КРАТКИЕ СООБЩЕНИЯ

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CHANGES IN THE ADRENAL MEDULLA OF MARAL DOES DURING THE YEAR = ИЗМЕНЕНИЯ СТРУКТУРЫ МОЗГОВОГО ВЕЩЕСТВА НАДПОЧЕЧНЫХ ЖЕЛЕЗ САМОК МАРАЛА В ТЕЧЕНИЕ ГОДА

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Описаны особенности гистологического строения и кариометрические параметры мозгового вещества надпочечных желез небеременных самок марала в течение года. Установлено, что в осенний период синтетическая активность норадреналинпродуцирующих клеток у холостых самок марала наименьшая по отношению к другим сезонам года. Сравнение полученных результатов с литературными данными позволяет выявить половые различия в деятельности железистых клеток мозгового вещества надпочечников маралов, что указывает на различные механизмы регуляции обмена веществ у самцов и самок весной (период активного роста рогов у самцов) и разную степень стрессируемости их организмов осенью (период полового размножения).

Ключевые слова: *надпочечники, структура мозгового вещества, марал, адреналин, норадреналин, функциональная активность, сезонные изменения.*

Biological characteristics of animals are linked with the dynamics of hormonal activity and allow them to adapt to environmental conditions. The structure of the mammalian adrenal gland varies from species to species. Studying the changes in its structure during the annual cycle in mammals of temperate latitudes allows us to more accurately determine the mechanisms of adaptation. Due to the production of catecholamines, the adrenal medulla provides stress response. There are data on changes in the activity of adrenal glands when the ambient temperature changes [1]. It should be noted that marals are characterized by broad adaptive reactions. The structure of the adrenal

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glands of maral bucks and pregnant does, as well as the adrenal cortex of non-pregnant does have been described in detail [2]. When it comes to the seasonal aspect and other deer species, only the dynamics of the blood levels of hormones produced by the adrenal cortex has been described [3–5]. In addition, there is literature data on sex-related differences in the structural and functional features of deer adrenal glands [6]. Therefore, the topic of this research is highly relevant. The aim of the paper was to study the tissue structure, as well as the morphometric and karyometric parameters of the adrenal medulla of maral does during different periods of the year.

As the material for the research we used adrenal glands obtained from non-pregnant maral does aged 5–8 years living on the free-range maral farms of the Altai Republic. The glands were taken from animals in January, March, June, and October after a special shooting (one shot to the head) in accordance with Directive 2010/63/EU. Fragments of the middle parts of the adrenal glands were fixed in Carnoy's solution and embedded in paraffin. The histological sections of the glands were made using a microtome and then stained with haematoxylin and eosin [7–9]. The parameters for determining the functional activity of cells were selected on the basis of classical morphometric methods according to G.G. Avtandilov [10]. The thickness of the medulla, the diameter of epinephrine-producing cells (E cells) and norepinephrine-producing cells (N cells), as well as the volume of their nuclei were determined. The nuclear-cytoplasmic (N:C) ratio of the cells was calculated using the following formula:

$$\text{N:C ratio} = d^2_{n\ \max} / d^2_{c\ \max},$$

where $d_{n\ \max}$ is the maximum diameter of the cell nucleus and $d_{c\ \max}$ is the maximum diameter of the cytoplasm of the same cell [10].

To calculate the studied parameters, we used a MicroMed microscope with a camera and an adapter with Toup Vien software. We compared the values of the morphometric parameters obtained from animals in winter with spring, in spring with summer, and in summer with autumn (see Table). Statistical analysis of the results of the study was carried out using the software package STATISTICA 10.0 for Windows. The significance of the differences between the groups was determined applying the nonparametric Mann–Whitney *U* test. The differences were considered significant at $p < 0.05$.

