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Comparative quantitative ultrastructural study of pinealocytes in eight mammalian species

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Abstract

OBJECTIVES: The studies related to quantitative ultrastructural features of mammalian pinealocytes, especially in comparative aspects, are relatively rare. Quantitative ultrastructural studies in many mammalian species are lacking. Because of the existence of differences in diurnal melatonin profiles in various species it seemed interesting to compare the ultrastructure of pinealocytes in various mammalian species, both laboratory and domestic animals. MATERIAL AND METHODS: The pineal glands of the following mammalian species have been examined: mouse, rat, Syrian hamster, gerbil, sheep, horse, pig and European bison. For each species 4 adult animals (2 males and 2 females) were used. Cross-sectional areas of the pinealocyte and its nucleus and the relative volumes of the following cytoplasmic organelles were analyzed: mitochondria, lysosomes, Golgi apparatus, granular endoplasmic reticulum, and lipid droplets. The relative volumes of membrane-bound bodies (MBB) in the pig pinealocytes, pigment granules in the horse pinealocytes, and calcareous concretions in the gerbil pineal gland were also analyzed. In addition, numerical density of dense-core vesicles and "synaptic" ribbons was estimated. RESULTS: Ultrastructure of pinealocytes in examined species shows many common features, and existing differences are of quantitative nature only. The observed qualitative differences include MBB in the pig, abundance of pigment granules in the horse, and calcareous concretions in the gerbil. Relative volumes or the numbers of some cell structures (mitochondria, Golgi apparatus, lipid droplets, dense-core vesicles and "synaptic" ribbons) show distinct interspecie differences, whereas those of other cell structures (granular endoplasmic reticulum, lysosomes) are similar in all studied species. CONCLUSIONS: The ultrastructural features of pinealocytes in all examined species confirm earlier suggestions of high activity of these cells. No distinct correlation has been found between the type of melatonin secretion and ultrastructural patterns of pinealocytes. It should be stressed, however, that the ultrastructure of pinealocytes in the present study was studied during the daytime, whereas differences in melatonin secretion are observed at night.

ORIGINAL ARTICLE

Introduction

The number of papers devoted to pineal morphology, and especially its ultrastructure, is very high. The ultrastructure of the pinealocyte has been examined in various species in many natural and experimental conditions [cf e.g. 1–9]. However, the studies related to quantitative ultrastructural features of mammalian pinealocytes, especially in comparative aspects are relatively rare. Quantitative ultrastructural studies in many mammalian species are lacking. Therefore, it seemed interesting to compare the ultrastructure of pinealocytes in eight different mammalian species, both laboratory and domestic animals, especially because of the existence of differences in diurnal melatonin profiles in various species [10, 11].

Material and methods

The animals.

The pineal glands of the following mammalian species have been examined: mouse (BALB/c; 3-month-old), rat (Wistar; 3-month-old), Syrian hamster (2-month-old), gerbil (3-month-old), sheep (1- to 3-year-old), horse (3- to 5-year-old), pig (8- to 12-month-old) and European bison (2- to 4-year-old). For each species 4 animals (2 males and 2 females) were used. The laboratory animals (mice, rats, Syrian hamsters, gerbils) received standard laboratory food and tap water ad libitum, domestic animals (sheep, horses, pigs) were fed with food typical for each of these animals, whereas European bisons were fed in natural reservation conditions.

Laboratory animals.

Laboratory animals were housed in a room with controlled illumination (LD 12:12; light on at 06:00h) and temperature (22±2°C). The animals were killed by decapitation between 9h and 10h, in the spring. The pineal glands were removed immediately after decapitation.

Domestic animals.

Domestic animals, housed in natural conditions, were killed in the slaughter house between 10h and 12h, in the spring. The pineal glands were removed 5 to 10 minutes after the animals' death.

European bison.

European bisons living free in the reservation were killed during selection shooting between 10h and 12h, in the early spring. The pineal glands were removed 7 to 12 minutes after the animals' death.

