AGE-RELATED MORPHODENSITOMETRIC CHANGES IN NEURONS OF THE SUPRAOPTICAL NUCLEUS OF THE HYPOTHALAMUS OF RATS DURING STRESS MODELING AND ITS PHARMACOLOGICAL CORRECTION

O.S. Agranov, R.Ye. Bulyk

Bukovinian State Medical University, Chernivtsi, Ukraine

Objective *– to study melatonin effect on the morphodensitometric parameters of neurons of the hypothalamus supraoptical nuclei in old rats subjected to immobilization stress.*

Material and methods. *The experiments were carried out on male white rats aged 19-23 months and weighing 300-320 g, divided into three series (two groups each) for investigation during the day and night periods. The first series of animals was kept for 30 days under the lighting regime of 12.00L:12.00D; the second series was under the same lighting regime and the animals were simulated with prolonged immobilization stress. Rats of the third series under conditions of immobilization stress were administered melatonin. Morphodensitometric analysis of the hypothalamus neurons of rats was performed using a computer system for digital image analysis of the VIDAS-386 series (Kontron Elektronik, Germany) in the visible spectrum. Quantitative parameters of the area of neurons, their nuclei and nucleoli, RNA content in the cytoplasm of cells, their nuclei and nucleoli were obtained in a semi-automatic mode using licensed software. To establish the probability of differences in values, the Student (t) test was used. Scientific research was conducted in compliance with the main provisions of Ukrainian Law No. 3447-IV "On Protection of Animals from Cruelty", the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (18.03.1986), EU Directive 2010/63/EU, Orders of the Ministry of Health of Ukraine No. 690 (23.09.2009), No. 944 (14.12.2009), and Order of the Ministry of Education and Science of Ukraine No. 249 (01.03.2012). The research protocol N2 was approved by the Biomedical Ethics Commission of [BSMU] (17.10.2024).*

The research was conducted within the framework of approved research project of the Department of Medical Biology and Genetics "Morphofunctional Reorganization of Structures of Nervous and Endocrine Systems in Different Periods of Postnatal Ontogenesis and Biochemical Mechanisms of Metabolism of Signaling Molecules, State of Oxidative and Antioxidant Systems under Conditions of Experimental Pathologies and Influence of Glutathione and Melatonin (Experimental Study)" (state registration number 0124U002513, implementation period 01.2024-12.2028).

Results*. The study of the morphodensitometric characteristics of neurons of the hypothalamus supraoptical nuclei revealed daily dynamics of the indices. Under standard light conditions a daily rhythm of morphofunctional activity of neurons of the hypothalamus supraoptical nuclei with a maximum activity at night (02.00) was recorded in rats. Keeping animals under conditions of immobilization stress caused more pronounced changes in the morphodensitometric parameters of neurons of the hypothalamus supraoptical nucleus at 02.00 than at 14.00. Thus, the area of the neuron nucleus constituted 265.61±6.327 μm² and was probably smaller (14.0%) concerning the same index in the control group of animals. These changes were accompanied by a decrease in the area of the nucleus and cytoplasm of the neuron, which constituted 167.64±4.707 μm² and was significantly smaller (24.0%) than that in animals kept under standard lighting conditions. The staying of animals under immobilization stress disturbed the circadian rhythm of the morphofunctional activity of neurons of the hypothalamus supraoptical nuclei. Their greater activity, in contrast to rats kept under normal lighting, was registered during the daytime observation period. In order to correct pharmacologically the identified disorders caused by immobilization stress, the animals were administered melatonin intraperitoneally. Thus, at 14.00 a veritable increase in the areas of the nucleus and neuron nucleolus of the hypothalamus supraoptical nucleus was registered, which constituted 111.79±7.095 μm² and 84.71±8.577 μm², respectively, against a background of a decrease in the area of its cytoplasm, concerning the animals that were subjected to immobilization stress without melatonin administration. During this period of time, the RNA concentration in the neuronal structures under study accordingly also increased. At 02.00, the areas of the nucleus, nucleolus, and cytoplasm of the hypothalamic supraoptical nucleus neuron, as well as the RNA concentration level in the nucleus and cytoplasm of the neuron were probably greater than in rats that were*