Sections of the adrenal glands of maral does are characterized by the usual structure for most mammals, namely, the cortex is under the connective tissue capsule, the medulla is at the centre of the gland, and the uneven connective tissue layer is located between the cortex and the medulla. There are two distinct types of cells in the medulla: on the periphery, the cells are large, arranged in the form

MORPHOMETRIC PARAMETERS OF THE ADRENAL MEDULLA OF NON-PREGNANT MARAL DOES

МОРФОМЕТРИЧЕСКИЕ ПАРАМЕТРЫ МОЗГОВОГО ВЕЩЕСТВА НАДПОЧЕЧНЫХ ЖЕЛЕЗ НЕБЕРЕМЕННЫХ САМОК МАРАЛА

Parameter	Winter	Spring	Summer	Autumn
Absolute thickness, μm	2347.85 ± 79.99	2456.44 ± 63.23	2290.58 ± 54.82**	2019.28 ± 23.67
Diameter of E cells, μm	14.56 ± 1.53	13.91 ± 2.40	15.09 ± 1.73	13.37 ± 1.53
Volume of E cell nuclei, μm^3	127.23 ± 32.08	138.11 ± 42.23	131.58 ± 43.43	156.15 ± 65.21
N:C ratio of E cells	0.185 ± 0.035	0.022 ± 0.063	0.176 ± 0.039	0.248 ± 0.061
Diameter of N cells, μm	12.93 ± 1.44	12.46 ± 1.51	13.21 ± 1.48*	10.62 ± 1.04
Volume of N cell nuclei, μm^3	117.87 ± 33.41	111.92 ± 28.97	128.28 ± 28.23	91.46 ± 23.38
N:C ratio of N cells	0.223 ± 0.048	0.235 ± 0.054	0.226 ± 0.048	0.278 ± 0.860

Note. The difference from the subsequent group is statistically significant: * – when $p < 0.05$, ** – when $p < 0.01$.

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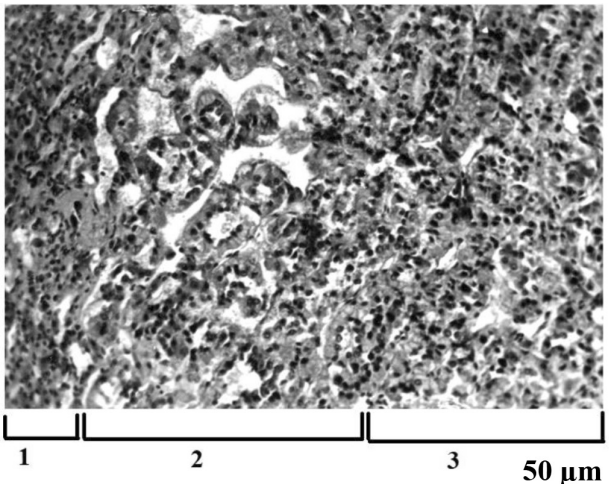
of strands, with their nuclei occupying an eccentric position – these are the E cells; in the central part, the cells are smaller and rather tightly adjacent to each other – these are the N cells (Fig.).



Adrenal gland of a 7-year-old maral during winter, stained with haematoxylin and eosin: 1 – adrenal cortex; 2 – epinephrine-producing cells; 3 – norepinephrine-producing cells

Надпочечная железа 7-летнего марала в зимний период (окраска гематоксилин-эозином): 1 – мозговое вещество; 2 – адреналинпродуцирующие клетки; 3 – норадреналинпродуцирующие клетки

The results of the study of the morphometric parameters are presented in the table.

In winter, the absolute thickness of the medulla in adult non-pregnant maral does is 2347.85 ± ± 79.99 μm, the rows of E cells are not very smooth, but are clearly separated from the N cells. The N cells form dense clusters of polygonal cells; the vascularization is high.

In spring, the thickness of the medulla remains unchanged. The histological pattern on the sections is similar to that in winter; the difference is that the level of vascularization and the lumen of blood vessels decrease. The morphometric parameters under study do not differ significantly from those in the previous season.

The structural organization and the morphometric parameters of the medulla in summer remain the same as in spring.

