Neuroendocrinology Letters ISSN 0172–780X

ORIGINAL ARTICLE

The age-related quantitative ultrastructural changes in pinealocytes of gerbils

Jacek Swietoslawski

Laboratory of Electron Microscopy, Chair of Pathomorphology, Medical University of Lodz, Lodz, Poland

Correspondence to: Jacek Swietoslawski, M.Sc., Laboratory of Electron Microscopy, Chair of Pathomorphology, Medical University of Lodz, 92-216 Lodz, Czechoslowacka 8/10, Poland
TEL/FAX: +48 42 675 7613
E-mail: mikrojs2@polbox.com

Submitted: *September 23, 1999*
Accepted: *October 28, 1999*

Key words: **pineal gland; pinealocyte; gerbil; ultrastructure; aging**

*Neuroendocrinology Letters 1999; **20**:391–396 pii: NEL200699A05*

Abstract

OBJECTIVES. Relatively few ultrastructural studies of the pineal gland of aging animals have been published. The pineal gland of the gerbil is especially interesting in respect to aging because of its progressive calcification with age, and this species has been considered as an excellent model for research on aging. Therefore, the aim of the present study was to examine the quantitative ultrastructure of pinealocytes of the gerbil in three different age groups.

METHODS. Three groups of animals were included in the study: 1-month-old, 3-month-old, and 14-month-old. Cross-sectional areas of the pinealocyte and its nucleus and relative volumes of the following cell organelles: mitochondria, lysosomes, Golgi apparatus, granular endoplasmic reticulum and calcareous concretions as well as the number of dense-core vesicles and "synaptic" ribbons were analyzed.

RESULTS. No age-dependent changes were observed in the size of pinealocytes and their nuclei. The relative volume of mitochondria and the number of dense-core vesicles increased progressively with age, and that of lysosomes was lowest in the 1-month-old animals and increased at age of 3 and 14 months, whereas a decrease in the relative volume of granular endoplasmic reticulum was observed in 3- and 14-month-old gerbils in comparison with 1-month-old animals. No difference was observed in relative volume of Golgi apparatus and in the number of "synaptic" ribbons. The most striking change was observed in the formation of calcareous concretion within the pineal with age. The pineal gland of 1-month-old gerbils was essentially devoid of these structures, their number and size in 3-month-old animals were moderate, and increased dramatically in 14-month-old animals.

CONCLUSION. The ultrastructural features of the gerbil pinealocyte in all examined age groups point to high metabolic activity of these cells.

Introduction

Decline in pineal melatonin secretion with age has been proven in many studies in different species, including humans [1]. This has been also shown in such rodents as hamster [2, 3] and rat [4, 5]. However, relatively few ultrastructural studies of the pineal gland of aging animals have been published. The pineal gland of the gerbil is especially interesting in respect to aging because of its progressive calcification with age [6], and this species has been considered as an excellent model for research on aging [7]. Therefore, the aim of the present study was to examine the quantitative ultrastructure of pinealocytes of the gerbil in three different age groups.

Material and Methods

Twelve male gerbils of three different age groups (1-, 3- and 14-month-old; 4 animals per each group) housed in a room with controlled illumination (LD 12:12; light on at 06:00h) and temperature (22±2°C) were used in the experiment. Standard laboratory food and tap water were available ad libitum. The animals were killed by decapitation between 09:00h and 10:00h. The pineal glands were removed immediately after decapitation and immersion-fixed in 3.5% glutaraldehyde in 0.1 M cacodylate buffer, post-fixed in 1% osmium tetroxide, and embedded in Epon. Thin sections were stained with uranyl acetate and lead citrate, and examined under a JEM 100B electron microscope.