Keywords: *supraoptical nucleus, hypothalamus, morphometry, densitometry, immobilization stress, melatonin.*

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E-mail: *sagranov03@gmail.com*

subjected to immobilization stress without melatonin administration. At the same time, melatonin did not correct the neurons'rhythm activity of the supraoptical nucleus, which remained the same as in animals exposed to immobilization stress and inversive concerning the animals that were under normal light conditions.

Conclusions. *1. Under standard light conditions, a daily rhythm of morphofunctional activity of neurons of the hypothalamus supraoptical nuclei with a maximum of activity at night is registered in rats. 2. Keeping animals under immobilization stress provoked a disturbance of the rhythm of morphofunctional activity of the neurons under study and caused more pronounced changes in the morphodensitometric parameters of their structural components at 02.00 than at 14.00, accompanied by a significant decrease in the area of the nucleus, nucleolus and cytoplasm of the neuron concerning similar values in the control group of animals. 3. The administration of melatonin to rats, exposed to immobilization stress, had a positive effect and contributed to the increase in the functional activity and correction of deviations in morphodensitometric parameters of the studied neurons of the supraoptical nucleus of the hypothalamus of rats, mostly at 02.00, when the body normally produces the most melatonin.*

ВІКОВІ МОРФОДЕНСИТОМЕТРИЧНІ ЗМІНИ НЕЙРОНІВ НАДЗОРОВОГО ЯДРА ГІПОТАЛАМУСА ЩУРІВ ПРИ МОДЕЛЮВАННІ СТРЕСУ ТА ЙОГО ФАРМАКОЛОГІЧНІЙ КОРЕКЦІЇ

О.С. Агранов, Р.Є. Булик

Bukovinian State Medical University, Chernivtsi, Ukraine

Key words: *надзорове ядро, гіпоталамус, морфометрія, денситометрія, іммобілізаційний стрес, мелатонін.*

Клінічна та експериментальна патологія. 2026; Т.25, № 2 (96). С. 84-91.

Мета роботи *– вивчити вплив мелатоніну на морфоденситометричні параметри нейронів надзорових ядер гіпоталамуса старих щурів, що зазнали дії іммобілізаційного стресу.*

Матеріал і методи. *Експерименти виконано на самцях білих щурів віком 19-23 міс. масою 300-320 г, яких розподілено на три серії (у кожній по дві групи) для дослідження в денний та нічний періоди доби. Тварин першої серії утримували 30 діб за умов режиму освітлення 12.00С:12.00Т; друга серія – за аналогічного світлового режиму з моделюванням тривалого іммобілізаційного стресу. Щурам третьої серії в умовах іммобілізаційного стресу вводили мелатонін. Морфоденситометричний аналіз нейронів гіпоталамуса щурів проводили з використанням комп'ютерної системи цифрового аналізу зображення VIDAS-386 (Kontron Elektronik, Німеччина) у видимому спектрі. Кількісні параметри площі нейронів, їхніх ядер та ядерець, а також вміст РНК отримували в напівавтоматичному режимі за допомогою ліцензованого програмного забезпечення. Для встановлення вірогідності відмінностей значень використовували t-критерій Стьюдента. Дослідження виконані з дотриманням основних положень Закону України № 3447-IV «Про захист тварин від жорстокого поводження», Конвенції Ради Європи про охорону хребетних тварин, що використовують в експериментах та інших наукових цілях (від 18.03.1986 р.), Директиви Європейського Союзу 2010/63/EU та наказів МОЗ України № 690 від 23.09.2009 р., № 944 від 14.12.2009 р. і наказу МОН № 249 від 01.03.2012 р. Протокол №2 наукового дослідження затверджений Комісією з питань біомедичної етики БДМУ від 17.10.2024 року. Дослідження виконували в рамках науково-дослідної роботи кафедри медичної біології та генетики «Морфофункціональні перебудови структур нервової та ендокринної систем у різні періоди постнатального онтогенезу та біохімічні механізми метаболізму сигнальних молекул, стан оксидантної та антиоксидантної систем за умов експериментальних патологій і впливу глутатіону та мелатоніну (експериментальне дослідження)» (державний реєстраційний номер 0124U002513, термін виконання 01.2024 р.–12.2028 р.).*

