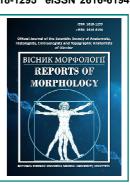
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# Morphometric features of rat pinealocytes in conditions of chronic ethanol intoxication

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Ethanol has chronobiological effects, which are associated with inhibition of melatonin synthesis and secretion and disruption of the sleep-wake cycle. Ethanol is known to cause sleep fragmentation due to frequent awakenings, prolong wakefulness, and reduce the duration of the slow sleep phase. At the same time, changes in the morphology of the pineal gland under chronic exposure to ethanol remain poorly studied. Of particular interest are changes in the basic morphometric parameters of pinealocytes, because they are a marker of the functional state of the pineal gland. The aim of the study was to morphometrically study the features of morphological changes in rat pinealocytes in the physiological norm and in chronic ethanol intoxication. To achieve this goal, we used 20 laboratory male rats: a control group and an experimental group. The control group was under standard vivarium conditions. For the experimental group, alcohol intoxication was modeled by injecting a 40% ethanol solution at the rate of 12 mg/kg of body weight intragastric 4 times a day. The morphometric parameters of pinealocytes were studied on day 30 from the start of the study. According to the results of morphometric measurements, a significant increase in the parameters of light pinealocytes and a decrease in the parameters of dark cells were established. It was determined that the average values of the cytoplasm area of light pinealocytes increase by 54.55% (p<0.05), the area of the nucleus increases by 61.32% (p<0.05), and the area of the nucleolus by 32.84% (p<0.05) compared with the control group. The area of the cytoplasm of dark pinealocytes decreases by 27.2% (p<0.05), and the area of the nucleus by 37.33% (p<0.05). Changes in the ratio of light and dark pinealocytes were also noted. An increase in the number of active light cells by 8.17% was found. The detected morphometric changes indicate high functional activity of cells, which has a compensatory nature in response to apoptosis of pinealocytes.

Key words: pineal gland, chronic ethanol intoxication, pinealocytes, vacuolar dystrophy.

### Introduction

In recent years, there has been a significant increase in the number of cases of intoxication due to alcohol abuse, due to a sharp increase in the level of alcoholism, an increase in the number of low-quality alcoholic beverages. According to official statistics, the level of alcohol consumption in our country is one of the highest compared to all other countries in the world. There are reports in the literature that the level of alcohol consumption increased significantly during the COVID-19 pandemic and was due to increased stress, depression, boredom and the availability of alcoholic beverages [2, 10]. The World Health Organization (WHO) considers alcohol abuse as a global medical and social problem of the XXI century [16, 17].

It is known that ethanol and its metabolites have a toxic

effect on almost all organs and systems of the body. The greatest impact as a result of ongoing chemical reactions aimed at the excretion of ethanol and detoxification of the body are the liver and kidneys. Changes in the morphology of various organs of visceral systems are described in detail in the literature [1, 16, 17, 18, 19, 22]. At the same time, the effect of ethanol on the morphofunctional state of various parts of the brain has not been studied enough. The vast majority of publications are devoted to changes in the morphology of the cerebellum under the influence of ethanol [14, 20, 21, 24].

There is information that ethanol has chronobiological effects that are associated with inhibition of melatonin synthesis and secretion and affects the sleep-wake cycle,

in particular causes fragmentation of the cycle due to frequent awakenings, prolongs wakefulness, reduces the duration of the slow phase of sleep [3, 5, 7, 12, 23]. However, changes in the morphology of the pineal gland under the influence of ethanol remain poorly understood [15]. Of particular interest are the changes in the basic morphometric parameters of pinealocytes, because they are a marker of the functional state of the pineal gland during the experiment.

The aim of our work was to study the morphometric features of rat pinealocytes in conditions of chronic ethanol intoxication.

#### Materials and methods

The results of this work are a fragment of the research topic of the Department of Anatomy, Clinical Anatomy, Operative Surgery, Pathomorphology and Forensic Medicine of the Petro Mohyla Black Sea National University of MES of Ukraine "The role of environmentally hazardous factors in the development of diseases of civilization", state registration № 0120U002026.

The experimental studies involved 20 adult male Wistar rats, weighing 180-220 g. The animals were kept in standard vivarium conditions. Experimental animals were divided into 2 groups: control and experimental. The control group included 6 animals, the experimental group 14. The first group consisted of intact rats, which were under normal conditions without the influence of additional factors. The second group of animals was simulated alcohol intoxication by administering 40% ethanol solution at a rate of 12 mg/kg body weight intragastrically 4 times a day [19, 25, 26]. On day 30 after the start of the experiment, rats were euthanized with thiopental anesthesia at a rate of 25 mg/kg body weight.