For quantitative estimation 10 micrographs at magnification of x 3,000, and 30 micrographs at magnification of x 10,000 were taken from each gland using slightly modified systematic random sampling method [8]. Every upper right corner of the grid aperture in which pinealocytes were present was photographed. Altogether, 480 prints were used for quantitative study. A digital analyzer connected on-line to an IBM-PC computer (Logitex, Poland) was used to obtain the morphometric data. For estimation of the cross-sectional areas of pinealocyte and its nucleus the prints were enlarged photographically to x 7,500, whereas for estimation of the relative volume of cell organelles the prints were enlarged photographically to x 25,000. The relative volume of the following cytoplasmic organelles was analyzed: mitochondria, lysosomes, Golgi apparatus and granular endoplasmic reticulum. In addition, numerical density of dense-core vesicles (expressed as number per 50 μm^2) was estimated. The relative volume of calcareous deposits was estimated after analyzing of the pineal gland sections covering 10 grid apertures (20,250 μm^2). For the quantification of "synaptic" ribbons (expressed as number per 20,000 μm^2) the tissue overlying 10 grid apertures, each measuring 45 x 45 μm^2, was scanned at x 15,000.

Statistical analysis of the data was performed using ANOVA and LSD (least significant difference) method according to Statgraphic plus V4 computer program. Additionally, the lineal correlation coefficient was determined.

Results

Generally, the ultrastructure of gerbil pinealocytes resembles that of other rodents. The details of the ultrastructure of the gerbil pinealocytes, predominant cell element in the pineal gland of this species, have been described by Welsh and Reiter [9] and will not be repeated here.

No age-dependent differences were found in the cross-sectional areas of the pinealocyte and its nucleus (Fig. 1) and in the relative volume of Golgi apparatus (Fig. 2) as well as in the number of "synaptic"

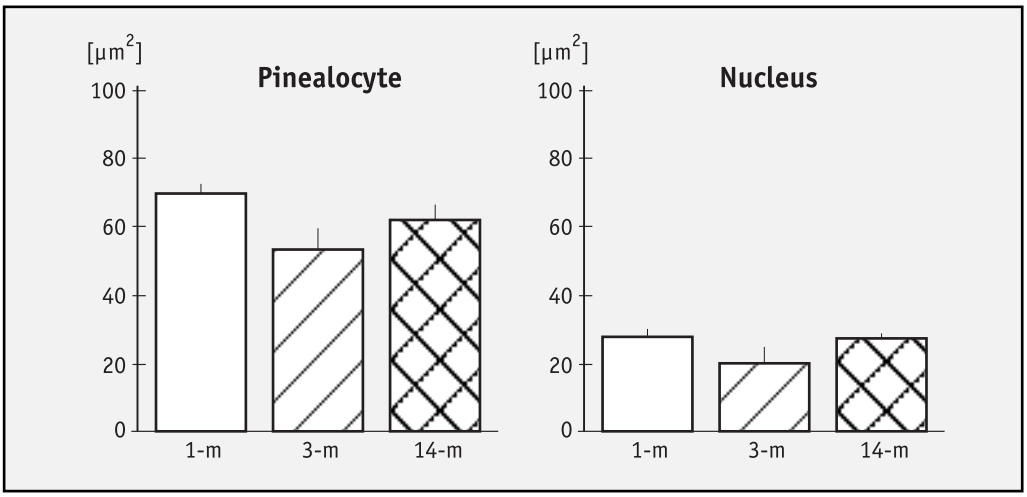

Fig. 1. Cross-sectional areas of the pinealocyte and its nucleus in 1-, 3- and 14-month-old gerbils.

