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Sex-related differences in the nuclear population of postpubertal rat pineal gland. A quantitative study

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Summary. Male and female parenchymal pineal cell types have been studied throughout postpubertal development to determine the existence of sex-related differences on a time basis. Six age groups (2, 3, 4, 8, 15 and 24 months) of eight rats (4 males and 4 females) were used in this study. Nuclei of both parenchymal pineal cell types were counted in 5 areas of 26.377 μm^2 per pineal gland on semithin sections. Nonparametric statistics of our results (Mann-Whitney U-test and Kruskal-Wallis H-test) demonstrated significant differences between male and female pinealocytes through the stages studied. In all age groups, the number of nuclei per unit area was larger in female rats. Pineoglial cells did not show significant sex-related differences.

Key words: Pineal gland, Pinealocytes, Sex differences, Morphometry, Rat

Introduction

Numerous studies report quantitative modifications of the pinealocytes and their nuclei in relation to different intra or extraglandular factors (for review see Vollrath, 1981). Circadian variations of the pineal morphological parameters are among the most extensively studied phenomena (Quay and Renzoni, 1966; Welsh et al., 1979; Dielh, 1981; Matsushima et al., 1990). Other natural cycles such as seasonal rhythms (Mogler, 1958; Legait et al., 1975; McNulty et al., 1980; Kachi and Quay, 1984) or strual rhythms (Lincoln, 1976; Przybylska et al., 1990) have also been studied. Finally, quantitative morphological differences related to age (Quay and Levine, 1957; Cassano et al., 1961; De Martino et al., 1962; Wallace et al., 1969; Blumfield and Tapp, 1970; Trakulrungsi and Yeager, 1977; Calvo and Boya, 1983, 1984a; Boya and Calvo, 1984; Hira et al., 1989) or even intraglandular regional differences have also been reported (Blumfield and Tapp, 1970; Vollrath, 1979; Boeckmann, 1980; Dielh, 1981; Jung and Vollrath, 1982; Heidbüchel and Vollrath, 1983).

Despite the well known influence of the pineal gland on the reproductive function, potential morphological differences in the pineal gland according to the sex of the animal have scarcely been studied (Vollrath, 1981). So, in rats, Legait et al. (1976), found a greater mean pineal gland volume among females. Santamarina and Venzke (1953) reported a smaller mean pineal gland weight among females. Vollrath (1986) also analyzed sex-related differences in glandular volume in the first postnatal developmental stages. Milcu et al. (1962) described more lipid droplets in male pineal glands. Peinado et al. (1990) observed a larger content of immunoreactive somatostatin in the pineal gland of female rats.

In this work, it has been attempted to assess the existence of a sex-related dimorphism for both parenchymal pineal cell types (pynealocytes and pineoglial cells) thoughout the postpubertal development. With this aim, a quantitative study of the pineal gland cell population was developed.

Materials and methods

Forty-eight adult Wistar albine rats were kept under identical temperature (18° C), lighting (14L:10D) and feeding (ad libitum) conditions. Animals were separated into six groups of 8 rats (4 males and 4 females) according to age (2, 3, 4, 8, 15 and 24 month adult rats).

In order to avoid any potential chronobiological differences, all rats were sacrificed at 18:00 h in a period ranging between the months of April and May. Ether anesthetized rats were decapitated and pineal glands were fixed by immersion in cold 2% glutaraldehyde- 2% paraformaldehyde in 0.1 M (pH 7.4) phosphate buffer. After fixation, each gland was longitudinally scised in

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two halves, washed in 0.1M phosphate buffer, postfixed in 1% osmium tetroxide in the same buffer and embedded in Vestopal. Semithin sections were stained with the silver impregnation method of Klein et al. (1981).

Nuclei of both parenchymal cell types were counted on semithin sections in 5 different areas of 26.377 μm^2 for each pineal gland, using a computerized image analysis system (VIDS IV^R). The statistical analysis and the degree of significance of data from each age interval were performed using the nonparametric Mann-Whitney U-test (Mann and Whitney, 1947). The nonparametric Kruskal-Wallis H-test (Kruskal and Wallis, 1952) was used to assess the overall level of significance of the study. Data were expressed as the average nuclear number per interval and sex ± standard error of the mean (SEM).