In order to perform morphological studies, the pineal gland was isolated [8] and fixed in a 10% solution of neutral formalin, dehydrated in solutions of ethyl alcohol with increasing concentration (40-96°) and poured into paraffin. Sections with a thickness of 4-6 µm were made on a rotary microtome of the semi-automatic type of the "Microm" brand (Germany). For further light-optical examination, histological specimens were stained according to the conventional method with hematoxylin and eosin. Light microscopy and microphotography of histopreparations were performed using a microscope brand "Carl Zeiss" (Germany) at a magnification of objective lens x20, ocular lens x10.

The number of pinealocytes in the field of view of the microscope was counted on the histological preparations of the pineal gland obtained in this standard way. The field of view of the microscope in our studies (objective lens x20, ocular lens x10) was taken as a conventional unit of area in which the number of pineal cells was counted. Cell counting was performed by analogy with the counting chamber and used Egorov's rule, namely pinealocytes were counted in the field of view and on the border of the

upper and right sectors, and cells located on the lower and left border of the microscope field of view were not counted. To obtain statistically significant data, the number of cells in 10 fields of view of the microscope was determined.

Morphometric measurements were performed using a micrometer eyepiece screw type MOV 1-16 at magnification (x40). 10 histological preparations of the studied organ of each experimental group were analyzed. Histological preparations of the control and experimental groups were studied in parallel.

Large and small diameters of the nucleus and cytoplasm of both cell types were measured. The parameters of the nucleoli of pinealocytes were determined in one direction, which can be explained by the rounded shape of these cell structures. The calculation of the area of the nucleus and cytoplasm was performed using the formula:  $S=\pi rR$ , where: r is a small radius; R is a large radius. Since the nucleoli have a rounded shape, their area was determined using a well-known mathematical formula to calculate the area of structures that have the form of a circle:  $S=\pi r^2$ .

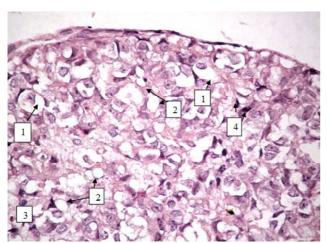
The determined quantitative morphometric data, which characterized the parameters of pinealocytes and their changes, were entered into the electronic journal of studies and subjected to statistical analysis using conventional methods of variation statistics, namely Student's t-test. Mathematical and statistical processing of the obtained numerical results was performed on a personal computer using the standard software "STATISTICA 6" for computer equipment with the Windows operating system. For all indicators, arithmetic mean values, standard errors of arithmetic mean and standard deviation were calculated, which are denoted in the work by the corresponding symbols  $M\pm m$  and  $\sigma$ . The difference was considered significant when the difference of numerical parameters between the intact and experimental series at the level of not less than p<0.05. This level of reliability is common in mathematical and statistical research in the biological and medical fields.

All manipulative interventions on experimental animals were carried out in strict compliance with the provisions of the 1997 Convention on Bioethics of the Council of Europe, the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, and the general ethical principles of animal experiments. (September 2001), the Law of Ukraine "On Protection of Animals from Cruelty" (2006) and other international agreements and current national legislation in the field of medical and biological research.

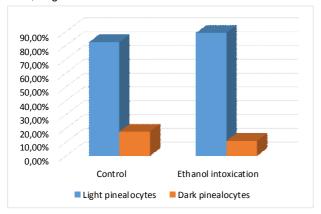
#### Results

Microscopic examination of the pineal gland of rats revealed that the overall structure of the parenchyma of the organ is typical. The parenchyma is formed by two types of pinealocytes, which form the lobes of the organ. At the same time, remodeling of pinealocytes was revealed, which

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**Fig. 1.** A fragment of the parenchyma of the rat pineal gland under conditions of ethanol intoxication. HE. Oc. x10, ob. x20. 1 - T1P in a state of vacuolation; 2 - vacuoles filled with cytoplasmic fluid; 3 - T2P; 4 - glial cells.



**Fig. 2.** Graphic ratio of T1P and T2P of the pineal gland parenchyma in experimental animals under the influence of ethanol compared with the control group of animals.

**Table 1.** Morphometric parameters of T1P in the control group of animals and under the influence of ethanol.

Group of animals	Cytoplasm area, μm² (M±m, n=50)	Nuclei area, μm² (M±m, n=50)	Nucleoli area, μm² (M±m, n=50)
Control	45,28±1,32	16,83±0,71	2,040±0,100
Ethanol intoxication	69,98±2,85*	27,15±1,27*	2,710±0,220*

Notes: \* reliably with control (p<0,05).

accordingly affected their morphometric parameters. It was found that cell remodeling was manifested by their edema, enlightenment and vacuolation of the cytoplasm of the vast majority of T1P (Type 1 pinealocytes, light pinealocytes). A large number of vacuoles of different sizes were found on histological specimens, which indicates the death of overloaded cells (Fig. 1). Due to such changes, the cells are located in the parenchyma sparsely.