Quantitative analysis.

The pineal glands of all animals were 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 5 to 7 micrographs at magnification of x 3,000, and 30 to 35 micrographs at magnification of x 10,000 were taken from each gland using slightly modified systematic random sampling method [12]. Every upper right corner of the

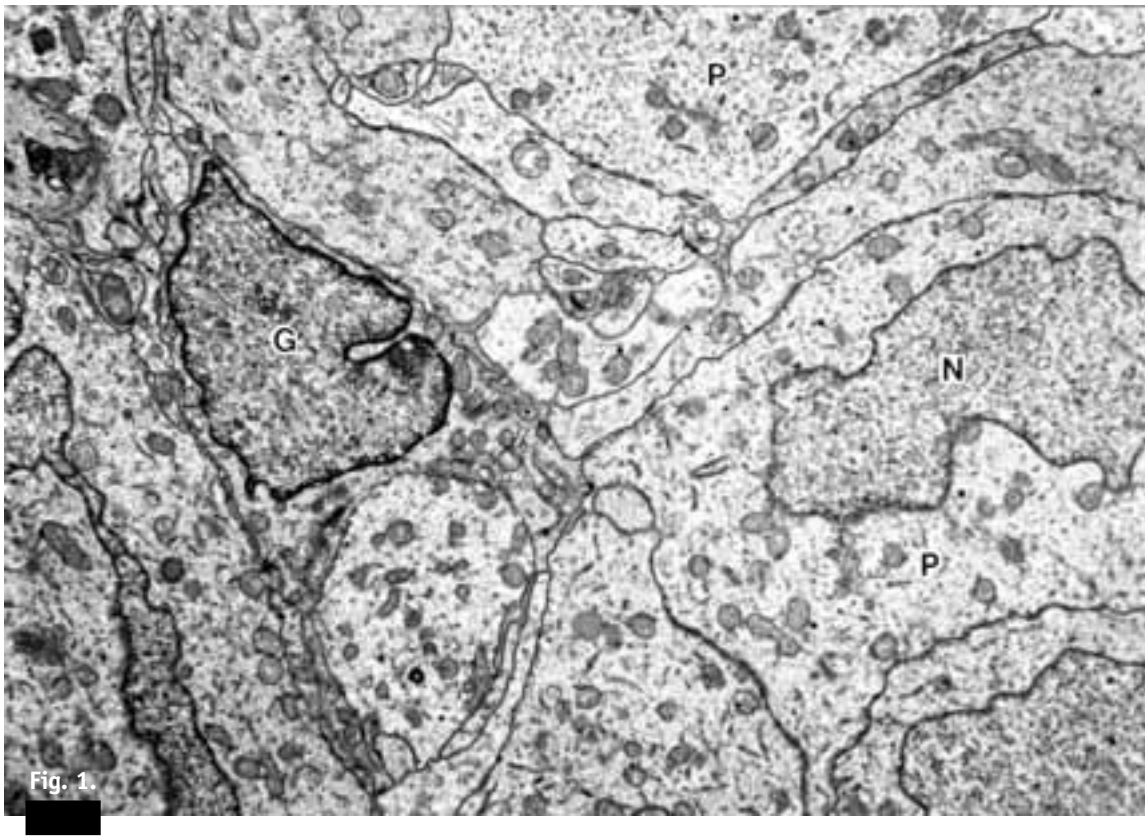


Fig. 1.

grid aperture in which pinealocytes were present was photographed. Altogether, 1,375 prints were used for quantitative study. A digital analyzer connected online to an IBM-PC computer (Logitex, Poland) was used to obtain the morphometric data. For estimation of the cross-sectional areas of the 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 were analyzed: mitochondria, lysosomes, Golgi apparatus, granular endoplasmic reticulum, and lipid droplets. In addition, numerical density of dense-core vesicles (expressed as number per 50 μm^2 of cell body cytoplasm) was estimated. For the quantification of "synaptic" ribbons (expressed as number per 20,000 μm^2 of pineal tissue) the tissue overlying 10 grid apertures, each measuring 45 x 45 μm^2, was scanned at x 15,000.

