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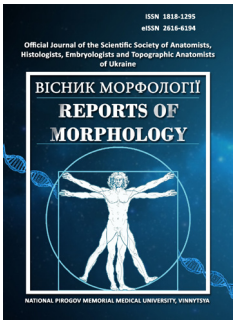

Topographical-anatomic features of the pineal gland in laboratory rats of the Wistar line

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The pineal gland is an organ of the central endocrine system that coordinates the activity of peripheral endocrine glands, immune and nervous systems, regulates biological rhythms, ensures adaptation processes and triggers the stress response. The pineal gland is inherent in almost all vertebrates, but there are different variations in the shape and location of the pineal gland in the brain of animals even within the same species. Most often, laboratory rats of the Wistar line are use for laboratory research, and for the correct interpretation of the results obtained, species-specific anatomical features and possible individual variations in the location of the pineal gland in normal laboratory rats should be taken into account. The aim of the work was to study the features of the anatomical structure and topography of the pineal gland in white laboratory rats of the Wistar line. The pineal glands of 24 sexually mature male Wistar laboratory rats were examine. Morphological, morphometric and statistical research methods were use. During the study, individual variations in the topography, shape, and blood supply of the pineal gland in laboratory rats were identify. It has been establish that in some animals the pineal gland was located in the groove between the posterior edges of the occipital lobes of the cerebral hemispheres, was richly supply with blood by a large number of blood vessels, and had either an oval or a conical shape. In other animals, the pineal gland was located in the groove between the upper anterior corpora quadrigemina. In this case, the pineal gland was supply with blood by a small number of blood vessels, and the shape of the pineal gland with such localization in the brain was rounded. Individual variations in the linear dimensions of the epiphysis have been establish. When studying morphometric parameters, it was found that the transverse size of the pineal glands was the most stable, while the vertical size was more labile, which probably caused individual differences in the volumes of the pineal glands in the studied rats. Thus, the obtained results of the study on individual variations in the anatomical, topographic and morphometric structure of the pineal gland in intact laboratory Wistar rats can be the basis for assessing pathological changes in the organ when conducting experimental studies in medical and biological fields.

Keywords: *brain, pineal gland, anatomy, topography, laboratory rats, individual variations.*

Introduction

The pineal gland, or epiphysis cerebri, is an organ of the central endocrine system that has close morpho-functional connections with the central nervous system and coordinates the activities of peripheral endocrine glands, the immune and nervous systems. [12, 25]. It is known that the pineal gland is inherent in almost all vertebrates, but in different classes of animals, there are peculiarities of its structure, which is associated not only with the climatic conditions of residence, lifestyle, and weight of animals, but also with evolutionary changes, from the photoreceptor organ in fish to the neuroendocrine organ in mammals [18, 22, 30, 31]. In all animal species, the main functions of the pineal gland are the regulation of biological rhythms, in particular the sleep-wake cycle, reproductive cycles, triggering the neuroendocrine response to stress of various origins, and ensuring adaptive processes in the body [4, 8, 16].

In recent years, a very large number of scientific publications by both Ukrainian researchers and scientists from other countries have been devoted to the structure and functions of the pineal gland [28]. Increased interest in the pineal gland is due primarily to the wide spectrum of biological action of the hormone it synthesizes - melatonin and its successful use in various areas of medicine for the purpose of correction and prevention of diseases of various genesis [3, 20, 24, 29]. In addition, the pineal gland is a source of such important biological substances as tryptophan and serotonin, which regulate vital processes occurring in the body [15, 27]. At the same time, it should be noted that the pineal gland is the least studied endocrine gland, which is due to the small size of the organ and topographic features that complicate its atraumatic removal [4, 9, 19]. In this regard, the morphological features of the pineal gland of various animal species are still poor described in literary sources. In addition, according to literature data, there are different variations in the shape and location of the pineal gland in the brain of vertebrates, even within the same species [10]. Considering the fact that animals, especially laboratory rats, are objects that are most often used for experimental research in biological and medical fields, which is due to the similarity of the structural organization of human and animal organs, for the correct interpretation of the obtained research results, one should take into account not only the species-specific features of the anatomical structure of this organ, but also possible individual variations of its location in laboratory rats of the Wistar line in the norm.

The purpose of the study was to study the features of the anatomical structure and topography of the pineal gland in white laboratory rats of the Wistar line.

