Original article

Morphologic analysis of mice's pineal gland

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Abstract

The pineal gland or pineal body is an endocrine gland that constitutes an important part of the neuroendocrine system, due to the secretion of melatonin, a hormone responsible for the seasonal organization of several physiologic and behavioral events of an individual's life. Experimental researches using animals such as rats, mice and rabbits are often found in the extensive specific literature but aspects related to the morphology of mice's pineal gland are few. Concerning its small size, the present paper performed a microscopic analysis of serial median sagittal sections of the pineal gland of 13 (thirteen) *Swiss* mice. The pineal gland of *Swiss* mice was found to be in the median plane below the splenium of the corpus callosus, superior and dorsal to the habenular commissure, and rostral to the rostral colliculi. The pineal gland is closely related to the third ventricle and presents itself with a characteristic tonsillar shape with a stalk. Two types of different cells were identified in the gland, that is, astrocytes and pinealocytes, spreading randomly all over the glandular tissue. Calcifications of the pineal gland were not found in any of the observed animals.

Keywords: pineal gland, mouse, morphology.

1 Introduction

The pineal gland or pineal body, formerly named epiphysis, has been known as a single structure since ancient times, being mentioned in Galen's anatomical studies (THIEBLOT, 1947). Nevertheless, until the 19th century it was considered rather a target for philosophic and/or anthropologic studies than anatomic and/or physiologic ones.

It was only in 1958 that Lerner et al. isolated a pineal hormone, which they named melatonin, and this fact encouraged new researches on the pineal gland in many scientific fields.

So, the synthesis and excretion of melatonin determine the physiologic role of the gland, which is to signalize to the internal medium by alternating the diary presence and absence of its principal hormone (melatonin) in blood flow and in several other body fluids. In this way, the pineal gland gives rhythm to many neuroendocrine functions that it modulates, for instance, determining the sleep-awake cycle and the reproductive and metabolic activities of several species (BARTNESS and GOLDMAN, 1989).

Due to the relation of this photoreceptor organ with circadian and reproductive cycles, the importance of the pineal gland on sexual behavior and gonad maturation has been extensively studied and begetting knowledge based more in functional observations than in morphologic bases (SILVINO, BOMBONATO, MIGLINO et al., 2000). Although biologic researches using experiment animals as rats, mice and rabbits are often, with an extensive literature that can be read, morphologic aspects of the pineal gland in these animals are not so easy to be found, indeed being quite scarce (SILVINO, BOMBONATO, MIGLINO et al., 2000).

Therefore, this paper has the objective of adding some data that could possibly complement other results of the literature and to be a more precise support material for performing new concepts and applying new techniques for pinealectomy and stereotaxia.

2 Material and methods

We used 13 (thirteen) male *Swiss* mice (*Swiss webster*) from the Central Biotery of the Federal University of Alfenas, MG (UNIFAL).

The brains of these animals were collected soon after their sacrifice and fixed in a 10% formaldehyde solution during three weeks.

Our study was performed in the Laboratory of Anatomy and Laboratory of Histology of the Federal University of Alfenas, MG (UNIFAL), according to ethical principles in animal experimentation and approved by the Ethics Committee (register 266/2010).

The pineal glands were withdrawn with nervous tissue safety margin for further processing by the usual classic histological technique: ethanol dehydration, xylol diaphanization and Histosec® (Merck) inclusion (BEHMER, TOLOSA and NETO, 1976). From this material we obtained 7 mm-width serial median sagittal sections stained by Violet cresil technique.

The photomicrographic documentation of the pineal gland was done by a photomicroscope Nikon Eclipse E-80i to which Nikon NIS-Elements were attached for morphometric analysis of the pineal gland and its cells.

3 Results

The pineal gland of *Swiss* mice is microscopic and this avoided the evaluation of its weight and macroscopic measures.

By analyzing the histological sections it was possible to see the gland placed in the median plane in all animals. It was visualized next to the habenular commissure, rostral to the rostral colliculi, closely related to the third ventricle and with the stalk inserted in the intercommissural region. Its usual aspect can be of a characteristic tonsillar shape with a stalk (Figure 1), having a minute size when compared to other structures of the nervous system.

The pineal gland is located below the splenium of the corpus callosus, inferior to the third ventricle and superior and dorsal to the habenular commissure, to which it is intimately related (Figure 2).

In all extension the gland is coated by a ciliated cylindrical epithelium (Figure 3) arising from the pia-mater. Meningeal septa then pass to the interior of the gland and divide it in incomplete lobules of different sizes, giving to it the aspect of a tonsilla (Figure 4).