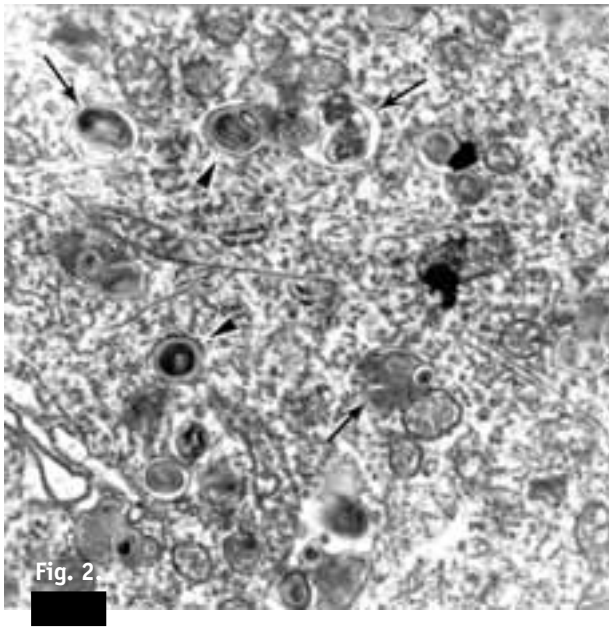
The relative volumes of membrane-bound bodies (MBB) in the pig pinealocytes and pigment granules in the horse pinealocytes were also analyzed. In the gerbil pineal gland the relative volume of calcareous deposits was estimated after analyzing 20,250 μm^2 of the pineal gland sections.

Statistical analysis.

Statistical analysis of the data was performed using nonparametric Mann-Whitney U test.

Results

Ultrastructure of pinealocytes in examined species shows many common features, and existing differences are of quantitative nature only. Typical submicroscopic morphology of these cells is presented in Figure 1. The ultrastructure of mammalian pinealocytes in natural conditions has been described several times, also in large reviews [2–4, 7–9, 13], and therefore it is not repeated here. The presence of membrane-bound bodies (MBB) in the pig pinealocytes (Fig. 2), numerous pigment granules in the pinealocytes of the horse (Fig. 3), and calcareous concretions in the pineal gland of the gerbil (Fig. 4) were the only distinct qualitative differences observed in the present study.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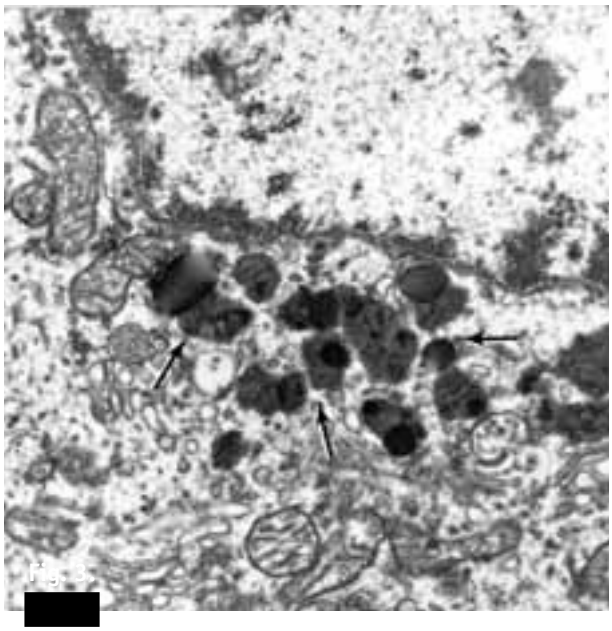


