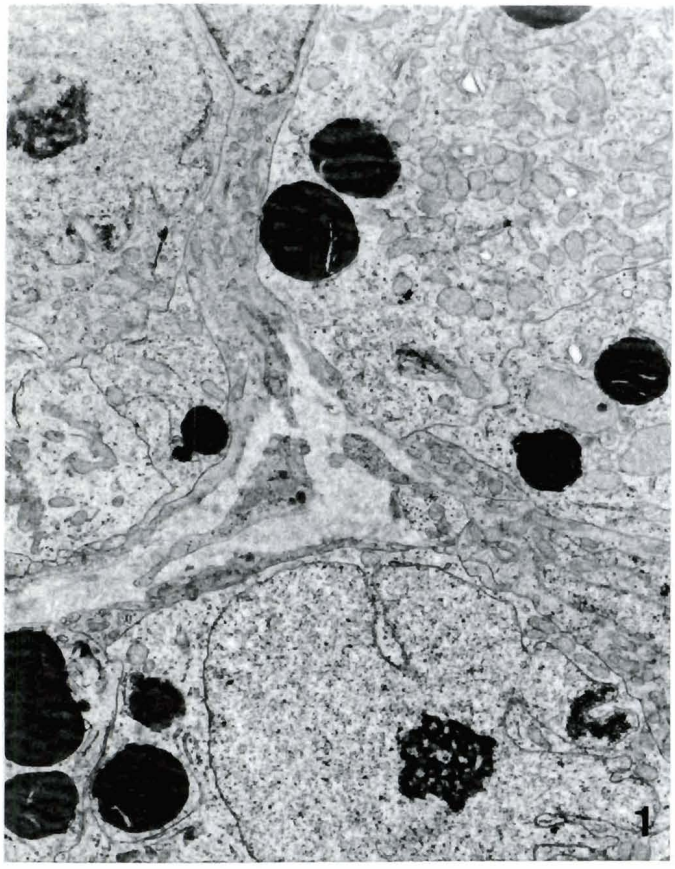
The relation between pineal stroma and parenchyma was modified by sympathetic denervation. Although pineal gland volume was not quantified in this study, Quay (1971) found no changes in pineal volume after GX. The proportion parenchyma/stroma decreased in denervated pineal glands. Connective tissue spaces of pineal gland were usually enlarged in experimental groups but no increases of connective tissue cells nor

Fig. 1. Control rat. Pinealocytes showing several large lipid droplets. × 19,500

Fig. 2. Decentralized rat. 60 postoperative days. Low magnification of the pineal parenchyma showing clusters of pineal glial cells (asterisks). × 3,900

Fig. 3. Ganglionectomized rat. 7 postoperative days. Strong decrease in the amount and size of lipid droplets in pinealocytes. × 13,500