Analyzing the serial histological sections, one could observe pinealocyte-like cells spreading all over the gland and piercing adjacent structures. We also noted that these cells had large round nuclei with remarkable loose chromatin and acidophilic cytoplasm with few granules (Figure 5).

Yet, we visualized cells with elongated strong basophilic nuclei. These cells had extensions which stretched out between the pinealocytes, an image compatible to astrocytes. No calcifications were found in the pineal glands of the observed animals.

In relation to the vasculature, we noticed a single high-diameter artery entering the gland stalk, showing a remarkable quantity of erythrocytes within (Figures 5 and 6).

The superficial area of the pineal gland in the observed animals ranged from 14.378×10^3 to 51.955×10^3 mm^2 with mean and pattern standard deviation of $29.008 \times 10^3 \pm 11.260 \times 10^3$ mm^2; the number of

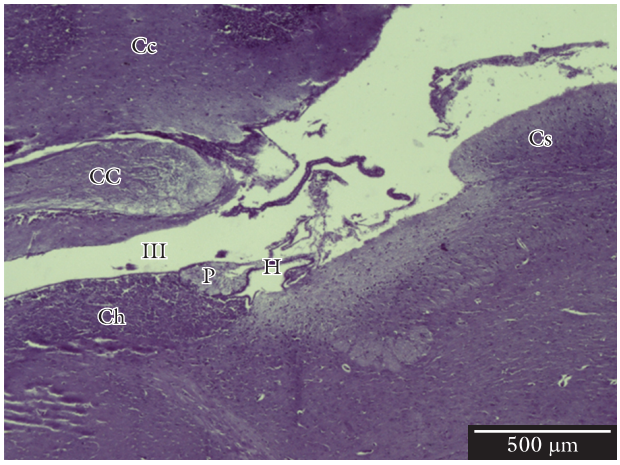

Figure 1. Photomicrography of mice's pineal gland (P) showing the tonsilar shape and the stalk (H). Habenular commissure (Ch); Corpus Callosus (CC); Third ventricle (III); Cerebral cortex (Cc); Rostral colliculus (Cs). Violet cresil, 40×.

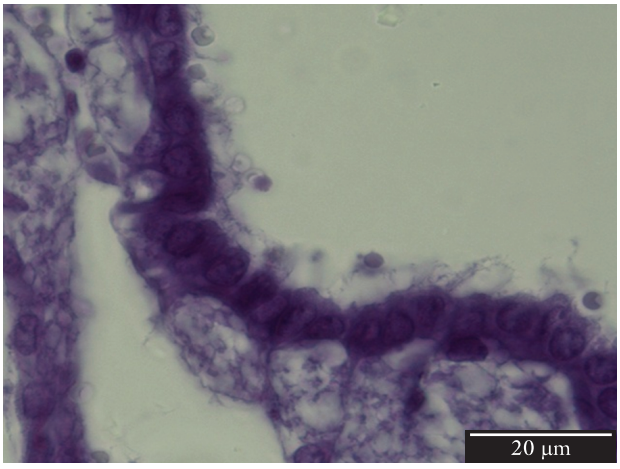

Figure 3. Photomicrography of the ciliated cylindrical epithelium coating the pineal gland. Violet cresil, 1000×.

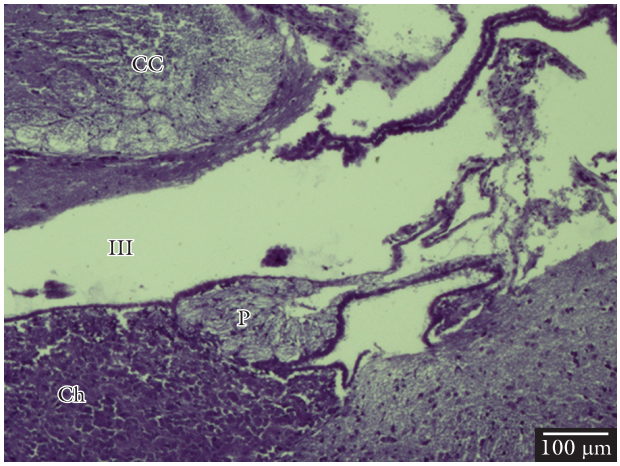

Figure 2. Photomicrography of mice's pineal gland (P) showing the tonsilar shape and the stalk (H). Habenular commissure (Ch); Corpus Callosus (CC); Third ventricle (III). Violet cresil, 100×.