Results

Results are shown in Tables 1 and 2. The usual aspect of semithin sections used for counting is shown in Figs. 1-4. Table 1 shows average values for pinealocytes, the main pineal cell type, in each age period studied. Results for the second cell type are reflected in Table 2. The second pineal cell type, known as the interstitial cell (Vollrath, 1981; Karasek, 1983) or type II pinealocyte according to our previous works (Calvo and Boya, 1983, 1984a,b; Boya and Calvo, 1984), are redenominated as pineoglial cell in the present work according to their stated content in different glial cell markers (Schachner et al., 1984; Calvo et al., 1988).

The statistical analysis of results after the application of the Mann-Whitney U-test, demonstrated significant sexual differences ($p < 0.05$) in the number of nuclei and the pinealocytes for all the intervals studied (Table 1). The mean number of nuclei of pinealocytes per surface unit through the period studied was 212.23 ± 4.45 for females, with respect to 162.43 ± 2.74 in males. The application of this same test to the values obtained for pineoglial cells yielded non significative sex-related differences, except for the 15 month old interval (Table 2). After the statistical Kruskal-Wallis H-test analysis for the entire post-pubertal life period, significative results were found for the sex factor both for pinealocytes ($p < 0.001$, Table 1) and for pineoglial cells ($p < 0.01$, Table 2).

Figures 5 and 6 show sexual differences in the average number of nuclei for each pineal cell type in each age interval. As shown in Fig. 5, the number of pinealocyte nuclei per unit area was always larger for