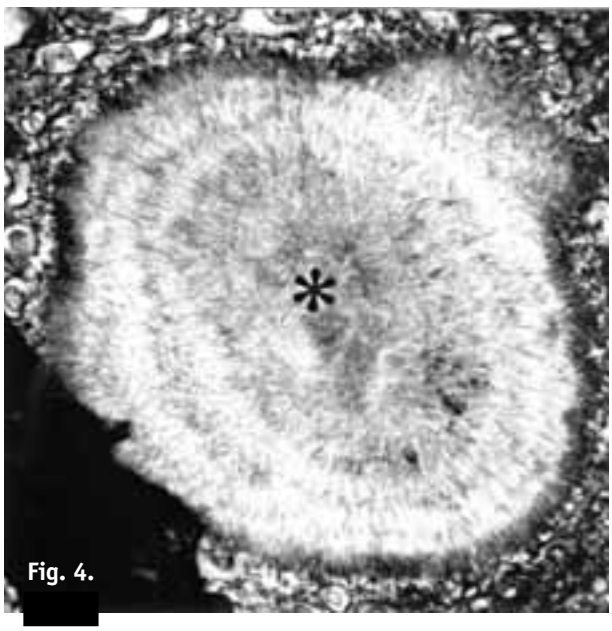


Fig. 4.

Fig. 1. Low power micrograph of the mouse pineal gland. P – pinealocyte, G – glial cell, N – pinealocyte nucleus; x 9500.
Fig. 2. The pinealocyte of the pig. Membrane-bound bodies of the type 1 (arrows) and of the type 2 (arrow heads); x 18,000.
Fig. 3. The pinealocyte of the horse. Numerous pigment granules (arrows); x 18,000.
Fig. 4. The pinealocyte of the gerbil. Calcareous concretion (asterix). x 20,000.