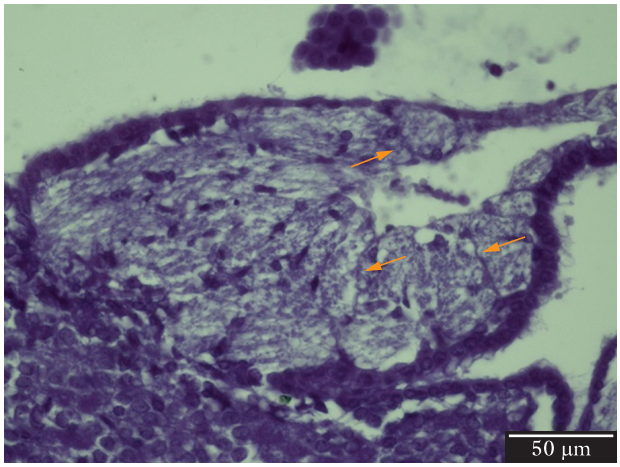

Figure 4. Photomicrography of mice's pineal gland showing the tonsilar shape. Note the connective tissue septa dividing the pineal gland into several lobules (yellow arrow). Violet cresil, 400×.

pinealocytes in a plotted gland area of 6400 mm² ranged from 3 to 8 cells, with mean and pattern standard deviation of 5.23 ± 1.69 pinealocytes.

4 Discussion

According to Schaffer, Symington and Bryce (1909), all animals have pineal gland except the amphioxus. Initially we intended to perform an analytical macroscopic and microscopic study of this gland in *Swiss* mice, but due to the minute size of the pineal gland in these animals we could only accomplished the microscopic analytical study.

We found the pineal gland placed in the median plane between the two brain hemispheres, rostral to the rostral colliculi, as reported by Didio (2002), Gartner and Hiatt (1999) and Vollrath (1981). However, there were no evidences of relationship of the gland with the diencephalon roof (epithalamus) in the observed animals, denying reports of the preceding authors.

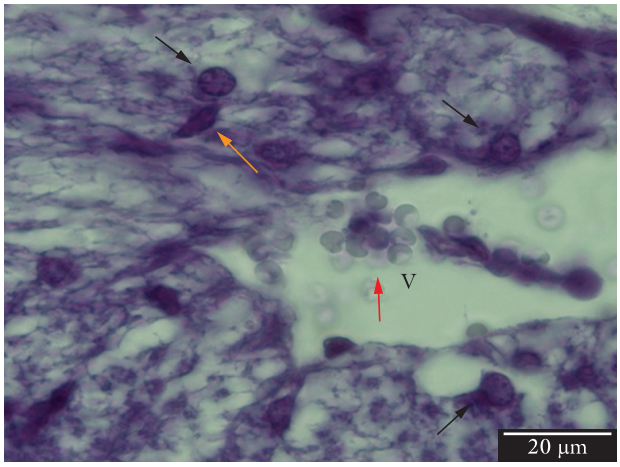

Figure 5. Photomicrography of mice's pineal gland showing pinealocytes (black arrow), astrocytes (yellow arrow), erythrocytes (red arrow) and a blood vessel (V). Violet cresil, 1000×.

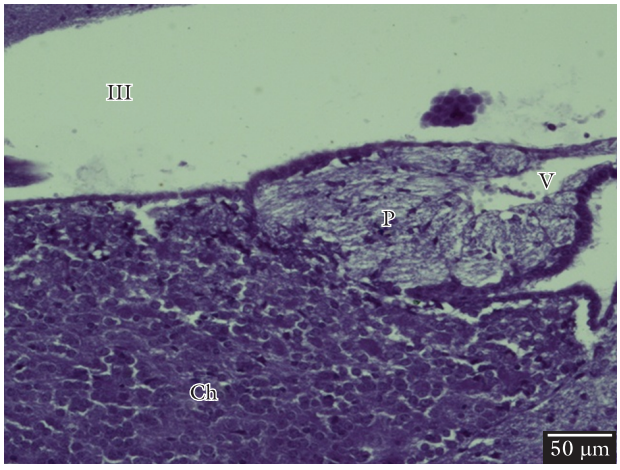

Figure 6. Photomicrography of mice's pineal gland (P) showing the tonsilar shape and the stalk (H). Habenular commissure (Ch); Third ventricle (III) and a blood vessel (V). Violet cresil, 400×.

In the histological sections of mice's brains it was possible to locate the pineal gland in a site similar to that of human beings, as described by Didio (2002), that is, in a depression between the superior colliculi and below the splenium of the corpus callosus, separated from it by the tela choroidea of third ventricle.