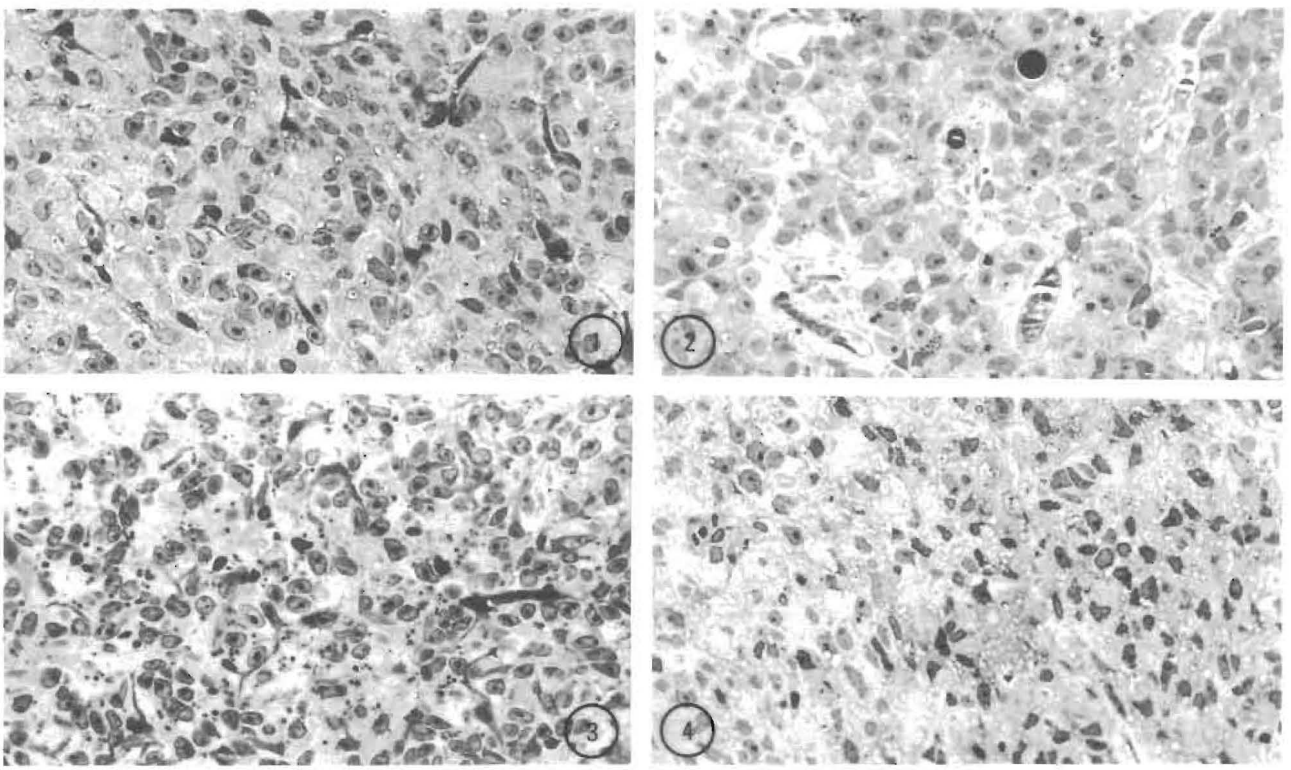

Fig. 1. Two-month-old male rat pineal gland. × 170

Fig. 2. Twenty four-month-old male rat pineal gland. × 170

Fig. 3. Two-month-old female rat pineal gland. × 170

Fig. 4. Twenty four-month-old female rat pineal gland. × 170

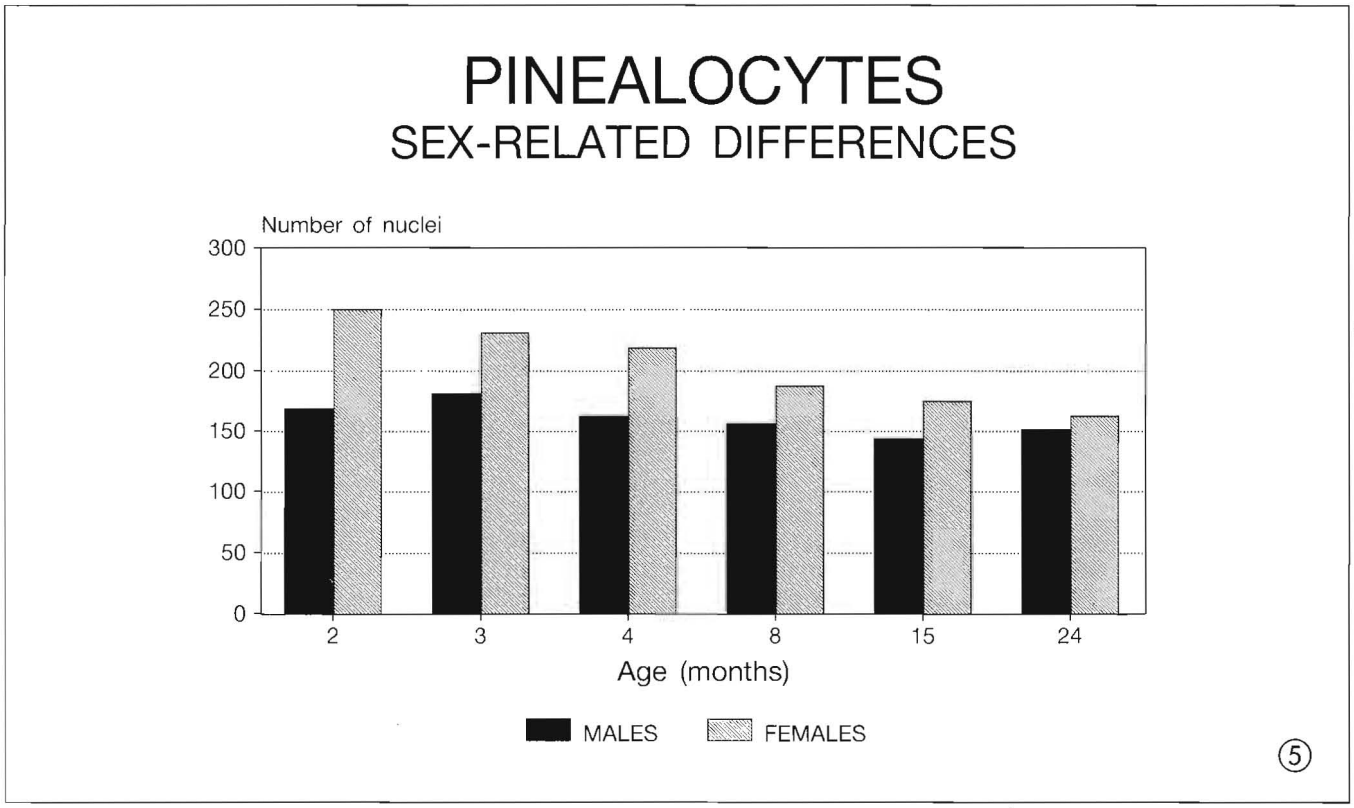

Fig. 5. Graph showing sexual differences in the number of pinealocyte nuclei per surface unit (26,377 μm^2) for the different ages studied.