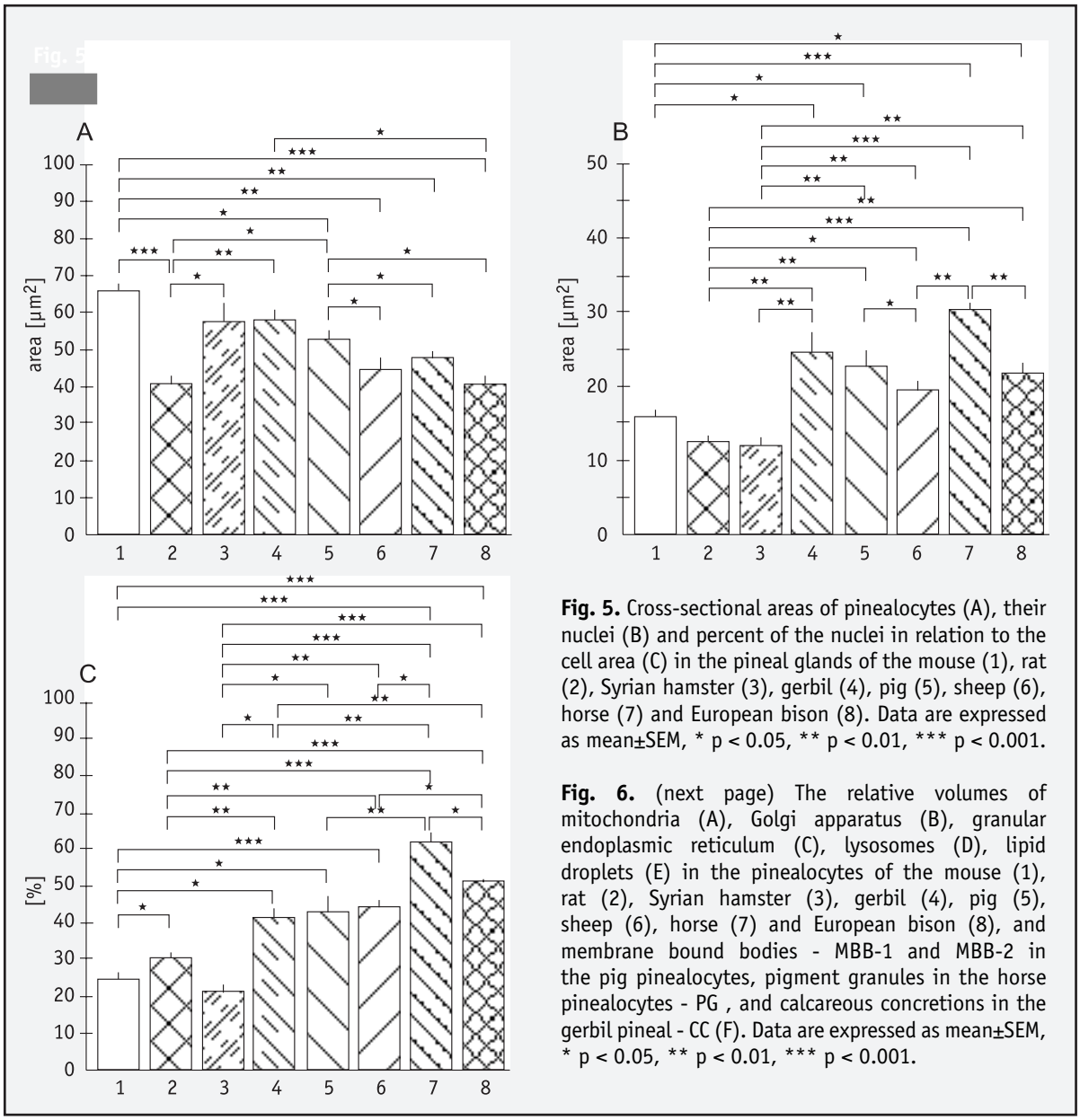


Fig. 5. Cross-sectional areas of pinealocytes (A), their nuclei (B) and percent of the nuclei in relation to the cell area (C) in the pineal glands of the mouse (1), rat (2), Syrian hamster (3), gerbil (4), pig (5), sheep (6), horse (7) and European bison (8). Data are expressed as mean±SEM, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Fig. 6. (next page) The relative volumes of mitochondria (A), Golgi apparatus (B), granular endoplasmic reticulum (C), lysosomes (D), lipid droplets (E) in the pinealocytes of the mouse (1), rat (2), Syrian hamster (3), gerbil (4), pig (5), sheep (6), horse (7) and European bison (8), and membrane bound bodies - MBB-1 and MBB-2 in the pig pinealocytes, pigment granules in the horse pinealocytes - PG , and calcareous concretions in the gerbil pineal - CC (F). Data are expressed as mean±SEM, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Size of the pinealocytes, expressed here as a cross-sectional area, was similar in all studied species, whereas size of pinealocyte nucleus differed from 12.1 μm^2 in the Syrian hamster to 29.8 μm^2 in the horse (Fig. 5). The nucleus constitutes from 21.4% (Syrian hamster) to 63.4% (horse) of the pinealocyte (Fig. 5).

Relative volumes of cell organelles are given in Figure 6.