According to Vollrath (1981), mammals' pineal body originates from the posterior and dorsal part of the diencephalon roof (epithalamus). In spite of their common origin, this does not avoid the finding of pineal glands with different shapes and positions in rodents.

Disregarding the pine-shaped human gland Dyce, Sack and Wensing (1997), many other shapes has been identified in several species, as this paper showed in mice's pineal glands which had a somewhat tonsillar shape with a stalk.

According to Branco, Guimaräes, Miglino et al. (1997) and Vollrath (1981) this tonsillar-like aspect differs from the general bat-shape found in rodents. The works of Gomes (2003) showed dog's pineal glands shaping from conic to tongue-like (concerning the human tongue).

In animals often used for pineal gland experiments, like mature *Wistar* rats, it has the characteristics of a deep pine-shaped complex with the stalk inserted in the subcommissural region and extending superficially until it lies close to the confluence of the superior sagittal and transverse sinuses (BOECKMANN, 1980). In our study we found mice's pineal gland to have the same characteristics of that from rats, differing only in the tonsillar shape.

As described by Leeson and Leeson (1976) and Gomes et al. (2008) in dogs (*Canis familiaris*), mice's pineal glands are also draped by a connective tissue capsule arising from the pia-mater. Meningeal septa then pass to the interior of the gland and forms incomplete lobules.

In relation to the vasculature, we noticed a single high-diameter artery entering the gland stalk, with a remarkable quantity of erythrocytes within, what indicates that this organ is richly irrigated, as described by Machado (2006).

Nevertheless, in the work published by Marques, Carvalho, Mançanares et al. (2010), it has been demonstrated a great amount of vessels equally distributed among the cells in all parts of the glandular parenchyma of the pineal gland of crab-eating raccoons (*Procyon cancrivorus*). These vessels travelled along the septa to the interior of the gland, as also depicted in the pineal gland of capuchin monkeys (BARROS, 2006).

As stated by Vollrath (1981), pinealocytes and astrocytes are the principal cells types of the pineal gland, arranged in a follicular manner with narrow or large spaces. In an adult cat's pineal gland Boya, Calvo and Rancaño (1995) these were the only cells observed, as it was in the present study, in which it was not possible to identify and differentiate other cells. Besides pinealocytes and pinealocytes, other cellular elements such as mastocytes, fibroblasts, fibrocytes and collagen fibers has already been reported previously in buffalo's Carvalho, Ambrósio, Miglino et al. (2007) and capuchin monkeys' pineal glands (BARROS, 2006).

The pinealocytes had large round nuclei with a remarkable loose chromatin and a weak basophilic cytoplasm, while typical astrocytes intermingled them. These characteristics are similar to those described by Banks (1992).

Although the morphologic aspects of the cells founded in our observations are compatible to those described for pinealocytes and astrocytes, we believe that further researches using specific immunohistochemical techniques are necessary in order to clarify completely their identification.

The so-called brain sand or "acervuli" (GARTNER and HIATT, 1999) was not found in any of the analyzed pineal glands. This absence was also reported in dogs (GOMES, 2003) and crab-eating raccoons (*Procyon cancrivorus*) (MARQUES, CARVALHO, MANÇANARES et al., 2010).

Using the classification proposed by Blin and Maurin (1956), that considers the position of the gland in relation to the splenium of the corpus callosus, we can describe *Swiss* mice's pineal gland to be of the subcallosal type; however, these same authors describe rodents' pineal glands to be of the supracallosal type.

The classification of Oksche (1965) is based on the shape of the pineal gland of the studied animal and typifies it into elongated, conic or pyriform forms. In doing so, we can also classify *Swiss* mice's pineal gland as pyriform (pear-shape).

Due to the great complexity of the pineal gland, especially in rodents (VOLLRATH, 1979), this author proposed a new classification (VOLLRATH, 1981) that seems to fit most of the morphologic comparisons among mammals. This classification, instead of considering the gland relationship with the corpus callosus, regards the relation of the gland with the third ventricle, its shape and arrangement. According to this classification, *Swiss* mice's pineal glands are said to be of abC iv type, because the pineal gland of these animals has a minute size, it is closely related to the third ventricle and its length is twice as its width.

5 Conclusion

Swiss mice's pineal gland is microscopic in size and showed to be present in all analyzed animals. It is located in median sagittal sections between the two brain hemispheres, rostral to the rostral colliculi, superior and dorsal to the habenular commissure and having a tonsillar-like shape, as seen in the same sections. Cells similar to pinealocytes and astrocytes are also identified.

Swiss mice's pineal glands can be classified as abC iv type, pyriform and subcallosal.

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