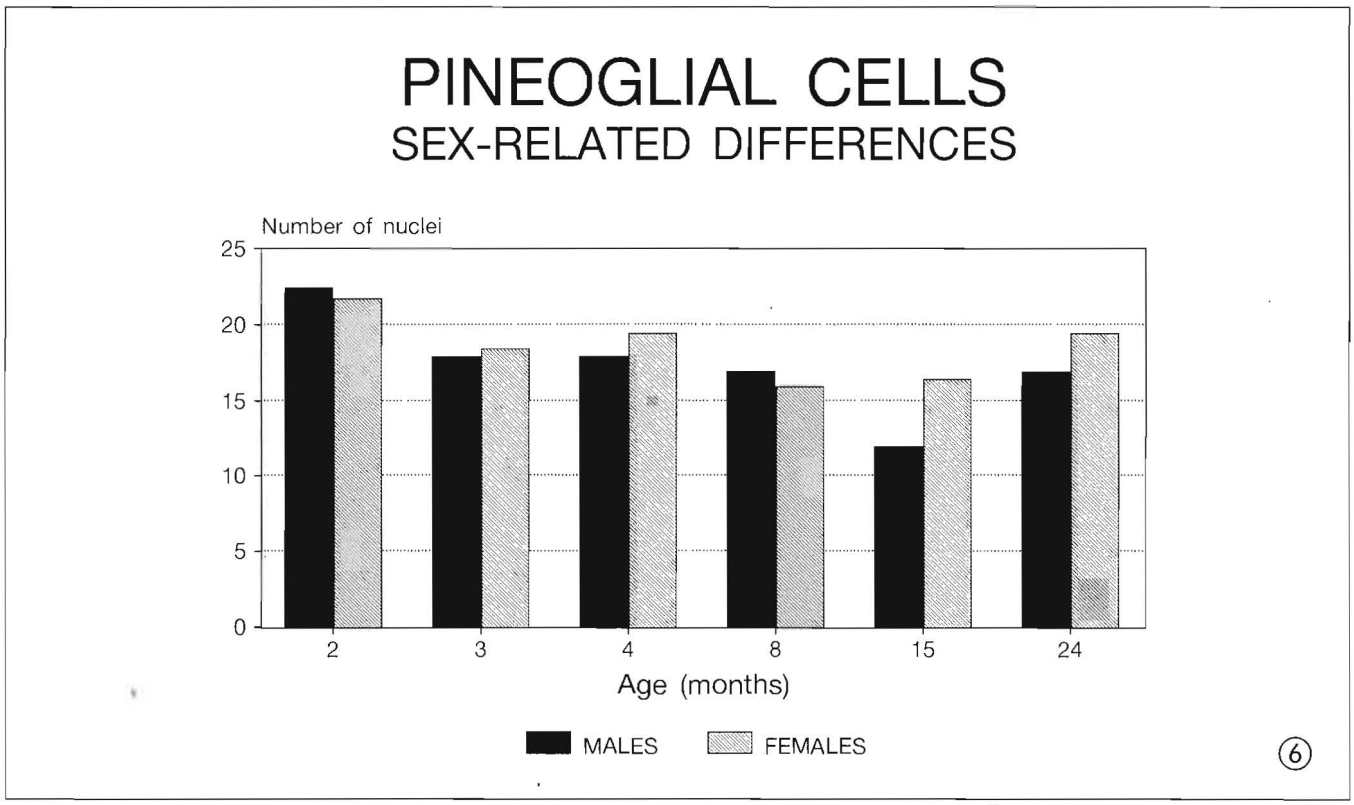

Fig. 6. Graph showing sexual differences in the number of pineoglial nuclei per surface unit (26,377 μm^2) for the different ages studied.

Table 1. Number of pinealocyte nuclei per surface unit during postpubertal development. Morphometric analysis.*

Age (months)	MALES	FEMALES	U***
2	171.87 ± 5.92	251.08 ± 6.78	$p < 0.05$
3	182.00 ± 6.68	230.83 ± 3.82	$p < 0.05$
4	164.00 ± 5.06	220.75 ± 7.65	$p < 0.05$
8	158.75 ± 6.50	192.87 ± 4.85	$p < 0.05$
15	145.12 ± 2.35	177.25 ± 3.79	$p < 0.05$
24	152.87 ± 4.91	167.62 ± 2.88	$p < 0.05$
M**	162.43 ± 2.74	212.23 ± 4.45	
H****	$p < 0.001$		

Table 2. Number of pineoglial nuclei per surface unit during postpubertal development. Morphometric analysis.*