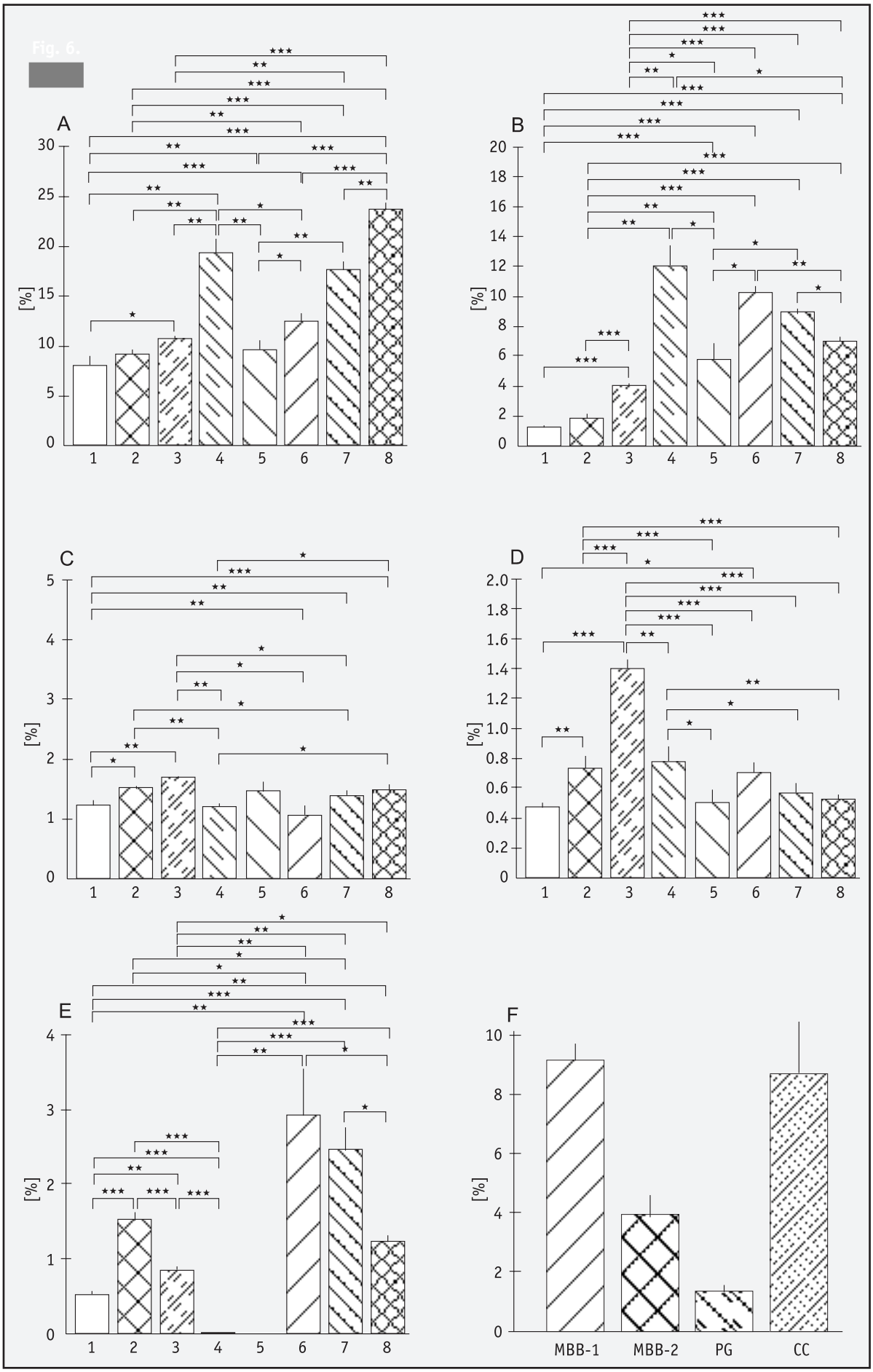
Relative volumes of some cell organelles (granular endoplasmic reticulum, lysosomes) were relatively stable in most species, whereas other (mitochondria, Golgi apparatus) showed distinct differences among species (Fig. 6). Differences were also observed in the relative volume of lipid droplets (Fig. 6) and in the number of dense-core vesicles and "synaptic" ribbons (Fig. 7).

Mitochondria are the most abundant cell organelles in all species examined, with relative volume from 8.2% to 23.5%, whereas that of Golgi apparatus differed from 1.2% to 12.0%. The smallest difference was observed in the relative volume of granular endoplasmic reticulum (1.1%–1.6%) and that of lysosomes (0.5%–1.4%). Lipid droplets were observed only sporadically in the gerbil and the pig, and their relative volume in remaining species differed between 0.5% and 3.0% (Fig. 6).