Fig. 4. Ganglionectomized rat. 60 postoperative days. Widely dilated perivascular space. No nerve fibres can be seen. × 13,500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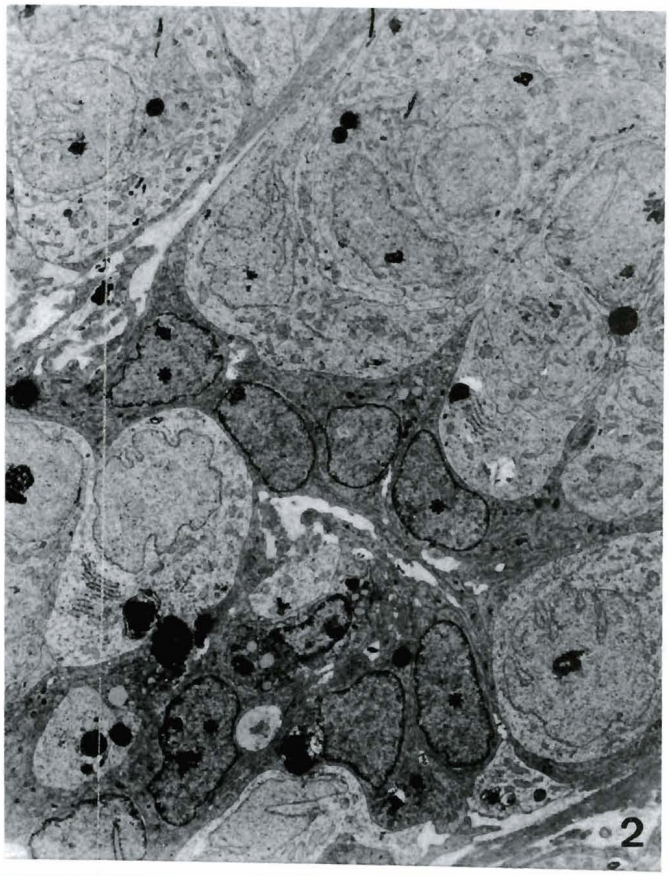
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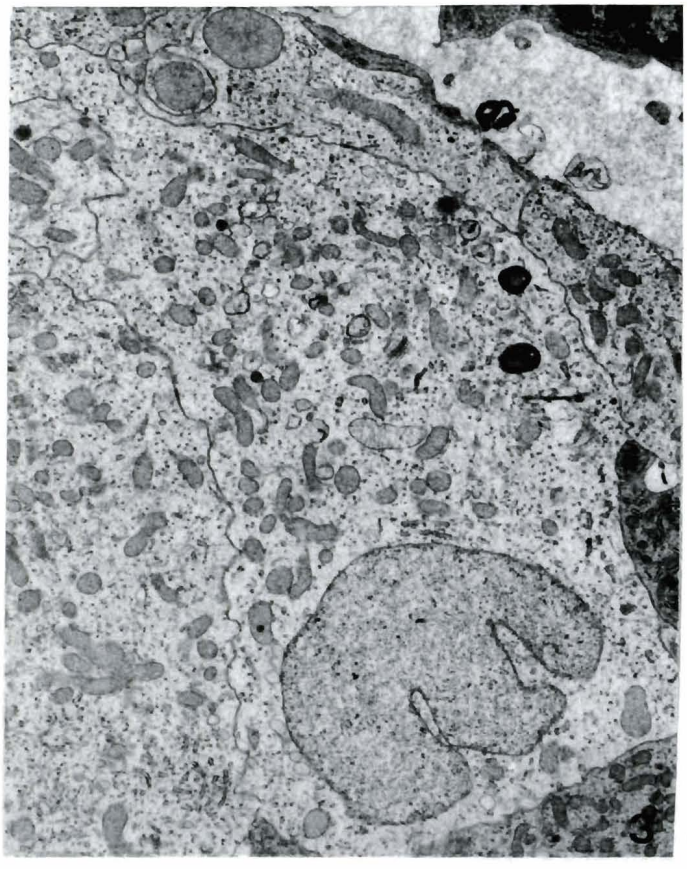
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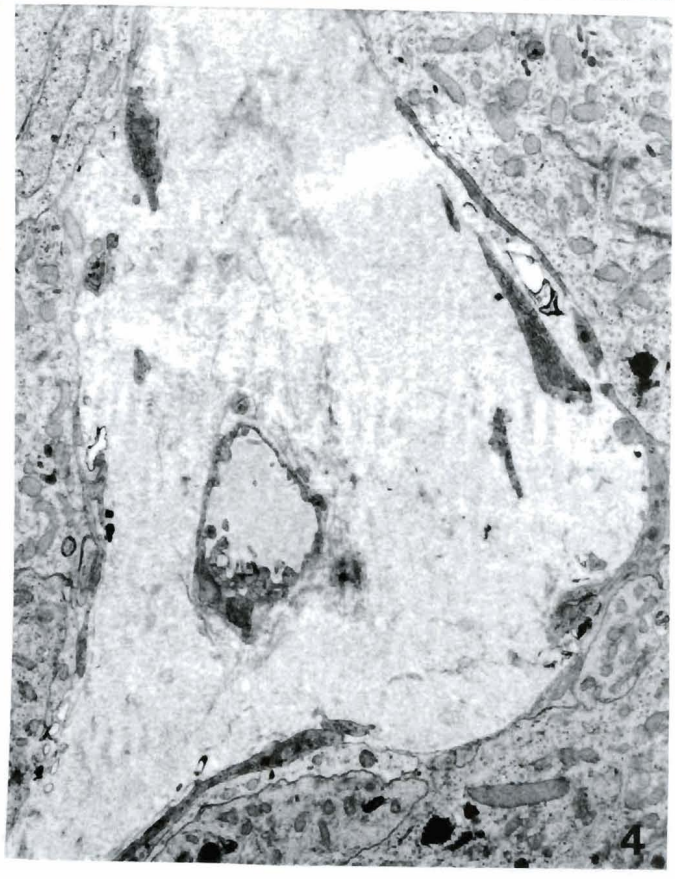
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Table 1. Effects of sympathetic denervation on pineal gland morphometric measurements*