Результати. *Вивчення морфоденситометричних характеристик нейронів надзорових ядер гіпоталамуса виявило добову динаміку показників. За стандартного світлового режиму в щурів реєстрували добовий ритм морфофункціональної активності нейронів надзорових ядер гіпоталамуса з максимумом активності в нічний час (02.00 год). Утримання тварин в умовах іммобілізаційного стресу викликало більш значущі зміни морфоденситометричних параметрів нейронів надзорового ядра гіпоталамуса о 02.00 год, ніж о 14.00 год. Зокрема, площа ядра нейрона становила 265,61±6,327 мкм^2 і була вірогідно меншою* *(на 14,0 %) за аналогічний показник у контрольній групі тварин. Вказані зміни супроводжувалися зменшенням площі ядерця та цитоплазми нейрона (остання становила 167,64±4,707 мкм^2), що було вірогідно менше (на 24,0 %) відносно тварин, яких утримували за стандартного режиму освітлення. Перебування тварин в умовах іммобілізаційного стресу порушувало добовий ритм морфофункціональної активності нейронів надзорових ядер гіпоталамуса. Більшу їх активність, на відміну від щурів, які перебували за звичайного освітлення, реєстрували у денний період спостереження. З метою фармакологічної корекції виявлених порушень, спричинених іммобілізаційним стресом, тваринам внутрішньоочеревинно вводили мелатонін. Так, о 14.00 год зареєстровано вірогідне зростання площ ядра і ядерця нейрона надзорового ядра гіпоталамуса, які становили 111,79±7,095 мкм^2 та 84,71±8,577 мкм^2 відповідно, на тлі зниження площі його цитоплазми, щодо тварин, які зазнали дії іммобілізаційного стресу без уведення мелатоніну. У цей часовий проміжок також зростала концентрація РНК у досліджуваних нейронних структурах. О 02.00 год площі ядра, ядерця, цитоплазми нейрона надзорового ядра гіпоталамуса, а також рівень концентрації РНК у ядрі та цитоплазмі нейрона були вірогідно більшими, ніж у щурів, яких піддали дії іммобілізаційного стресу без уведення мелатоніну. Водночас, мелатонін не корегував ритму активності нейронів надзорового ядра, він залишався таким, як і у тварин, що занали дії іммобілізаційного стресу та інверсним щодо тварин, які перебували за звичайного світлового режиму.*

Висновки. *1. За стандартного світлового режиму в щурів реєструється добовий ритм морфофункціональної активності нейронів надзорових ядер гіпоталамуса з максимумом активності в нічний час. 2. Утримування тварин в умовах іммобілізаційного стресу спричинило порушення ритму морфофункціональної активності досліджуваних нейронів і викликало більш виражені зміни морфоденситометричних параметрів їх структурних компонентів о 02.00 год, що супроводжувалося вірогідним зменшенням площі ядра, ядерця та цитоплазми нейрона щодо аналогічних величин контрольної групи тварин. 3. Уведення мелатоніну щурам, які зазнали дії іммобілізаційного стресу мало позитивний ефект і сприяло зростанню функціональної активності та корекції відхилень морфоденситометричних параметрів досліджуваних нейронів надзорового ядра гіпоталамуса щурів здебільшого о 02.00 год, коли в нормі в організмі продукується найбільше мелатоніну.*

Introduction

Motional activity is an important property of animals and human being, it is one of the conditions for their normal existence and development. Restriction of motional activity (hypokinesia, or immobilization) is a powerful stress factor that causes various pathological processes [1-3].