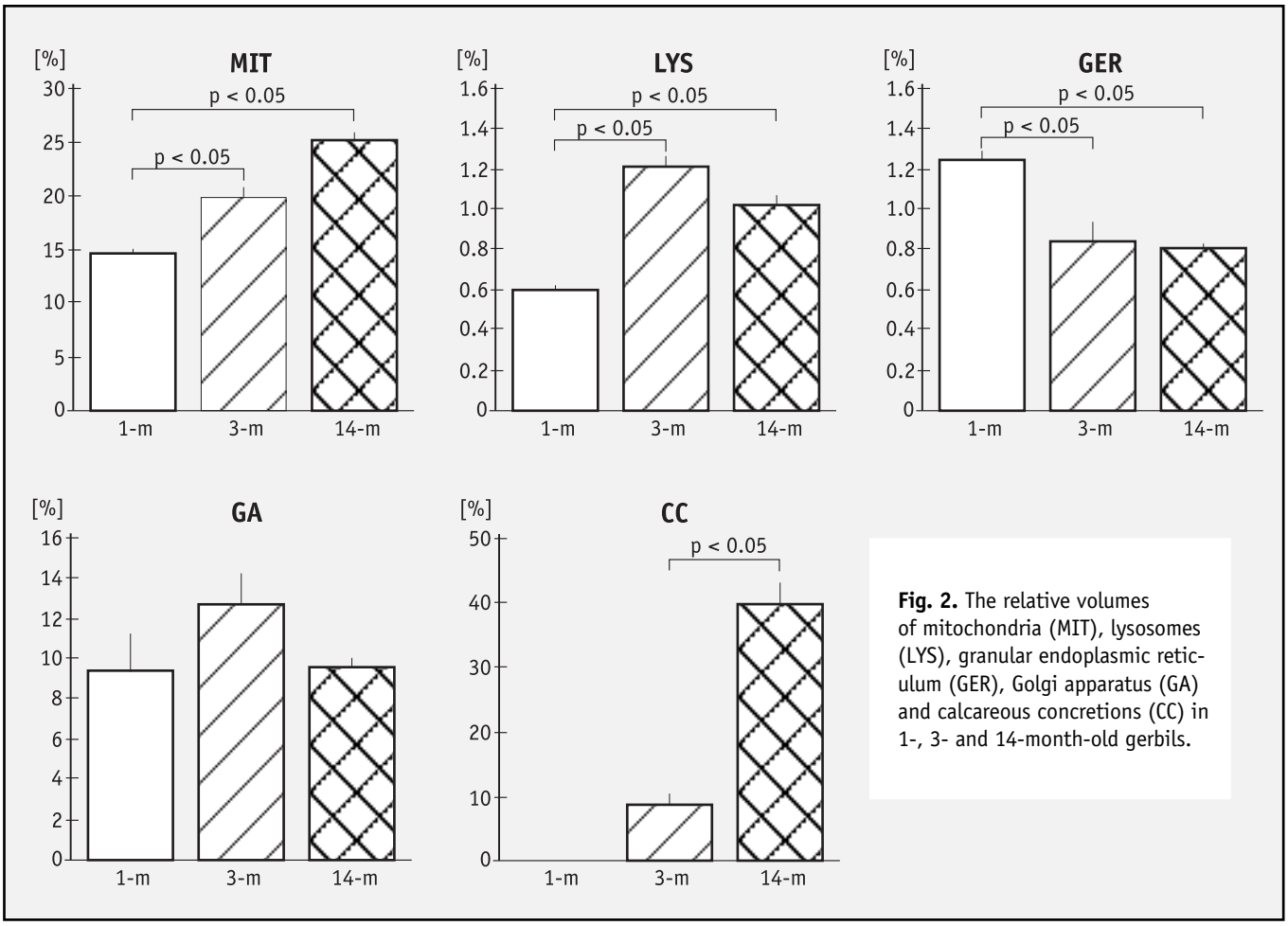

Fig. 2. The relative volumes of mitochondria (MIT), lysosomes (LYS), granular endoplasmic reticulum (GER), Golgi apparatus (GA) and calcareous concretions (CC) in 1-, 3- and 14-month-old gerbils.

ribbons (Fig. 3). The relative volume of mitochondria (Fig. 2) and the number of dense-core vesicles (Fig. 3) increased progressively with age, and the relative volume of lysosomes was lowest in the 1-month-old animals and increased at age of 3 and 14 months, whereas a decrease in the relative volume of granular endoplasmic reticulum was observed in 3- and 14-month-old gerbils in comparison with 1-month-old animals (Fig. 2). The most conspicuous observation concerns calcareous concretions. The pin-eal gland of 1-month-old gerbils is essentially devoid of these structures, and their relative volume increased significantly in 3-month-old, and especially in 14-month-old animals (Fig. 2). The increase between 3rd and 14th months of age was due to increased number of concretions as well as their size.