Age (months)	MALES	FEMALES	U***
2	22.37 ± 1.61	21.66 ± 0.97	N.S.
3	17.62 ± 1.62	18.16 ± 0.77	N.S.
4	17.87 ± 1.61	19.25 ± 0.98	N.S.
8	16.75 ± 1.22	15.75 ± 1.30	N.S.
15	11.75 ± 0.70	16.12 ± 0.51	$p < 0.05$
24	16.87 ± 1.85	19.62 ± 1.66	N.S.
M**	17.21 ± 0.73	18.68 ± 0.46	
H****	$p < 0.01$		

* Values expressed as the average nuclei number ± S.E.M. for each age. Area = 26,377 μm^2.
M** = average nuclei number ± S.E.M. for the whole time interval studied.
U*** = significance level for each age interval according to the Mann-Whitney U-test. N.S. = not significative.
H**** = overall level of significance for the whole postpubertal period according to the Kruskal-Wallis H-test.

females than for males during postpubertal life, almost equating at the 8th month. This progressive equivalence took place mainly due to a reduction (33.24%) in the number of pinealocyte nuclei in females between the ages of 2 months and two years old. Nuclei of pinealocytes in male pineal glands only showed an 11.56% decrease for the same time interval.

Glial cells followed a more irregular developmental pattern, as shown in Fig. 6. Mean values were larger for females, though differences were not statistically significant when separately analyzing each period. The average number of glial cell nuclei per surface unit in all the animals studied showed larger values for females (18.68 ± 0.46) than for males (17.21 ± 0.73). Fig. 6 shows a decrease in the glial cell nuclei number up to the first postnatal year, with further small increases for this cell type during the second year.

Discussion

Results of the present work show sex-related differences in the pineal gland parenchymal cells, specially for pinealocytes, throughout the whole adult life period. The use of plastic-embedded semithin sections stained with silver allowed an accurate identification of both pineal cell types. Our study was started in 2-month-old rats, which, after having acquired reproductive capacity, can be considered as adult animals and whose well defined and identifiable cell types are morphologically similar to those of the adult gland (Calvo and Boya, 1983). To avoid intraglandular topographic variations already described in the rat (Dielh, 1981; Heidbüchel and Vollrath, 1983), five areas, including both peripheral and central regions of the pineal gland, were analyzed in each animal.

No attemps were made to quantify connective tissue spaces, whose limits cannot be accurately identified under light microscopy. Notwithstanding, areas with large connective tissue spaces were avoided for countings. Nuclei of connective tissue cells and endothelial cells identifiable as smaller and darker than parenchymal cell nuclei were disregarded.

Other factors influencing the pineal nuclear volume and size (and therefore their number per surface unit) are the photoperiod and the circardian rhythm. The average nuclear volume is larger towards the half of the light period and smaller after several hours of darkness (hamster: Vollrath, 1979; gerbil: Welsh et al., 1979; rat: Lew et al., 1984; chinese hamster: Hira et al., 1989). The size of pinealocytes and their nuclei also seems to change during the year, increasing in winter and decreasing in summer (mouse: Legait et al., 1975; ground squirrel: McNulty et al., 1980). Considering these factors, we sacrificed animals when variations are considered to be minimal (18:00 h in April and May).

According to our results, the number of nuclei per surface unit decreases from the second postnatal month. So, in 24-month-old rats, the number of parenchymal cell nuclei averaged 67% for females and 88% for males, with respect to values found in 2-month-old rats. Calvo and Boya (1984a) have described a decrease in the number of pineal cell nuclei during the first six postnatal months, although this decrease was not quantified. Cassano et al. (1961) and De Martino et al. (1962), also described a lineal increase in the nuclear volume of the rat pinealocytes from the pubertal phase to senescence. All these results suggest that the hypertrophy of pineal cells continues throughout the animal life. In addition, the continuous increase of the pineal stroma (Calvo and Boya, 1984b; Boya and Calvo, 1984) particularly in aging rats, could also contribute to explaining the progressive decrease in the cell content of the pineal organ.