The vital activity of all organisms on Earth is also influenced by biological rhythms (biorhythms) - (diurnal, seasonal, etc.) fluctuations in the intensity and nature of certain biological processes and phenomena that contribute to the adaptation of organisms to cyclical changes in the environment. Today, biorhythmicity is recognized as one of the main properties of all living organisms. It is an important mechanism for regulating functions that ensure the ability of organisms to maintain homeostasis and adapt to environmental changes [4-6].

The alternation of the circadian (diurnal) cycle of day and night is the most important regulator of various physiological rhythms in all living organisms [7, 8]. The invention of electricity and artificial lighting more than a hundred years ago radically changed both the light regime and the duration of light exposure, including on humans. The impact of light at night, often called light pollution, has increased and has become an essential part of the modern lifestyle, accompanied by a number of serious behavioral and health disorders.

The main rhythmic controller of the body's functions is considered to be the neuroendocrine brain structure - the pineal gland (epiphysis), which is found in all vertebrates. Together with the suprachiasmatic nucleus (SCN) of the hypothalamus, this gland is a part of the so-called biological clock of the body, what plays a key role in the mechanisms of "internal timekeeping" [9]. In addition to that, the SCN of the hypothalamus plays the role of a central oscillator that regulates the adjustment of metabolic and energy rhythms to the rhythms of lighting as an exogenous energy source [3].

The coding of information about the light regime is carried out by means of the main hormone of the pineal gland – melatonin [10]. By influencing the functional activity of the hypothalamic-pituitary-adrenal and reproductive systems, melatonin is involved in the regulation of the circadian and seasonal rhythms. It has been shown that melatonin secretion is subjected to clear daily variations with a minimum value during the day and a maximum around 02.00 [8]. Violation of the light regime (long-term lighting, constant darkness) is a determining stressor that resulted in the development of desynchronosis [11, 12].

The hypothalamus, as the highest subcortical center of the autonomic (vegetative) nervous system, has a powerful regulatory effect on all vital functions of the body, including maintaining the homeostatic balance of a living system, which is disturbed as a result of the activity of stressors, in particular immobilization, constant lighting

[13]. Due to the important role of the large-cell neurosecretory supraoptical nuclei of the hypothalamus, it is relevant to study the nature of their response to the effects of immobilization stress and melatonin on the organism of experimental animals in the implementation of the adaptive capabilities of the organism [14, 15].

The aim of the research

Study melatonin effect on the morphodensitometric parameters of neurons of the supraoptical nuclei of the hypothalamus of old rats exposed to immobilization stress.

Material and methods of the research

Experimental studies were carried out on non-linear white male rats aged 19-23 months and weighing 300-320 g. The animals were divided into 3 series of studies (each into two groups), in which the biomaterial was collected at 14.00 and 02.00. The chosen experimental periods were determined by different functional activity of the pineal gland at the indicated time periods of the day. The rats were kept in standard vivarium conditions with free access to food and water.

Animals of the 1st series (control group) were kept for 30 days under standard light conditions (light from 08.00 to 20.00, illumination by fluorescent lamps at the cage level of 1000 lux). Rats of the 2nd series, which were under the same lighting conditions as the animals of the 1st series, were simulated with prolonged immobilization stress by keeping them in special plastic cages-pencils for 6 hours daily for 30 days running. Rats of the 3-rd series were under the same experimental conditions as the rats of the 2nd series. They were daily injected with melatonin intraperitoneally at 19:00 (Sigma, USA, degree of purification –99.5%) at a dose of 10.0 mg/kg of animal body weight.