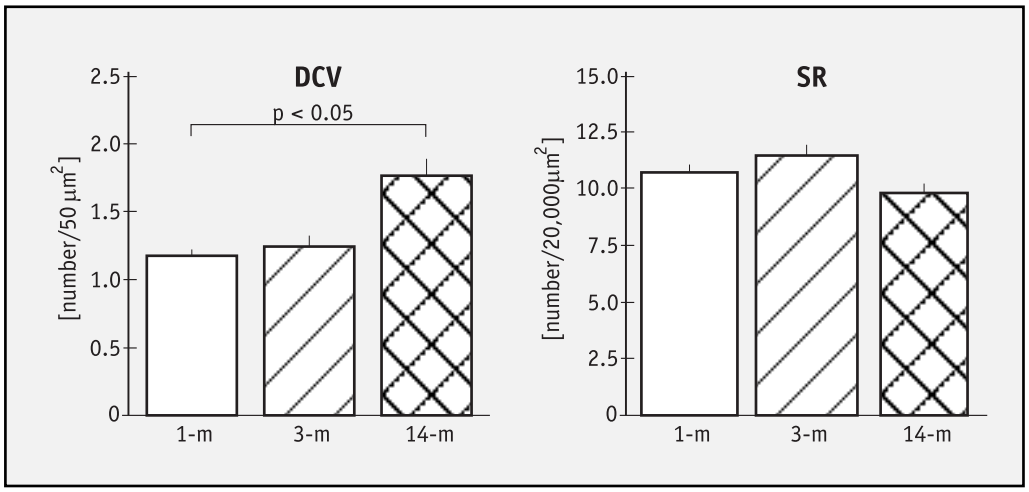

Fig. 3. The numerical density of dense-core vesicles (DCV) and "synaptic" ribbons (SR) in 1-, 3-, and 14-month-old gerbils.