It was found that the absolute majority in the histological sections of the pineal glands of both the control and experimental groups were T1P. However, it is necessary to note changes in the ratio between T2P (Type 2 pinealocytes,

dark pinealocytes) and T1P. It was determined that in the animals of the control group the percentage of T1P from the total number of pinealocytes was 82.35±0.68%, and the percentage of T2P was 17.65±0.73%. Under conditions of chronic exposure to ethanol, the number of T1P increased compared to the control group, and T2P - decreased. It was found that the percentage of T1P in the experimental group was 89.08±0.91%, and the percentage of T2P decreased to 10.92±1.21% (Fig. 2). Such changes in the ratio of cells indicate the transformation of T2P in the T1P, which occurs due to the loss of functioning overloaded cells.

Given the fact that the number of T1P is dominant for both intact and experimental groups, a sample of 50 for T1P and 30 for T2P was chosen to determine and analyze the morphometric parameters of pinealocytes.

In the comparative analysis of the average cytoplasm, nuclei and nucleoli of T1P in rats of the control and experimental groups, probable differences were found.

It was determined that the average values of the cytoplasm area of T1P in the physiological norm were  $45.28\pm1.32~\mu\text{m}^2$ , nuclei -  $16.83\pm0.71~\mu\text{m}^2$ , nucleoli  $2.040\pm0.100~\mu\text{m}^2$  (Table 1). On the 30 day of the study of the effects of ethanol, the average area of the cytoplasm of T1P was  $69.98\pm2.85~\mu\text{m}^2$ , which was significantly higher (54.55%) than in animals of the control group (p<0.05). The area of the nucleus similarly exceeded the corresponding parameter of the control group by 61.32% (p<0.05). Its average values were 27.15±1.27  $\mu\text{m}^2$ . The average area of the nucleolus significantly increased by 32.84% compared with the control group and was equal to 2.710±0.220  $\mu\text{m}^2$  (Fig. 3). Such changes in the parameters indicate an increase in the synthetic activity of T1P.

When comparing the morphometric data of T2P of both groups, a significant decrease in the average morphometric parameters of rats exposed to ethanol intoxication was observed (Table 2).

On the 30 day of the experiment, the area of the cytoplasm of T2P decreased by 27.2% (p<0.05) compared with the intact group and was 20.85±1.94  $\mu$ m². The average

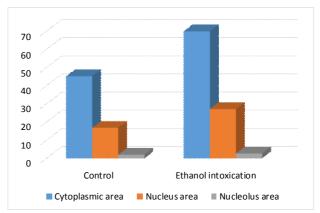


Fig. 3. Comparison of mean values of T1P morphometric parameters in control and experimental groups, µm<sup>2</sup>.

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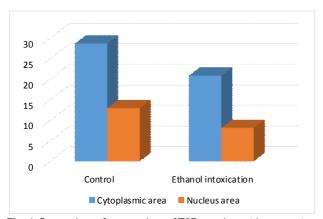


Fig. 4. Comparison of mean values of T2P morphometric parameters in control and experimental groups,  $\mu m^2$ .

**Table 2.** Morphometric parameters of T2P in the control group of animals and under the influence of ethanol.

Group of animals	Cytoplasm area, μm² (M±m, n=30)	Nuclei area, μm² (M±m, n=30)	Nucleoli area, μm² (M±m, n=30)
Control	28,64±1,65	12,94±0,62	Not determined
Ethanol intoxication	20,85±1,94*	8,110±0,800*	Not determined

Notes: \* reliably with control (p<0,05).

values of the nuclei area were  $8.110\pm0.800~\mu\text{m}^2$ , which is less than the control group by 37.33%~(p<0.05) (Fig. 4).

# Discussion

According to the literature, changes in the morphometric parameters of the cell are significant in the morphofunctional assessment of the cell state. Thus, the processes of transcription and transformation of ribosomal RNA (R-RNA) are associated with the nucleus and nucleoli, and the size of the nucleoli correlates with the intensity of cellular protein synthesis [11]. When comparing the morphometric parameters of pinealocytes of the group of animals that were in conditions of ethanol intoxication, with the control group of animals, a significant increase in all parameters of T1P was found. According to the literature and studies of other authors, such changes in the size of T1P indicate an increase in the processes of synthesis and, accordingly, the functional activity of the pineal gland [3]. At the same time, given the general decrease in the number of cells and their sparse location in the parenchyma, the increase in morphometric parameters, namely the area of the nucleus, nucleus and cytoplasm of T1P compared to similar parameters of control animals indicate high functional activity of cells in compensatory response. Our karyometric measurements of T2P correlate with the results obtained by J. Martinez-Salvador and co-

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[1] Abdulkadir, A., Mbajiorgu, E.F., & Nyirenda, T. (2018). Effects of concurrent chloroquine and ethanol administration on the rat kidney morphology. The Pan African Medical Journal, 18, 29authors in the study of the effect of ethanol on the morphology of the pineal gland and indicate a decrease in the average area of the nucleus and nucleolus of both the peripheral and central zones of the parenchyma of the organ [15].