Few studies analyze sex-related differences in the pineal gland (Vollrath, 1981). Santamarina and Venzke (1953) found a lesser average weight in female rat pineal (0.7 mg.) with respect to males (0.9 mg). Legait et al. (1976) reported a larger average glandular volume in females, though this difference was not statistically significant. The differences in the number of parenchymal pineal cells according to the sex of the animal found in this study can be explained in terms of the different hormonal environment for males and females. Females showed higher number of cells per surface unit (i.e. smaller cells) than males. The average number of cells was also more variable with time in females with respect to males. This could be interpreted as a delay in cell maturation and a slower cell ageing in the female pineal gland. It has been shown that estrogens act delaying the development of pinealocyte organoids (Clementi et al., 1965a,b) whereas androgens favour pinealocyte cell hypertrophy (Clementi et al., 1965b).

Glial cell types show a lesser degree of sexual variability, as interferred from the nonparametric statistics of the results in our study. For each phase studied, results were not statistically significant. However, when the whole period was analyzed through the Kruskal-Wallis H-test a low significance level ($p < 0.01$, Table 2) could be demonstrated. The decrease found in the number of pineoglial cells per surface unit along the first postnatal year could be a mere consequence of the cell hypertrophy of pinealocytes. Furthermore, the slight increase in pineoglial cells in older rats could be explained by the pineal gland involution described in these rats (Johnson, 1980; Boya and Calvo, 1984). In this way, the pineoglial cells would respond to the glandular atrophy with a form of pseudogliosis similar to that observed in the aged nervous tissue. Large clusters of glial cells were frequently seen near the wide connective tissue spaces in old pineal glands (Boya and Calvo, 1984).

References

Blumfield M.G. and Tapp E. (1970). Measurements of pineal parenchymal cells and their nuclei in the albino rat at different ages. Acta Morphol. Neerl. Scand. 8, 1-8.

Boeckmann D. (1980). Morphological investigation of the deep pineal of the rat. Cell Tissue Res. 210, 283-294.

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. (1984a). Postnatal evolution of the rat pineal gland. Light microscopy. J. Anat. 138, 45-53.

Calvo J. and Boya J. (1984b). 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 the glial cells in the rat pineal gland: a light and electron microscopic immunohistochemical study. Anat. Rec. 220, 424-428.

Cassano C., Torsoli A., Peruzy A.D. and De Martino C. (1961). Studi su l'epifisi. Indagini nell'animale da esperimento e nell'uomo. Folia Endrocrinol. Pisa. 14, 755-790.

Clementi F., Müller E. and Zanoboni A. (1965a). Pineal function and modifications of its ultrastructural aspects. Proc. 2nd. Int. Congr. Endocrinology. Excerpta Med. Int. Congr. Series. 83, 364-374.

Clementi F., Fraschini F., Müller E. and Zanoboni A. (1965b). The pineal gland and the control of electrolyte balance and of gonadotropic secretion. Functional and morphological observations. Prog. Brain Res. 10, 585-603.

De Martino C., Peruzy A.D., Pavoni P. and Caponi M. (1962). Captazione del ^{32}P ed aspetti istologici della ghiandola pineale nelle varie etá. Folia Endocrinol. Roma. 15, 363-369.

Diehl B.J.M. (1981). Time-related changes in size of nuclei of pinealocytes in rats. Cell Tissue Res. 218, 427-438.

Heidbüchel U. and Vollrath L. (1983). Morphological findings relating to the problem of cortex and medula in the pineal glands of rat and hamster. J. Anat. 136, 723-734.

Hira Y., Sakai Y. and Matsushima S. (1989). Comparisons of sizes of pinealocyte nuclei and pinealocytes in young and adult chinese hamster *(Cricetulus griseus)* under different photoperiod conditions. J. Pineal Res. 7, 411-418.

Johnson J.E. (1980). Fine structural alterations in the aging rat pineal gland. Exp. Aging Res. 6, 189-211.

Jung D. and Vollrath L. (1982). Structural dissimilarities in different regions of the pineal gland of Pirbright white

guinea-pigs. J. Neural Transm. 54, 117-128.

Kachi T. and Quay W.B. (1984). Seasonal changes in glycogen level and size of pinealocytes of the white-footed mouse, *Peromyscus leucopus*: a semicuantitative histochemical study. J. Pineal Res. 1, 163-174.

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.

Klein W., Jurecka W. and Böck P. (1981). Osmium dependent argentaffin staining of lysosomes. Histochemistry 73, 447-457.

Kruskal W.H. and Wallis W.A. (1952). Use of ranks in one-criterion variance analysis. J. Am. Stat. Assoc. 47, 583-621.