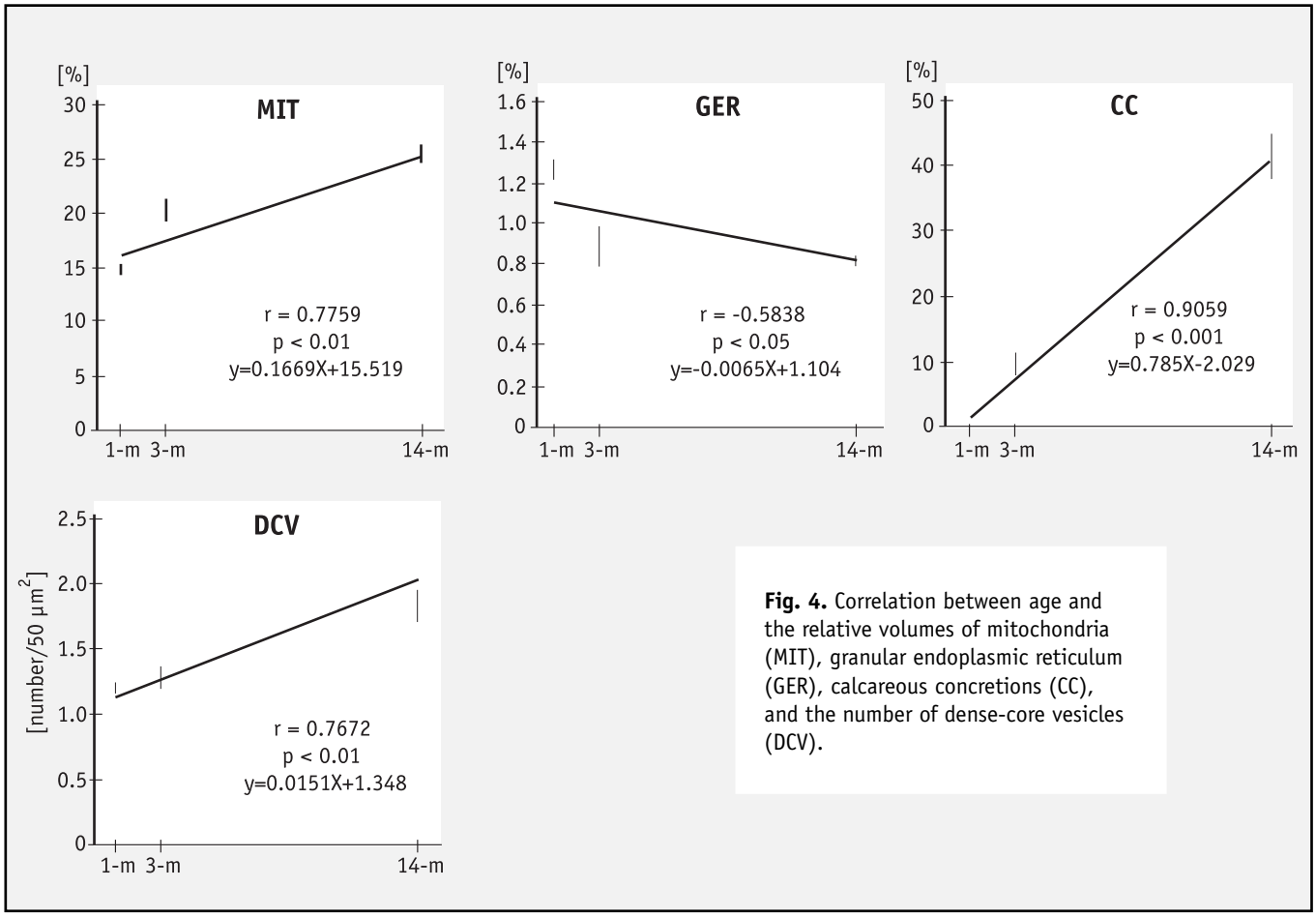

Fig. 4. Correlation between age and the relative volumes of mitochondria (MIT), granular endoplasmic reticulum (GER), calcareous concretions (CC), and the number of dense-core vesicles (DCV).

There was positive correlation between age and relative volumes of mitochondia and calcareous concretions, and number of dense-core vesicles, and negative correlation between age and relative volume of granular endoplasmic reticulum (Fig. 4).

Discussion

The ultrastructural studies on the changes in pinealocytes during aging are very rare. The most commonly studied species in this respect is the rat. However, even in this species there are no quantitative, morphometric data. In a qualitative study Johnson [10] reported increases in rat pinealocyte dense-core vesicles, nuclear inclusions, and dense bodies similar in appearance to lipofuscin deposits as well as in deep nuclear invaginations in old animals. The presence of abundant dense (lipofuscin) bodies in aged rat pinealocytes has been also observed by Allen et al. [11]. Age-associated increase in dense bodies containing acid phosphatase has been observed in the rat pinealocytes [12]. Also Karasek [13] reported increased number of lysosomes and lipid droplets in 12-month-old rat in comparison with 21-day-old animals.

Quantitative comparison of pinealocyte ultrastructure in 3-month-old and 28-month-old white-footed mice revealed decreases in the area of the Golgi apparatus and in the number of dense-core vesicles in old animals, with no differences in the areas of mitochondria, granular endoplasmic reticulum and lysosomes. No apparent changes between both age groups have been observed also in pinealocyte nuclear or cytoplasmic areas [14].

It should be stressed that gerbil is a rodent with relatively long lifespan, with maximum survival of about 50 months [15], and reproductively competent even until after 2 years [16]. It has been shown that plasma LH and prolactin levels in this species were similar in three different age groups (3–4-, 11–13- and 20–25-month-old), and FSH concentrations tended to increase with age [17]. Therefore, only our first studied group (1-month-old) was immature, and the remaining two groups were sexually active.

The observed ultrastructural features of pinealocytes indicate high metabolic activity of these cells in all studied groups. The relative volume of mitochondria and number of dense-core vesicles increased with age. The relative volume of lysosomes was higher in older animals, similarly to increase in these organelles observed in the rat [11–13]. The relative volume of Golgi apparatus remained stable, whereas granular endoplasmic reticulum was the only studied cell organelle where relative volume

decreased with age.

Our results are in agreement with similar diurnal melatonin secretion patterns in 2- and 15-month-old gerbils [18], indicating similar pineal activity in both age groups.

The most striking change was observed in the formation of calcareous concretion within the pineal. The pineal gland of immature gerbils was essentially devoid of these structures, their number and size in 3-month-old animals was moderate, and increased dramatically in 14-month-old animals.