The number of dense-core vesicles in the cell body was highest in the mouse (6.4/50 μm^2) and the Syrian hamster (5.1/50 μm^2), somewhat lower in the rat (3.1/50 μm^2), and in the remaining species was between 1–1.6/50μm^2) (Fig. 7).

The number of "synaptic" ribbons showed the great difference among species being highest in the Syrian hamster (33.1/20,000 μm^2), absent in the mouse, low in the pig (1.2/20,000 μm^2), whereas in

Fig. 6.



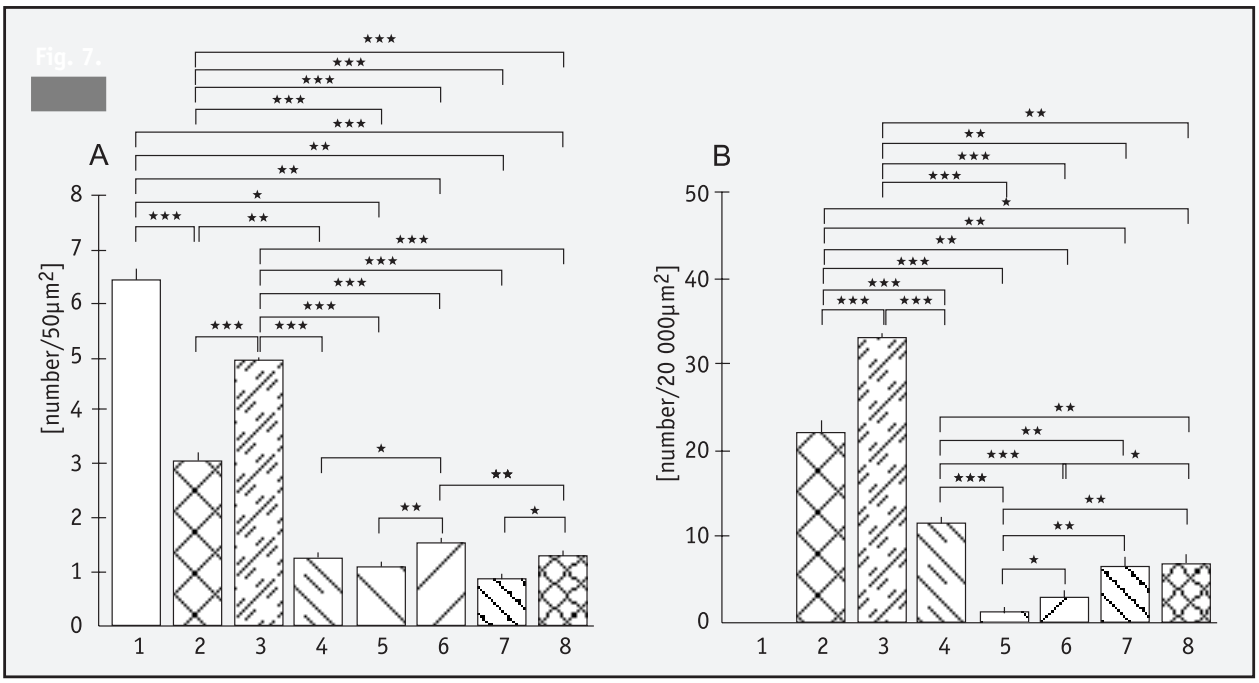


Fig. 7. The numerical density of the dense-core vesicles (A) and "synaptic" ribbons (B) in the pinealocytes of the mouse (1), rat (2), Syrian hamster (3), gerbil (4), pig (5), sheep (6), horse (7) and European bison (8). Data are expressed as mean±SEM, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

the other species it varied between 2.9–22.1/20,000 μm^2 (Fig. 7).

The relative volumes of the cell structures typical for some examined species, i.e. MBB, pigment granules and calcareous concretions are given in Figure 6.