Information on the effect of ethanol on changes in the morphometric parameters of pinealocytes in the literature we have studied is limited [15]. However, our studies correlate with data from other researchers who studied changes in the morphology of the pineal gland in response to the influence of pathological factors of endogenous and exogenous origin [4, 6, 9, 13].

This experimental article differs from those published in this field in that it is the first to describe and analyze data on the effect of ethanol on changes in the quantitative ratio and morphometric features of rat pinealocytes. The conducted mathematical and statistical analysis allowed to systematize the obtained experimental data and to present the comparative characteristics of the studied structures in the norm and under the influence of ethanol intoxication.

The obtained morphological data can be used later in research during the development of drugs aimed at correcting the negative effects of alcohol intoxication, treatment of alcohol poisoning and increase the body's adaptive capacity. The obtained results allow to expand and deepen the knowledge about the effect of ethanol on different parts of the brain. In the future it is planned to investigate the condition of the vascular bed of the pineal gland in ethanol intoxication.

#### Conclusions

- 1. As a result of histological examination, it was found that 30-day modeling of ethanol intoxication is accompanied by pronounced changes in the morphometric parameters of pinealocytes.
- 2. Remodeling of pinealocytes in the form of their edema and vacuolation of the cytoplasm was established, which had an impact on the morphometric parameters of T1P.
- 3. The ratio of T1P and T2P changes, namely: the number of active T1P increases by 8.17%.
- 4. Significantly increase the parameters of T1P and decrease T2P. It was determined that the average values of the cytoplasm area of T1P increase by 54.55% (p<0.05), the area of the nucleus increases by 61.32% (p<0.05), and the area of the nucleolus by 32.84% (p<0.05) compared with the control group. The area of the cytoplasm of T2P decreased by 27.2% (p<0.05), and the area of the nucleus by 37.33% (p<0.05). The detected morphometric changes indicate high functional activity of cells, which has a compensatory nature in response to apoptosis of pinealocytes.

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# МОРФОМЕТРИЧНІ ОСОБЛИВОСТІ ПІНЕАЛОЦИТІВ ЩУРІВ В УМОВАХ ХРОНІЧНОЇ ІНТОКСИКАЦІЇ ЕТАНОЛОМ *Пшиченко В.В., Черно В.С.*

Етанолу притаманні хронобіологічні ефекти, які пов'язані з пригніченням процесів синтезу і секреції мелатоніну та порушенням циклу "сон-неспання". Відомо, що етанол спричиняє фрагментацію сну через часті пробудження, подовжує неспання, зменшує тривалість повільної фази сну. Водночас зміни морфології епіфізу в умовах хронічного впливу етанолу лишаються малодослідженими. Особливий інтерес викликають зміни основних морфометричних параметрів пінеалоцитів, оскільки вони є маркером функціонального стану епіфізу. Метою дослідження було вивчити особливості морфологічних змін пінеалоцитів щурів в умовах фізіологічної норми і при хронічній інтоксикації етанолом. Для досягнення мети нами було використано 20 лабораторних самців-щурів: контрольна та дослідна групи. Контрольна група перебувала за стандартних умов віварію. Для дослідної групи моделювали алкогольну інтоксикацію шляхом введення 40% розчину етанолу з розрахунку 12 мг/кг маси тіла внутрішньошлунково 4 рази на добу. Вивчення морфометричних параметрів пінеалоцитів проводили на 30 добу від початку дослідження. За результатами морфометричних вимірювань встановлено достовірне підвищення параметрів світлих пінеалоцитів та зменшення показників темних клітин. Визначено, що середні значення площі цитоплазми світлих пінеалоцитів збільшуються на 54,55% (р<0,05), площа ядра збільшується на 61,32% (р<0,05), а площа ядерця на 32,84% (p<0,05) у порівнянні з контрольною групою. Площа цитоплазми темних пінеалоцитів зменшується на 27,2% (p<0,05), а площі ядра на 37,33% (р<0,05). Відмічено і зміни у співвідношенні світлих та темних пінеалоцитів. Встановлено збільшення кількості активних світлих клітин на 8,17%. Виявлені морфометричні зміни вказують на високу функціональну активність клітин, що має компенсаторний характер у відповідь на апоптоз пінеалоцитів.

Ключові слова: епіфіз, хронічна інтоксикація етанолом, пінеалоцити, вакуольна дистрофія.

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