In autumn, the absolute thickness of the medulla in non-pregnant maral does decreases compared with the summer values. In the central part of the medulla and among the E cells, cortical cells are identified. Blood supply intensity does not change. The size of the E cells and the volume of their nuclei are practically the same as in summer. However, the E cell layer is thinner than that in summer. The diameter of N cells and the volume of their nuclei in autumn are significantly smaller than in summer. The changes revealed in the cytological parameters of N cells indicate a decrease in their synthetic activity in contrast with summer.

The analysis of the changes in the lifestyle of non-pregnant maral does in summer and autumn revealed the following: in summer, their level of motor activity is higher, the animals eat a lot and gain weight. Most likely, muscle activity is provided by the action of norepinephrine (among other factors), epinephrine playing a lesser role in this case. In addition, the changes in the hormone-producing activity of cells can be caused by a stress factor, namely, quite high ambient temperatures in summer compared to those in spring and autumn.

Thus, the lowest synthetic activity of N cells in non-pregnant maral does is observed in autumn, compared to other seasons of the year. At the same time, the morphometric parameters that characterize E cells remain unchanged throughout the year.

Noteworthy, literature data indicate sex-related differences in the morphofunctional activity of the cortex and the medulla, for example, in rats [11]. According to our results, the dynamics of structural changes in the adrenal medulla of maral does and bucks differ. Adult maral bucks show signs of increased activity of E cells in spring and destructive changes in the medulla in autumn [12]. In contrast, no signs of destruction are observed in the medulla of maral does in autumn. The obtained data indicate that the early response to cold described for many species of mammals is not typical of marals, which is expressed in an increase in the activity of the glandular cells of the adrenal glands, including the medulla [13].

Thus, the results obtained demonstrate sex-related differences in the activity of glandular

cells of the adrenal medulla, which indicates different mechanisms of metabolic regulation in bucks and does in spring, when maral bucks' horns grow actively, and different degrees of stress during the autumn period of sexual reproduction.

Conflict of interest. Authors declare no conflict of interest.

Список литературы

1. *Алябьев Ф.В., Арбыкин Ю.А., Серебров Т.В., Яушев Т.Р., Вогнерубов Р.Н., Мельникова С.Ю., Воронков С.В., Логвинов С.В.* Морфофункциональные изменения внутренних органов и некоторых биохимических показателей в динамике общего переохлаждения организма // Сиб. мед. журн. (Томск). 2014. Т. 29, № 2. С. 71–74.

2. *Грибанова О.Г., Овчаренко Н.Д.* Сезонные изменения морфометрических показателей коркового вещества надпочечников у самцов и самок марала (*Cervus elaphus sibiricus*) // Морфология. 2020. Т. 157, № 2-3. С. 62.

3. *Bubenik G.A., Brown R.D.* Seasonal Levels of Cortisol, Triiodothyronine and Thyroxine in Male Axis Deer // Comp. Biochem. Physiol. A Physiol. 1989. Vol. 92, № 4. P. 499–503. DOI: 10.1016/0300-9629(89)90356-3

4. *Nilssen K.J., Bye K., Sundsfjord J.A., Blix A.S.* Seasonal Changes in T_3, FT_4, and Cortisol in Free-Ranging Svalbard Reindeer (*Rangifer tarandus platyrhynchus*) // Gen. Comp. Endocrinol. 1985. Vol. 59, № 2. P. 210–213. DOI: 10.1016/0016-6480(85)90371-5

5. *Reyes E., Bubenik G.A., Schams D., Lobos A., Enriquez R.* Seasonal Changes of Testicular Parameters in Southern Pudu *Pudu puda* in Relationship to Circannual Variation of Its Reproductive Hormones // Acta Theriol. 1997. Vol. 42, № 1. P. 25–35.