Materials and methods

The results of this work are a fragment of the research topic of the Department of Morphology and Public Health of the Petro Mohyla Black Sea National University of the Ministry of Education and Science of Ukraine “The influence of environmentally hazardous factors on the mechanisms of development of civilization diseases and their correction with physiologically active substances”, state registration number 0124U002163.

An anatomical and experimental study was conducted on 24 intact male laboratory Wistar rats weighing 180-220 g, who had reached sexual maturity. The choice of males for the study was due to the lack of fluctuations in the level of melatonin in the blood plasma compared to females, in which the concentration of the melatonin hormone, the morphological and functional state of the pineal gland are determined by the phase of the sexual cycle. All experimental animals were kept in standard vivarium conditions and had free access to food and drinking water. Artificial light sources not used in the vivarium, since the pineal gland responds to changes in light levels with morpho-functional restructuring. The study was carried out under natural lighting typical during the autumn-winter period.

The object of the study was the pineal gland. In order to remove the pineal gland, laboratory rats were fixed on a dissecting board using special straps [13]. After fixation, the animals were removed from the experiment by single-step decapitation under intraperitoneal thiopentane anesthesia at a rate of 25 mg/kg body weight. Excess wool on the animals’ heads was removed at the incision site using scissors [9]. After completion of the decapitation procedure, the skull of the animals was scalped with subsequent removal of the cranial vault along with the dura mater. Then the brain, along with the pia mater, was separated from the base of the skull and the pineal gland was isolated. Next, the isolated pineal glands was subjected to morphometric measurements, namely, the length and width of the organ (mm) were determined. Caliper were used to conduct organometric measurements of the sizes of the studied glands (mm). Then, the pineal gland was fixed in a 10 % neutral formalin solution. After fixation, dehydration was performed in alcohols of increasing concentration, after which the studied material was embedded in paraffin and sections 4-5 μm thick were made on a semi-automatic rotary microtome “Microm” type (Germany). The histological specimens thus obtained were stained with hematoxylin and eosin according to standard methods. After embedding the specimens in Canadian balsam, histological preparations of the pineal gland were studied at a magnification of eyepiece ×10 and objectives ×4, ×10 of a microscope of the “Carl Zeiss” brand (Germany). Photo documentation of the research results was performed using a Canon digital SLR camera.

All stages of the study were carried out in accordance with the current requirements of the general principles of work with experimental animals in accordance with the following standards: Council of Europe Convention on Bioethics (1997); European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, General Ethical Principles of Experiments on Animals, approved by the First National Congress of Ukraine on Bioethics (2001); Law of Ukraine “On the Protection of Animals from Cruelty” (2006) and other international treaties and current national legislation in the field of biomedical research. The study was conducted according to a protocol approved by the Bioethics Commission of the Petro Mohyla Black Sea State University (Protocol No. 4 dated June 24, 2024).

We entered the determined quantitative morphometric data characterizing the linear dimensions of the pineal glands into the electronic research journal and subjected it to statistical analysis. For all indicators, arithmetic means, standard mistakes of the arithmetic mean, and standard deviation were calculated. Statistical calculations were performed on a personal computer using standard software “STATISTICA 6” for computers with the Windows operating system.

Results

The pineal gland in animals belonging to the mammalian

class is located in the median plane between the upper anterior corpora quadrigemina. According to the results of our morphological studies, it was found that the shape and location of the pineal gland in the brain in laboratory Wistar rats had some individual variations. Thus, in most experimental animals, the narrowed apex of the pineal gland was located in the groove between the posterior edges of the occipital lobes of the cerebral hemispheres, above and caudal to the cerebellum and in direct contact with the tissue of the third ventricle of the brain. The other, opposite part of the pineal gland was directed towards the cerebral hemispheres. Therefore, we divided the pineal gland into three conditional parts. We designated the distal pole, directed towards the cerebral hemispheres, as the base, and the proximal pole, which faces the third ventricle, as the apex. The middle part of the organ, located between the poles, was called the body. With the described variant of location in the brain, two possible forms of the pineal gland were encountered on longitudinal sections made through the center of the studied organ. Thus, in some animals, the pineal gland appeared as a light red formation and had an elongated oval shape with two pointed edges (Fig. 1).