Legait H., Roux M., Dussart G., Richoux J.P. and Contet-Andonneau J.-L. (1975). Donnés morphométriques sur la glande pinéale du Loir (*Glis glis*) et du Lerot *(Elyomis quercinus)* au cours du cycle annuel. C.R. Soc. Biol. 169, 132-136.

Legait H., Bauchot R., Stephan H. and Contet-Andonneau J.-L. (1976). Etude des corrélations liant le volume de l'epiphyse aux poids somatique et encéphalique chez les rongeurs, les insectivores, les chiropteres, les prosimiens et les simiens. Mammalia 40, 327-337.

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.

Lincoln G.A. (1976). Seasonal changes in the pineal gland related to the reproductive cycle in the male hare, *Lepus europaeus*. J. Reprod. Fertil. 46, 489-491.

Mann H.B. and Whitney D.R. (1947). On a test of whether one of two random variables is stochastically larger than the other. Ann. Mathem. Stat. 18, 50-60.

Matsushima S., Sakai Y. and Hira Y. (1990). Effect of photoperiod on pineal gland volume and pinealocyte size in the chinese hamster, *Cricetulus griseus*. Am. J. Anat. 187, 32-38.

McNulty J.A., Dombrowski T.A. and Spurrier W.A. (1980). A seasonal study of pinealocytes in the 13-lined ground squirrel, *Spermophilus tridecemlineatus*. Reprod. Nutr. Dev. 20, 665-672.

Milcu S.M., Petrea I., Sahleanu V. and Holban R. (1962). Altrastructura epifizei dupa castrate çi dupa administrarea hormonilor sexuali. Stud. Cercet. Endocr. 13, 617-622.

Mogler R.K.-H. (1958). Das endokrine System des syrischen Goldhamster (*Mesocricetus auratus auratus* Waterhouse) unter Berüksichtigung des natürlichen und experimentellen Winterschlafs. Z. Morphol. Okol. Tiere. 47, 267-306.

Peinado M., Fajardo N., Hernández G., Puig-Domingo M., Viader M., Reiter R.J. and Webb S.M. (1990). Immunoreactive somatostatin diurnal rhythms in rat pineal, retina and harderian gland: effects of sex, season, continuous darkness and estrous cycle. J. Neural Transm. Gen. Sect. 81, 63-72.

Przybylska B., Wyrzykowski Z., Wyrzykowska K. and Karasek M. (1990). Ultrastructure of pig pinealocytes in various stages of the sexual cycle: a quantitative study. Cytobios 64, 7-14.

Quay W.B. and Levine B.E. (1957). Pineal growth and mitotic activity in the rat and the effect of colchicine and sex hormones. Anat. Rec. 129, 65-67.

Quay W.B. and Renzoni A. (1966). Twenty-four hour rhythms in the pineal mitotic activity and nucleolar dimensions. Growth 30, 315-324.

Santamarina E. and Venzke W.G. (1953). Physiological changes in the mammalian pineal gland correlated with to reproductive system. Am. J. Vet. Res. 14, 155-162.

Schachner M., Huang S.-K., Ziegelmuller P., Bizzini B. and Taugner R. (1984). Glial cells in the pineal gland of mice and rats. A combined immunofluorescence and electron-microscopic study. Cell Tissue Res. 237, 245-252.

Trakulrungsi W.K. and Yaeger V.L. (1977). Effect of photoperiod on early changes in neonatal rat pineal gland. Experientia 33, 84.

Vollrath L. (1979). Comparative morphology of the vertebrate pineal complex. In: The Pineal gland of vertebrates including man. Progress in Brain Research Vol. 52. Kappers J.A. and Pevet P. (eds). Elsevier/North-Holland Biomedical Press. Amsterdam. pp 25-38.

Vollrath L. (1981). The pineal organ. Handbuch der mikroskopischen Anatomie des Menschen. VI/7. Springer Verlag. Berlin.

Vollrath L. (1986). The postnatal differentiation of the mammalian pineal complex. In: The pineal gland during development: from fetus to adult. Gupta D. and Reiter R.J. (eds). Croom Helm. London & Sidney. pp 56-64.

Wallace R.B., Altman J. and Das G.D. (1969). An autorradiographic and morphological investigation of the postnatal development of the pineal body. Am. J. Anat. 126, 175-184.

Welsh M.G., Hansen J.T. and Reiter R.J. (1979). The pineal gland of the gerbil, *Meriones unguiculatus*. II. Morphometric analysis over a 24-hour period. Cell Tissue Res. 204, 95-109.

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