6. *Мошкин М.П., Евдокимов Н.Г., Мирошниченко В.А., Позмогова В.П., Большаков В.Н.* Изменчивость кортикостероидной функции в популяциях обыкновенной слепушонки (*Ellobius talpinus*) // Успехи соврем. биологии. 1991. Т. 111, № 1. С. 95–100.

7. *Гущин Я.А.* Применение дополнительных гистологических методов окраски в доклинических исследованиях // Лаб. животные для науч. исследований. 2019. № 4. С. 7.

8. *Zolnikova I.F., Silkin I.I., Popov A.P., Tomitova E.A., Ovcharenko N.D.* Muskrat's (*Ondatra zibetica*) Endocrine Regulation Organs as Bioindicators for Evaluation of Ecological Conditions in Baikal Region // Eurasian J. Biosci. 2019. Vol. 13, № 2. P. 707–709.

9. *Kiernan J.A.* Dyes and Other Colorants in Microtechnique and Biomedical Research // Color. Technol. 2006. Vol. 122, № 1. P. 1–21. DOI: 10.1111/j.1478-4408.2006.00009.x

10. *Автандилов Г.Г.* Медицинская морфометрия. М.: Медицина, 1990. 382 с.

11. *Котельникова С.В., Котельников А.В.* Сезонные особенности функционального состояния надпочечников белых крыс разного пола в норме и при воздействии солью кадмия // Вестн. Астрахан. гос. техн. ун-та. 2008. № 3(44). С. 178–181.

12. *Овчаренко Н.Д., Грибанова О.Г.* Сезонная динамика структурно-функционального состояния надпочечных желез благородного оленя (*Cervus elaphus sibiricus*, Artiodactyla, Cervidae) // Зоол. журн. 2016. Т. 95, № 4. С. 484–489. DOI: 10.7868/S0044513416040115

13. *Ленчер О.С.* Состояние гормональных и морфологических показателей активности надпочечников при холодовой адаптации // Науч. обозрение. Биол. науки. 2016. № 5. С. 5–11.

References

1. Alyab'ev F.V., Arbykin Yu.A., Serebrov T.V., Yaushev T.R., Vognerubov R.N., Mel'nikova S.Yu., Voronkov S.V., Logvinov S.V. Morfofunktsional'nye izmeneniya vnutrennikh organov i nekotorykh biokhimicheskikh pokazateley v dinamike obshchego pereokhlazhdeniya organizma [The Morphofunctional Changes in Internal Organs and Biochemical Indicators During Whole-Body Hypothermia]. *Sibirskiy meditsinskiy zhurnal (Tomsk)*, 2014, vol. 29, no. 2, pp. 71–74.

2. Gribanova O.G., Ovcharenko N.D. Sezonnye izmeneniya morfometricheskikh pokazateley korkovogo veshchestva nadpochechnikov u samtsov i samok marala (*Cervus elaphus sibiricus*) [Seasonal Changes in the Morphometric Parameters of Adrenal Cortex in Males and Females of the Red Deer (*Cervus elaphus sibiricus*)]. *Morfologiya*, 2020, vol. 157, no. 2-3, p. 62.

3. Bubenik G.A., Brown R.D. Seasonal Levels of Cortisol, Triiodothyronine and Thyroxine in Male Axis Deer. *Comp. Biochem. Physiol. A Physiol.*, 1989, vol. 92, no. 4, pp. 499–503. DOI: 10.1016/0300-9629(89)90356-3

4. Nilssen K.J., Bye K., Sundsfjord J.A., Blix A.S. Seasonal Changes in T_3, FT_4, and Cortisol in Free-Ranging Svalbard Reindeer (*Rangifer tarandus platyrhynchus*). *Gen. Comp. Endocrinol.*, 1985, vol. 59, no. 2, pp. 210–213. DOI: 10.1016/0016-6480(85)90371-5

5. Reyes E., Bubenik G.A., Schams D., Lobos A., Enriquez R. Seasonal Changes of Testicular Parameters in Southern Pudu *Pudu puda* in Relationship to Circannual Variation of Its Reproductive Hormones. *Acta Theriol.*, 1997, vol. 42, no. 1, pp. 25–35.