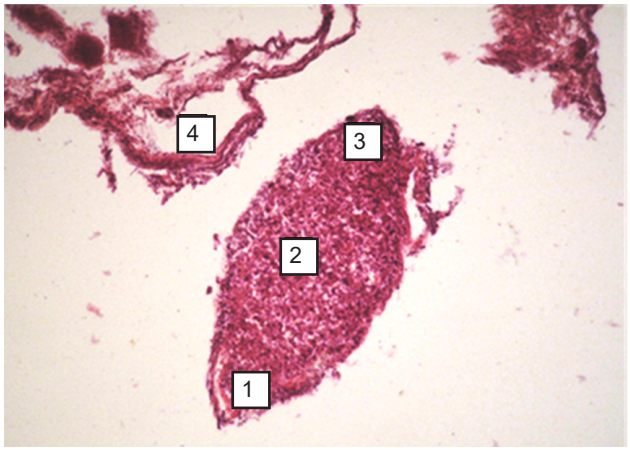

Fig. 1. Microphotograph of the pineal gland of laboratory Wistar rats. Oval shape of the pineal gland. 1 – narrowed apex; 2 – body; 3 – base of the epiphysis; 4 – extraorgan blood vessels. Hematoxylin and eosin staining. ×40.

In other laboratory animals, the pineal gland had a cone-shaped shape. At the same time, one of the ends of the studied organ, which faced the third ventricle, was pointed, while the opposite end, facing the cerebral hemispheres, was thickened and rounded (Fig. 2).

With this placement in the brain, it was noted that the pineal gland was surrounded by a large number of blood vessels of large and small caliber and had a red-gray color, which distinguished it from other parts of the brain. The detected vascular plexuses were a direct continuation of the vascular plexus of the third ventricle of the brain and in most animals penetrated into the studied organ. However, it should be noted that the upper surface of the pineal gland was free and had no contact with blood vessels.

In the other part of the laboratory rats studied, the apical

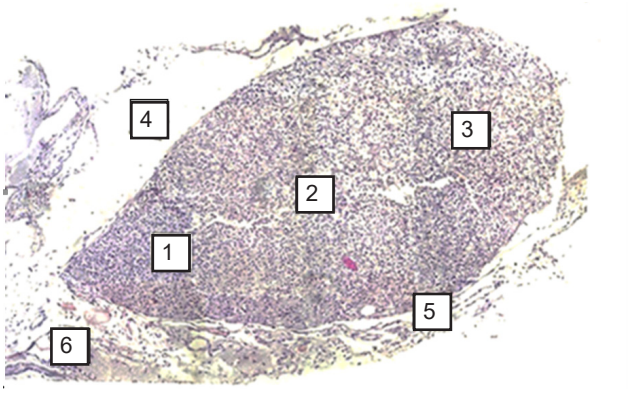

Fig. 2. Micrograph of the pineal gland of laboratory Wistar rats. Cone-shaped shape of the pineal gland. 1 – narrowed apex; 2 – body; 3 – rounded base; 4 – expanded subarachnoid space; 5 – narrowed subarachnoid space; 6 – extraorgan blood vessels. Hematoxylin and eosin staining. ×100.

part of the pineal gland was not identified in the sulcus between the occipital lobes of the cerebral hemispheres. In these cases, the pineal gland was found in the groove between the upper anterior corpora quadrigemina. At the same time, visualization of the pineal gland was complicated, since to detect it, it was necessary to lower the cerebellum and elevate the occipital lobes of the cerebral hemispheres. In some animals, the pineal gland was connected by a plate of the pia mater to the lower surface of the cerebral hemispheres. It should be noted that with such a topography of the pineal gland, there was no close connection with the vascular plexus, and the color of the pineal gland was light gray, which is explained by the smaller number of blood vessels in contact with this organ. It was found that one, at most two blood vessels approached the pineal gland, which tightly surrounded it, branched and penetrated the organ. It was established that the source of blood supply to the organ in this case was the vascular plexus located under the cover of the third ventricle of the brain. It should be noted that the shape of the pineal gland with this placement was rounded (Fig. 3).

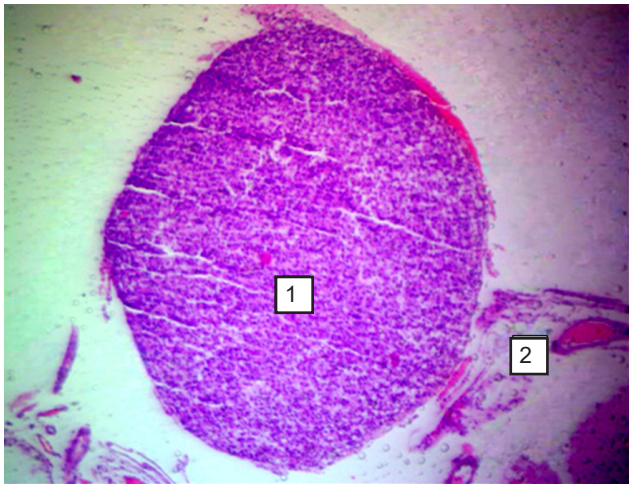

Fig. 3. Micrograph of the pineal gland of Wistar laboratory rats. Rounded shape of the pineal gland. 1 – pineal gland; 2 – extraorgan blood vessels. Hematoxylin and eosin staining. ×100.