The presence of calcareous concretions (corpora arenacea, acervuli, brain sand) in the human pineal gland has been well known for a long time [19, 20]. However, concretions have also been found in the pineal of other mammalian species, including ox, sheep, donkey and horse [19], cow [21, 22], rhesus monkey [21, 23], baboon [24], pig [25, 26], and also in common laboratory animals: rat [11, 23, 27, 28], in white guinea pig [29], and especially abundantly in gerbil [6, 9, 30–32]. Human and gerbil acervuli are composed of hydroxyapatite or carbonate apatite with traces of strontium and other oligoelements, and their matrix consists of glycoproteins and proteoglycans of unknown nature [32]. The results of our study are in agreement with general observations that the pineal concretions are absent at birth and increase progressively with age [6, 33]. Although several observations have been made concerning the number of calcareous concretions in the gerbil pinealocytes under various experimental conditions [34–37], especially when pineal sympathetic innervation is manipulated, the functional meaning of the concretions is still unclear. It seems probable, however, that concretions should not be correlated with atrophic processes in the pineal gland but could be somehow related to pineal secretory processes [21, 38] or may represent traces of the histophysiological history of the pineal gland and be indicative of the intensity of secretory processes [39].

Acknowledgments

The author expresses his appreciation to Prof. Dr. Michal Karasek for his stimulating interest and help in the present work. The study is a part of doctoral thesis and was supported by a grant from the Medical University of Lodz, No. 502-11-399.

REFERENCES

1 Reiter RJ. The ageing pineal gland and its physiological consequences. BioEssays 1992; **14**:169–75.
2 Reiter RJ, Richardson BA, Johnson LY, Ferguson BN, Dinh DT. Pineal melatonin rhythm: reduction in aging Syrian hamster. Science 1980; **210**:1372–3.
3 Pang SF, Tang PL. Decreased serum and pineal concentrations of melatonin and N-acetylserotonin in aged male hamsters. Hormone Res 1983; **17**:228–34.
4 Reiter RJ, Craft CM, Johnson LY, King TS, Richardson BA, Vaughan GM, et al. Age-associated reduction in nocturnal pineal melatonin levels in female rats. Endocrinology 1981; **109**:1295–7.
5 Pang SF, Tang F, Tang PL. Negative correlation of age and the levels of pineal melatonin, pineal N-acetylserotonin, and serum melatonin in male rats. J Exp Zool 1984; **229**:41–7.
6 Japha JL, Eder TJ, Goldsmith ED. Calcified inclusion in the superficial pineal gland of the Mongolian gerbil, *Meriones unguiculatus*. Acta Anat (Basel) 1976; **95**:533–44.
7 Cheal ML. The gerbil: a unique model for research on aging. Exp Aging Res 1986; **12**:3–21.
8 Weibel RR. Stereological methods. Vol.1. London: Academic Press; 1979.
9 Welsh MG, Reiter RJ. The pineal gland of the gerbil, *Meriones unquiculatus*. In: An ultrastructural study. Cell Tissue Res 1978; **193**:323–36.
10 Johnson JE, Jr. Fine structural alterations in the aging rat pineal gland. Exp Aging Res 1980; **6**:189–211.
11 Allen DJ, DiDio LJA, Gentry ER. The aged rat pineal gland as revealed in SEM and TEM. Age 1982; **5**:119–26.
12 Bondareff W. Electron microscopic study of the pineal body in aged rats. J Gerontol 1965; **20**:321–7.
13 Karasek M. Zaleznosc ultrastruktury szyszynki szczura od wieku. [(The dependence of white rat pineal gland ultrastructure on age.) (In Polish with English abstract)] Endokrynol Pol 1974; **25**:275–87.
14 King TS, Karasek M, Petterborg LJ, Hansen JT, Reiter RJ. Effects of advancing age on the ultrastructure of pinealocytes in the male white-footed mouse (*Peromyscus leucopus*). J Exp Zool 1982; **224**:127–34.
15 Vincent AL, Rodrick GE, Sodeman WA. The Mongolian gerbil in aging research. Exp Aging Res 1980; **6**:249–60.
16 Cheal M. Lifespan ontogeny of breeding and reproductive success in Mongolian gerbils. Lab Anim 1983; **17**:240–5.
17 Parkening TA, Collins TJ, Smith ER. Plasma and pituitary concentrations of LH, FSH and prolactin in aging Mongolian gerbils. Exp Gerontol 1984; **19**:359–65.
18 King TS, Richardson BA, Reiter RJ. Age-associated changes in pineal serotonin N-acetyltransferase activity and melatonin content in male gerbil. Endocr Res Commun 1981; **8**:253–62.
19 del Rio-Hortega P. Pineal gland. In: Penfield W, editor. Cytology and Cellular Pathology of the Nervous System. Vol. 2, New York: Hoeber; 1932. p. 637–703.
20 Bargmann W. Die Epiphysis Cerebri. In: Möllendorf WV, editor. Handbuch der mikroskopischen Anatomie des Menschen. Band VI, 4. Berlin: Springer Verlag; 1943. p. 309–502.
21 Lukaszyk A, Reiter RJ. Histophysiological evidence for secretion of polypeptides by the pineal gland. Am J Anat 1975; **143**:451–64.
22 Kawamura N, Ishibashi T, Miyamoto H. Scanning electron microscopy of brain sand in the bovine pineal gland. Jap J Zootech Sci 1986; **57**:1043–1045.