	Control	DX 7 D	DX 60 D	GX 7 D	GX 60 D	P**
Connective tissue spaces[1]	437 ± 48	1283 ± 98	563 ± 83	715 ± 79	733 ± 106	0.001
Pineal glial cells[1]	309 ± 30	412 ± 87	591 ± 110	262 ± 47	319 ± 41	0.01
Pinealocytes[1]	7120 ± 69	5985 ± 124	6734 ± 176	6821 ± 91	6784 ± 133	0.001
Nucleus						
Total area[1]	1132 ± 68	1215 ± 63	1530 ± 89	1358 ± 44	1367 ± 69	0.01
Number[3]	11.6 ± 0.9	14.4 ± 0.8	14.3 ± 0.7	15.9 ± 0.5	13.1 ± 0.6	0.01
Perimeter[2]	60.6 ± 1.8	56.9 ± 4.1	58.3 ± 1.9	57.2 ± 0.9	56.5 ± 1.4	N.S.
Nucleolus						
Total area[1]	37.2 ± 3.8	22.9 ± 3.1	30.1 ± 3.4	40.2 ± 4.5	32.8 ± 3.0	0.001
Number[3]	4.6 ± 0.2	4.5 ± 0.2	5.3 ± 0.1	5.4 ± 0.1	5.8 ± 0.2	0.01
Cytoplasm[1]	5978 ± 85	4758 ± 92	5196 ± 105	5449 ± 66	5422 ± 124	0.001
Lipid droplets						
Total area[1]	261 ± 19	51.6 ± 6.5	67.4 ± 9.1	52.1 ± 5.3	45.2 ± 4.7	0.001
Number[3]	65.1 ± 3.9	24.5 ± 2.5	16.3 ± 2.2	31.7 ± 1.9	16.4 ± 2.8	0.001
Mean diameter[2]	2.10 ± .07	1.78 ± .04	2.03 ± 0.1	1.51 ± .09	1.89 ± .06	0.001

*Values are mean ± standard error of the mean. Total area of the micrograph = 7850 μm^2.
Measuring units: [1] = μm^2
[2] = μm
[3] = number of profiles per micrograph
** p = level of significance according to kruskal-Wallis H test

Table 2. Relationships among components of the pineal gland

	Control	DX 7D	DX-60D	GX 7D	GX-60D
Percentage of pineal components[1]					
Connective tissue spaces	5.56	16.73	7.14	9.18	9.35
Pineal glial cells	3.93	5.37	7.50	3.36	4.07
Pinealocytes: Nucleus	14.41	15.85	19.42	17.45	17.43
Cytoplasm	76.10	62.05	65.94	70.01	69.15
Parenchymal cell area/ connective tissue space area	17	4.98	13.01	9.9	9.69
Pinealocytes					
Mean nuclear area[2]	97.3	84.3	106.9	85.4	104.3
Mean nucleolar area[3]	8.08	5.08	5.68	7.44	5.75
Nuclear area/ cytoplasmic area	0.19	0.25	0.29	0.25	0.25
Percentage of pinealocyte cytoplasm occupied by lipid droplets[4]	4.36	1.08	1.29	0.95	0.83

[1]Surface of the pineal components expressed as a percentage of total area of the micrograph.
[2]Total surface area per micrograph of the pinealocyte nuclei / number of nuclei per micrograph.
[3]Total surface area per micrograph of the pinealocyte nucleoli / number of nucleoli per micrograph.
[4]Surface of lipid droplets expressed as a percentage of the pinealocyte cytoplasm surface area.

fibres was found. Thus, the smaller proportion parenchyma/stroma should indicate a decrease of parenchymal volume rather than an increase of pineal stroma. The great area of connective tissue spaces found in the DX group seven days after surgery was transitory, decreasing at the 60 day stage. Bearing in mind that the SCG innervate cephalic blood vessels, this initial high profile area may be merely a consequence of some transient disturbance in pineal circulation such as edema.