Discussion

The number of studies on the ultrastructure of pinealocytes in various mammalian species is high [cf 2, 3, 5–9]. It refers also to the species examined in the present study. Among the animals most frequently studied in natural conditions are the rat [e.g. 14–18] and the Syrian hamster [e.g. 19–22]. The pineal glands of the mouse [23–25], the gerbil [26, 27], the pig [28–31], the horse [32, 33] and the sheep [34, 35] have also been studied. The pineal gland of the European bison has not been studied yet.

However, quantitative ultrastructural studies of pinealocytes in various mammalian species are very rare. Among species examined in the present study there is lack of morphometric data in the horse and European bison. It should be stressed that morphometric analysis performed in the present study refers to features of pinealocytes in photophase because the animals were killed between 10:00h and 12:00h.

Ultrastructure of pinealocytes in examined species shows many common features, and existing differences are of quantitative nature only. The observed qualitative differences include MBB in the pig, abundance of pigment granules in the horse, and calcareous concretions in the gerbil.

Membrane-bound bodies have been described in the pig pinealocytes earlier, and their involvement in the secretory processes of these cells was suggested [28, 36]. The presence of numerous pigment granules in the horse pinealocytes has also been described earlier [32, 37]. Calcareous concretions are typically present in the pineal gland of the human [38] but their occurrence has been also described in the pineal of other mammalian species, and especially abundantly in the gerbil [27, 39].

The ultrastructural features of pinealocytes observed in the present study confirm earlier suggestions of high activity of these cells. Pinealocytes contain numerous organelles indicating their high metabolic activity (as mitochondria), as well indicating high secretory activity (as Golgi apparatus).

It should be underlined that the size of pinealocytes is similar in all examined species but differences exist in the cell/nucleus ratio. The smallest amount of the cytoplasm is observed in the horse pinealocytes (approx. 37% of the cell area), and the largest amount is present in the Syrian hamster pinealocytes (approx. 79% of the cell area).

Relative volumes or the numbers of some cell structures (mitochondria, Golgi apparatus, lipid droplets, dense-core vesicles, and "synaptic" ribbons) show distinct interspecies differences, whereas those of other cell structures (granular endoplasmic reticu-

lum, lysosomes) are similar in all studied species.

Results of our morphometric analysis of pinealocytes of the rat, gerbil, mouse, Syrian hamster, pig, and sheep are generally in agreement with studies by other authors (rat: [40–45]; gerbil: [46]; mouse: [47, 48]; Syrian hamster: [22, 49, 50]; pig: [51, 52]; sheep: [35]) although there are some differences in certain organelles.

As it has been already mentioned we present the quantitative data of pinealocytes of the horse and European bison for the first time, with the exception of "synaptic" ribbons number in the horse pinealocytes that was described by Karasek and Cozzi [33].

It should be stressed that we did not find any significant differences in the ultrastructure of pinealocytes between laboratory and domestic animals, as well as European bison living free on the reservation.

Reiter [11] distinguished three different patterns of melatonin secretion. In all species melatonin production is minimal during the day. In some mammals melatonin diurnal profile is characterized by a discrete peak in late-dark phase (type A). In type B there is gradual increase in melatonin synthesis after onset of darkness with peak occurring in mid-dark phase, and in type C onset of darkness is associated with rather rapid rise in melatonin synthesis and prolonged peak is present during majority of dark phase. It seems that no differences exist between pinealocyte ultrastructure and the type of pineal melatonin profile. Among species examined in the present study, type A is observed in the Syrian hamster [53, 54], gerbil [55, 56] and mouse [57], type B is characteristic for the rat [54, 58], whereas type C has been described in the sheep [59, 60] and horse [61]. Data on melatonin secretion in the pig are inconsistent [62–64], and the melatonin profile in the European bison has not been studied yet.

No distinct correlation has been found between the type of melatonin secretion and ultrastructural patterns of pinealocytes. It should be stressed, however, that ultrastructure of pinealocytes in the present study was studied during the daytime, whereas differences in melatonin secretion are observed at night.

Acknowledgments

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