It was found that the outer surface of the pineal gland was surrounded by a continuation of the pia mater and arachnoid membrane of the brain. At the same time, it was noted that the subarachnoid space in the apex region was the widest, but when moving to the main part, it gradually narrowed (see Fig. 2). It can be assumed that the width of the subarachnoid space varies depending on individual fluctuations in the size of the organ itself or on the degree of filling it with cerebrospinal fluid. On the outside, the pineal gland was covered by a thin capsule formed by connective tissue. The capsule was tightly attached to the pineal gland and contained blood vessels. In places, connective tissue membranes extended from the capsule into the organ, dividing the parenchyma into lobes of various sizes. The intercellular membranes contained numerous blood vessels and nerve fibers.

During further anatomical studies, variations in the linear dimensions of the pineal gland in experimental animals were established. Thus, in rats whose pineal gland had a conical shape, the average length was 1.141±0.912 mm, and the width in the widest part was 0.884±0.382 mm. For animals whose pineal gland was oval shape, the following average linear dimensions were characteristic: length was 1.263±0.722 mm, and width was 0.812±0.294 mm. With a rounded shape of the pineal gland, its average length was 1.023±0.872 mm, and its width was 0.911±0.363 mm. As the obtained digital data showed, the transverse size of the pineal glands was the most stable, while the vertical size was more labile, which probably determines the individual differences in the glands volumes.

Discussion

The presented study was aimed at supplementing data on the topographic and anatomical features of the pineal gland in laboratory Wistar line rats, since information on possible variations in the location of the pineal gland in the domestic and foreign literary sources we reviewed is sparse.

According to these literature sources, the pineal gland in different classes and species of animals has anatomical and physiological features that are determined by a number of factors, including climatic conditions of residence, lifestyle, animal's weight, circadian and seasonal rhythms [13]. In addition, significant sexual differences in the linear dimensions of the pineal gland within the species are noted [7, 11]. The results of our study indicate that the topography, shape, and size of the pineal gland in Wistar line rats differ and have individual anatomical variations within the species, which is consistent with the data of other authors' research results [10]. Morphological studies have shown that there are two main types of pineal gland localization in the rat brain [10]. It was found that in some laboratory rats, the pineal gland was located in the groove between the posterior edges of the occipital lobes of the cerebral hemispheres. A similar variant of the pineal gland location has been observed in other vertebrates [1, 2]. With this placement, the pineal gland had an oval or cone-shaped shape and a close connection with the choroid plexus of the third ventricle [12, 14, 17, 30]. At the same time, the blood supply to the organ was intensive, as it occurred due to a large number of blood vessels [25]. It should be noted that the intensive blood supply also affected the color of the pineal gland, which manifested itself as a red-gray formation [10]. In other animals, the pineal gland was located in the groove between the upper anterior corpora quadrigemina and was characterized by a rounded shape [14. 21, 26]. With such topographic features, a small number of blood vessels approached the pineal gland, which branched and penetrated into the organ parenchyma. With the described variant of the pineal gland's location in the brain, its color was light gray. It is also necessary to note the fact that with all the identified variations in the location of the pineal gland, there was always a connection with the vascular plexus of the third ventricle of the brain, which is also indicated by the data of studies by other authors [10, 23]. Differences in the color of the pineal gland in different placement options may be associated with a different number of blood vessels that contact the pineal gland and penetrate its parenchyma, as well as with the intensity of blood supply to the organ under study [14].

Externally, the pineal gland was surrounded by the soft meninges of the brain and was washed by cerebrospinal fluid [6]. A thin connective tissue capsule, which contained a large number of blood vessels, was tightly attached to the pineal gland [14]. Connective tissue membranes extended from the capsule into the organ, dividing the parenchyma into lobules. In general, the described histological features of the pineal gland were typical for this organ and corresponded to the literature data on the structure of the pineal gland in rats and other vertebrates [12, 14].