With regard to the pineal cell types, the area occupied by the pinealocytes decreased in both experimental groups. This probably accounts for the smaller parenchymal areas found. The profile areas of pineal glial cells also increased in experimental groups, essentially in the DX group.

Most of the parameters included in this study refer to pinealocytes, the main cell type of the rat pineal gland. Both the nucleus/cytoplasmic quotient and the number of pinealocyte nuclei increased in denervated rats. Seven days after damage, the mean area of these nuclei was however smaller than that of control rats, although it evolved to values close to those of the control group in the 60 day stage. All these changes seem to indicate that sympathetic denervation results in a marked decrease in cytoplasmic volume in pinealocytes. There was no easy explanation for the unchanged mean nuclear perimeter together with changing nuclear profile areas. This suggests that invaginations of the nuclear envelope may increase in decreasing nuclei. On the other hand, a small nuclear size could also increase the probability of tangential sections of the nuclei providing small values of the profile area together with large values of nuclear perimeter. Finally, the mean area of the nucleolus was also smaller in experimental groups.

Lipid droplets of pinealocyte were dramatically decreased in denervated rats. This decrease was evident not only in the total profile area, number and mean diameter of the droplets but also in the percentage of pinealocyte cytoplasm occupied by lipid droplets. On average, experimental groups showed values 4 to 5 times smaller than the control group.

Several animal species show circadian rhythms in some of the components of the pineal gland. However, the results obtained vary and are even contradictory not only among species (Welsh et al., 1979; Dombrowsky and McNulty, 1984; Matsushima et al., 1989) but also within the same species (Quay and Renzoni, 1966; Lew et al., 1984; Diehl, 1984). Despite the disagreements on the exact nature of the circadian morphological changes, the possibility exists that our results are a consequence of such rhythms. Nevertheless, this possibility could be ruled out for the following reasons. Firstly, animals of our study were kept under a controlled lighting schedule (14L:10D) and sacrificed at the same hour (11 a.m.) Secondly, numerous studies have demonstrated that the GX determines a loss of biochemical circadian rhythms in the pineal gland (Pellegrino de Iraldi et al., 1963; Fiske, 1964; Wurtman et al., 1964; Eichler and Moore, 1971; Klein et al., 1971; Moore and Rapport, 1971; Deguchi and Axelrod, 1972; Nagle et al., 1973; Gallardo and Tramezzani, 1975). Furthermore, Benson and Krasovitch (1977) reported a loss of the rhythm in the number of granular vesicles in mouse pinealocytes after GX.

According to our results, sympathetic denervation of rat pineal gland is followed by a hypofunction of pinealocytes. Similar findings have been previously reported in species other than rats after GX (Lin et al., 1973, 1975; Romijn, 1975; Welsh et al., 1979; Karasek et al., 1983). In a quantitative study on the effects of GX on rat pineal synaptic ribbons, King and Dougherty (1982) reported a contraction of Golgi complex and granular endoplasmic reticulum of pinealocytes. These findings are also interpreted as signs of hypoactivity of pinealocytes. However, these authors did not find other changes in pinealocyte organelles or nuclei.

The effects of DX and GX on pineal gland ultrastructure differed quantitatively. In addition to an increase in the populations of pineal glial cells, the pinealocytes of the DX group showed smaller cytoplasmic and nucleolus profile areas. On the contrary, although pinealocyte lipid droplets were decreased in both experimental groups, the DX group showed slightly higher values. The morphological effects of DX are largely unknown. Similar effects of GX and DX have been described in species other than the rat (ferret: Trueman and Herbet, 1970; rabbit: Romijn, 1975; mouse: Kachi and Ito, 1977). In the parameters studied, we have found the same qualitative responses, but quantitative differences were observed mostly seven days after operation. This may suggest that, perhaps through somewhat different mechanisms, both lesions result in a similar atrophy of pinealocytes. With regard to the DX mechanism of action, Parfitt et al. (1972) have studied co-cultures of pinealocytes and SCG neurons, and in vitro situation which resembles DX. From the results obtained, these authors concluded that SCG neurons, in the absence of stimuli are continuously secreting a ß-agonist which acts on pinealocytes.