23 Karasek M. Ultrastructure of the mammalian pineal gland: its comparative and functional aspects. Pineal Res Rev 1983; **1:**1–48.
24 Theron JJ, Biagio RP, Henning CN. Circadian changes in the ultracytochemical localization of calcium in the pinealocytes of the baboon (*Papio ursinus*). Adv Pineal Res 1989; **3**:33–8.
25 Heiniger HJ. Histologie der Epiphyse des Schweines hinsichtlich Geschlecht und Alter. Zeiss-Mitteilungen 1965; **3**:301–53.
26 Lewczuk B, Przybylska B, Wyrzykowski Z. Distribution of calcified concretions and calcium ions in the pig pineal gland. Folia Histochem Cytobiol 1994; **32**:243–9.
27 Erdinc F. Concrement formation in the rat pineal gland. Experientia 1977; **33**:514.
28 Diehl BJM. Occurence and regional distribution of calcareous concretions in the rat pineal gland. Cell Tissue Res 1978; **195**:359–66.
29 Jung D, Vollrath L . Structural dissimilarities in different regions of the pineal gland of Pirbright white guinea pigs. J Neural Transm 1982; **54**:117–28.
30 Reiter RJ, Welsh MG, Vaughan MK. Age-related changes in the intact and sympathetically denervated gerbil pineal gland. Am J Anat 1976; **146**:427–32.
31 Krstic R, Golaz J. Ultrastructural and X-ray microprobe comparison of gerbil and human pineal acervuli. Experientia 1977; **35**:507.
32 Krstic R. Pineal calcification: its mechanism and significance. J Neural Transm 1986; **21** [suppl]: 415–32.
33 Zimmerman RA, Bilaniuk LT. Age-related incidence of pineal calcification detected by computer tomography. Radiology 1982; **142**:659–62.
34 Vaughan MK, Spanel-Borowski K, Karasek M, Champney TH, Reiter RJ. Action of subcutaneous implants or injections of melatonin on reproductive and metabolic variables and pineal concretions in male gerbil. Biomed Res 1983; **4**:329–36.
35 Lewinski A, Vaughan MK, Champney TH, Reiter RJ, Smith NKR. Dark exposure increases the number of pineal concretions in male gerbil (*Meriones unguiculatus*). IRCS Med Sci 1983; **11**:977–8.
36 Champney TH, Joshi BN, Vaughan MK, Reiter RJ. Superior cervical ganglinectomy results in the loss of pineal concretions in the adult male gerbil (*Meriones unguiculatus*). Anat Rec 1985; **211**:465–8.
37 Vaughan MK, Joshi BN, Reiter RJ. Daily propranolol administration reduces pineal concretion formation in the Mongolian gerbil. Proc Soc Exp Biol Med 1986; **182**:372–4.
38 Welsh MG, Beitz AJ. Modes of protein and peptide uptake in the pineal gland of the Mongolian gerbil: an ultrastructural study. Am J Anat 1981; **162**:343–55.
39 Wurtman RJ, Axelrod J, Barchas JD. Age and enzyme activity in the human pineal. J Clin Endocrinol 1964; **24**: 299–301.

Neuroendocrinology Letters ISSN 0172–780X