Depending on the shape of the pineal gland, we also detected changes in its linear indicators. Thus, in the oval and conical shape, the length of the pineal glands exceeded the length of the pineal glands, which were characterized by a rounded shape. Instead, the width of the round-shaped pineal glands was greater. It should be noted that the shape of the pineal gland and its linear dimensions influenced the volume of the subarachnoid space, which was wide in the apex and narrowed as it moved towards the main part. The linear dimensions of the pineal glands we have established correlate with the data of other researchers and are within the physiological norm for rodents [12, 13].

Thus, our morphological and morphometric analysis of macropreparations and histological sections of the pineal gland of laboratory Wistar rats allowed us to characterize in detail its possible individual topographic and anatomical features.

This experimental article differs from those published in this field in that it is the first to describe and analyze possible individual variations in the location of the pineal gland in laboratory Wistar rats. The morphological analysis performed allowed us to systematize the obtained experimental data and present a comparative topographic and anatomical characteristic of the pineal gland in animals that are most often used in experimental studies.

Conclusions

1. The anatomical features of the pineal gland in laboratory rats of the Wistar strain have certain species and individual variations, which are determined by the peculiarities of the topography, shape, and blood supply of the organ, which must be taken into account when conducting experimental studies and interpreting the results obtained.

2. Two variants of the pineal gland location in the brain of laboratory rats have been established. In some animals, the pineal gland was located in the groove between the posterior edges of the occipital lobes of the cerebral hemispheres, was richly supplied with blood by a large number of blood vessels, and had either an oval shape with two pointed edges or a cone-shaped shape. In other animals, the epiphysis was located in the groove between the upper anterior corpora quadrigemina, blood supply was provided by a small number of blood vessels and the shape of the epiphysis with such localization was rounded.

3. The morphometric data obtained as result of the study on the linear dimensions of the epiphyses in intact laboratory Wistar rats can be the basis for assessing pathological changes in the organ in the experiment.

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ТОПОГРАФО-АНАТОМІЧНІ ОСОБЛИВОСТІ ЕПІФІЗУ У ЛАБОРАТОРНИХ ЩУРІВ ЛІНІЇ ВІСТАР

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Епіфіз – орган центральної ендокринної системи, який координує діяльність периферичних ендокринних залоз, імунної та нервової систем, регулює біологічні ритми, забезпечує процеси адаптації та запуск стрес-реакції. Епіфіз притаманний практично всім хребетним тваринам, але існують різні варіації форми та розташування епіфізу у головному мозку тварин навіть у межах одного виду. Найчастіше, для проведення лабораторних досліджень залучаються лабораторні щури лінії Wistar і для правильної інтерпретації отриманих результатів слід враховувати видові особливості анатомічної будови та можливі індивідуальні варіації розташування епіфізу у лабораторних щурів у нормі. Метою роботи було вивчення особливостей анатомічної будови та топографії епіфізу у білих лабораторних щурів лінії Wistar. Були досліджені епіфізи 24 статевозрілих самців лабораторних щурів лінії Wistar. Використовували морфологічні, морфометричні та статистичні методи дослідження. В ході проведення дослідження були виявлені індивідуальні варіації топографії, форми та кровопостачання епіфізу у лабораторних щурів. Встановлено, що в одних тварин епіфіз розташовувався у борозні між задніми краями потиличних часток півкуль головного мозку, рясно кровопостачався великою кількістю кровоносних судин та мав овальну або конусоподібну форму. В інших тварин епіфіз розташовувався у борозні між верхніми зоровими горбками чотиригорбикового тіла. При цьому кропопостачання епіфізу відбувалося невеликою кількістю кровоносних судин, а форма епіфізу при такій локалізації була округлою. Встановлені індивідуальні варіації лінійних розмірів епіфізу. При вивченні морфометричних параметрів було встановлено, що найбільш стабільним був поперечний розмір епіфізів, а вертикальний розмір був більш лабільним, що, вірогідно, і обумовило індивідуальні відмінності об'ємів епіфізів у досліджуваних щурів. Таким чином, отримані результати дослідження щодо індивідуальних варіацій анатомічної, топографічної та морфометричної будови епіфізу в інтактних лабораторних щурів лінії Вістар можуть бути основою при оцінці патологічних змін органу при проведенні експериментальних досліджень у медичних та біологічних науках.

Ключові слова: *головний мозок, епіфіз, анатомія, топографія, лабораторні шури, індивідуальні варіації.*

Author contribution

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Iovenko A. V. – writing and editing a review.
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