Comparisons of results obtained at 7 and 60 days showed an evolution of some of the parameters studied in both experimental groups. The proportion of pineal gland constituents was almost unchanged. As discussed above, the very large connective tissue spaces found at 7 days after DX were reduced at the 60 day stage. Mean nuclear area and number of nuclei seemed to tend toward values closer to control group values. Decreased area of nucleoli showed no changes, being even less at 60 days in the GX group. Lipid droplets maintained their low values. Lipid droplet population was however different. At 60 days after denervation, lipid droplets were scarcer, but larger than in 7 days stage.

References

Benson B. and Krasovich M. (1977). Circadian rhythms in the number of granulated vesicles in the pinealocytes of mice. Effect of sympathectomy and melatonin treatment. Cell Tissue Res. 184, 499-506.

Boya J. and Calvo J. (1984). Structure and ultrastructure of the

aging rat pineal gland. J. Pineal Res. 1, 83-89.
Calvo J. and Boya J. (1983). Postnatal development of cell types in the rat pineal gland. J. Anat. 137, 185-195.
Calvo J. and Boya J. (1984). Ultrastructure of the pineal gland in the adult rat. J. Anat. 138, 405-409.
Calvo J., Boya J., Borregón A. and García-Mauriño J.E. (1988). Presence of glial cells in the rat pineal gland: a light and electron microscopic immunohistochemical study. Anat. Rec. 220, 424-428.
Cardinali D.P., Vacas M.I. and Gejman P.V. (1981). The sympathetic superior cervical ganglia as peripheral neuroendocrine centers. J. Neural Transm. 52, 1-21.
Deguchi T. and Axelrod J. (1972). Induction and superinduction of serotonin N-acetyltransferase by adrenergic drugs and denervation in rat pineal organ. Proc. Natl. Acad. Sci. USA 69, 2208-2211.
Diehl B.J.M., Heldbüchel U., Welker H.A. and Vollrath L. (1984). Day/night changes of pineal gland volume and nucleolar size assesed over 10 consecutive days. J. Neuroal Transm. 60, 19-29.
Dombrowski T.A. and McNulty J.A. (1984). Morphometric analysis of pineal complex in the golden hamster over a 24-hour light: dark cycle. I. The superficial pineal in untreated and optically enucleated animals. Am. J. Anat. 171, 359-368.
Eichler V.B. and Moore R.Y. (1971). Pineal hydrxyindole-O-methyltransferase and gonadal responses to blinding or continuous darkness blocked by pineal denervation in the male hamster. Neuroendocrinol. 8, 81-85.
Fiske V.M. (1964). Serotonin rhythms in the pineal organ: control by the sympathetic nervous system. Science 146, 253-254.
Gallardo G.M.P. and Tramezzani J.H. (1975). Hydroxyindole-O-methyltransferase activity in the pineal gland of the rabbit. J. Neural Transm. 36, 51-57.
Kachi T. and Ito I. (1977). Neural control of glycogen content and its diurnal rhythm in mouse pineal cell. Am. J. Physiol. 232, 584-589.
Karasek M. (1983). Ultrastructure of the mammalian pineal gland: its comparative and functional aspects. In: Pineal Research Reviews. Vol. 1. Reiter R.J. (Ed.). Alan R. Liss. New York. pp 1-48.
Karasek M., Petterborg L.J., King T.S., Hansen R.J. and Reiter R.J. (1983). Effect of superior cervical ganglionectomy on the ultrastructure of the pinealocyte in the cotton rat (Sigmodon hispidus). Gen. Comp. Endocrinol. 51, 131-137.
King T.S. and Dougherty W.J. (1982). Effect of denervation on «synaptic» ribbon populations in the rat pineal gland. J. Neurocytol. 11, 19-28.