6. Moshkin M.P., Evdokimov N.G., Miroshnichenko V.A., Pozmogova V.P., Bol'shakov V.N. Izmenchivost' kortikosteroidnoy funktsii v populyatsiyakh obyknovennoy slepushonki (*Ellobius talpinus*) [Corticosteroid Function Variability in Mole Vole Populations (*Ellobius talpinus*)]. *Uspekhi sovremennoy biologii*, 1991, vol. 111, no. 1, pp. 95–100.
7. Gushchin Ya.A. Primenenie dopolnitel'nykh gistologicheskikh metodov okraski v doklinicheskikh issledovaniyakh [Additional Histological Methods of Staining in Preclinical Studies]. *Laboratornye zhivotnye dlya nauchnykh issledovaniy*, 2019, no. 4, p. 7.
8. Zolnikova I.F., Silkin I.I., Popov A.P., Tomitova E.A., Ovcharenko N.D. Muskrat's (*Ondatra zibetica*) Endocrine Regulation Organs as Bioindicators for Evaluation of Ecological Conditions in Baikal Region. *Eurasian J. Biosci.*, 2019, vol. 13, no. 2, pp. 707–709.
9. Kiernan J.A. Dyes and Other Colorants in Microtechnique and Biomedical Research. *Color. Technol.*, 2006, vol. 122, no. 1, pp. 1–21. DOI: 10.1111/j.1478-4408.2006.00009.x
10. Avtandilov G.G. *Meditsinskaya morfometriya* [Medical Morphometry]. Moscow, 1990. 382 p.
11. Kotel'nikova S.V., Kotel'nikov A.V. Sezonnye osobennosti funktsional'nogo sostoyaniya nadpochechnikov belykh krys raznogo pola v norme i pri vozdeystvii sol'yu kadmiya [The Seasonal Features of Functional Condition of Adrenal Glands of White Rats of Both Sexes in the Norm and Under Cadmium Salt Exposure]. *Vestnik Astrakhanskogo gosudarstvennogo tekhnicheskogo universiteta*, 2008, no. 3, pp. 178–181.
12. Ovcharenko N.D., Gribanova O.G. Sezonnaya dinamika strukturno-funktsional'nogo sostoyaniya nadpochechnykh zhelez blagorodnogo olenya (*Cervus elaphus sibiricus*, Artiodactyla, Cervidae) [Seasonal Dynamics of the State of Adrenal Glands in Red Deer (*Cervus elaphus sibiricus*, Artiodactyla, Cervidae)]. *Zoologicheskiy zhurnal*, 2016, vol. 95, no. 4, pp. 484–489. DOI: 10.7868/S0044513416040115
13. Lencher O.S. Sostoyanie gormonal'nykh i morfologicheskikh pokazateley aktivnosti nadpochechnikov pri kholodovoy adaptatsii [Hormonal and Morphological Parameters of Adrenal Activity During Cold Adaptation]. *Nauchnoe obozrenie. Biologicheskie nauki*, 2016, no. 5, pp. 5–11.

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CHANGES IN THE ADRENAL MEDULLA OF MARAL DOES DURING THE YEAR

This paper dwells on the histological structure and karyometric parameters of the adrenal medulla of non-pregnant maral does throughout the year. It was found that in autumn the synthetic activity of norepinephrine-producing cells in non-pregnant maral does is the lowest in relation to other seasons of the year. Having compared our results with the literature data, we identified sex-related differences in the activity of glandular cells of the adrenal medulla, which indicates different mechanisms of metabolic regulation in bucks and does during spring (period of active horn growth in bucks) and different levels of stress in autumn (period of sexual reproduction).

Keywords: *adrenal glands, structure of adrenal medulla, maral, epinephrine, norepinephrine, functional activity, seasonal changes.*

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