Klein D.C., Weller J.L. and Moore R.Y. (1971). Melatonin metabolism: neural regulation of pineal serotonin: acetyl coenzime A N-acetyltransferase activity. Proc. Natl. Acad. Sci. USA 68, 3107-3110.
Korf M.W. and Möller M. (1984). The innervation of the mammalian pineal gland with special reference to central pinealopetal projections. In: Pineal Research Reviews. Vol. 2. Reiter R.J. (ed). Alan R. Liss. New York. pp 41-86.
Lew G.M., Washko K. and Quay W.B. (1984). Quantitation of ultrastructural twenty-four hour changes in pineal nuclear dimensions. J. Pineal Res. 1, 61-68.
Lin H.S., Hwang R.B. and Tseng C.Y. (1975). Fine structural changes in the hamster pineal gland after blinding and superior cervical ganglionectomy. Cell Tissue Res. 158, 285-299.
Lin H.S., Tseng C.Y. and Hwang H.B. (1973). Effect of blinding and superior cervical ganglionectomy on the fine structure of the hamster pineal gland and adenohypophysis. Ant. Rec. 175, 508-509.
Matsushima S., Sakai Y. and Hira Y. (1989). Twenty four-hour changes in pinealocytes, capillary endothelial cells and pericapillary and intercellular spaces in the pineal gland of the mouse. Semiquantitative electron-microscopic observations. Cell Tissue Res. 255, 323-332.
Moore R.Y. and Rapport R.L. (1971). Pineal and gonadal function in the rat following cervical sympathectomy, pinealectomy or blinding. Neuroendocrinology 7, 361-374.
Nagle C.A., Cardinali D.P. and Rosner J.M. (1973). Retinal and pineal hydroxyindole-o-methyl transferases in the rat: changes following cervical sympathectomy, pinealectomy or blinding. Endocrinology 92, 1560-1565.
Parfitt A.G., Freschi J.E., Shain W.G., Schmechel D.A., Auerbach J.N. and Turner J.N. (1982). Pineal cells: culture and co-culture with neurons from the superior cervical ganglia. In: Melatonin rhythm generating system. Developmental aspects. Klein D.C. (ed). Karger. Basel. pp 62-83.
Pellegrino de Iraldi A., Zieher L.M. and De Robertis E. (1963). The 5-hydroxytryptamine content and synthesis of normal and denervated pineal gland. Life Sci. 2, 691-696.
Quay W.B. (1971). Effects of cutting nervii conarii and tentorium cerebelli on pineal composition and activity shifting following reversal of photoperiod. Physiol. Behav. 6, 681-688.
Quay W.B. and Renzoni A. (1966). Twenty-four-hour rhythms in pineal mitotic activity and nuclear and nucleolar dimensions. Growth 30, 315-324.
Romijn H.J. (1975). The ultrastructure of rabbit pineal gland after sympathectomy, parasympathectomy, continuous illumination, and continous darkness. J. Neural Transm. 36, 183-194.
Szurszewski J.H. (1982). Physiology of mammalian prevertebral ganglia. Ann. Rev. Physiol. 43, 53-68.
Trueman T. and Herbert J. (1970). Monoamines and acetylcholinesterase in the pineal gland and habenula of the ferret. Z. Zellforsch 109, 83-100.
Vollrath L. (1981). The pineal organ. Handbuch der mikroskopischen Anatomie des Menschen. VI/7. Springer Verlag. Berlin.
Weibel E.R. (1979). Stereological methods. Volume 1: Practical methods for biological morphometry. Academic Press. London.
Welsh M.G., Hansen J.T. and Reiter R.J. (1979). The pineal gland of the gerbil, Meriones unguiculatus. III. Morphometric analysis and fluorescence histochemistry in the intact and sympathetically denervated pineal gland. Cell Tissue Res. 204, 111-125.
Wurtman R.J., Axelrod J. and Fischer J.E. (1964). Melatonin synthesis in the pineal gland: effect of light mediated by the sympathetic nervous system. Science 143, 1328-1330.

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