After completion of the experiment, the animals were removed from the experiment the next day at 14.00 and 02.00 by means of decapitation under thiopental-sodium anesthesia (40.0 mg/kg intraperitoneally). The brain of the animals was immediately removed and placed in 10.0% formalin solution in 0.1 mole phosphate buffer (pH 7.2) for 20 hours at room temperature. After the standard procedure of dehydration and chloroform-paraffin impregnation, the brain was embedded in paraffin. Scientific research was conducted in compliance with the main provisions of Ukrainian Law No. 3447-IV "On Protection of Animals from Cruelty", the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (18.03.1986), EU Directive 2010/63/EU, Orders of the Ministry of Health of Ukraine No. 690 (23.09.2009), No. 944 (14.12.2009), and Order of the Ministry of Education and Science of Ukraine No. 249 (01.03.2012). The research protocol N2 was approved by the Biomedical Ethics Commission of BSMU (17.10.2024).

The research was conducted within the framework of approved research project of the Department of Medical Biology and Genetics "Morphofunctional Reorganization of Structures of Nervous and Endocrine Systems in Different Periods of Postnatal Ontogenesis and Biochemical Mechanisms of Metabolism of Signaling Molecules, State of Oxidative and Antioxidant Systems under Conditions of Experimental Pathologies and Influence of Glutathione and Melatonin (Experimental Study)" (state registration number 0124U002513, implementation period 01.2024-12.2028).

Histological sections 7 μm thick were deparaffinized in xylene, rehydrated in descending concentrations of ethanol (100%, 96%, 70%), washed three times in distilled water and stained for 48 h by the Einarson method in a solution of halocyanine-chromium alum, which allows the detection of nucleic acids (mostly RNA) in neurons, in order to study the morphometric characteristics of neurons of the hypothalamus supraoptical nucleus. Then the sections were washed three times in distilled water, dehydrated in ascending concentrations of ethanol (70%, 96%, 100%), xylene and placed in Canada balsam.

Morphometric analysis of hypothalamic neurons was carried out in a computer-based digital image analysis system VIDAS-386 (Kontron Elektronik, Germany) in the visible spectrum. The image, obtained on the AXIOSKOP microscope, was installed into the VIDAS-386 (Kontron Elektronik, Germany) computer system for digital image analysis using a COHU-4922 video camera (COHU Inc., USA). Image analysis was performed in a semi-automatic mode using the VIDAS-2.5 (Kontron Elektronik, Germany) application program package: the boundaries of the neuron body, its nucleus and nucleolus were interactively determined.

The experimental data obtained were processed on personal computers using the VIDAS-2.5 (Kontron Elektronik, Germany) application and statistical program package and EXCEL-2003 (Microsoft Corp., USA). For all indicators, the values of the arithmetic mean of the sample (x), its variance and the error of the mean (Sx) were calculated. To identify the probability of differences in the results of studies in the experimental and control groups of animals, the Student coefficient (t) was determined, after which the probability of the difference of the samples (p) and the confidence interval of the mean were found according to the Student distribution tables. Values were considered probable for which $p<0.05$.

Scientific research was carried out in compliance with the main provisions of the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty to Animals", the Council of Europe Convention for the Protection of Vertebrate Animals Used for Experiments and Other Scientific Purposes (dated 03/18/1986), the European Union Directive 2010/63/EU and orders of the Ministry of Health of Ukraine No. 690 dated 09/23/2009, No. 944 dated 12/14/2009 and order of the Ministry of Education and Science No. 249 dated 03/01/2012. The scientific research protocol was approved by the Biomedical Ethics Commission of the BSMU dated 02/15/2024.

Results and Discussion

When studying the morphometric parameters of the supervisory nucleus of the hypothalamus at 14.00, it has been found that the area of the neuron body constituted 277.72±4.358 μm², the nucleus – 75.51±1.280 μm², the nucleolus – 31.49±4.510 μm² and the cytoplasm – 202.21±4.230 μm² (Table 1). The conducted correlation analysis established a close relationship between the area of the neuron body and the nucleus (r=0.72), between the area of the nucleus and the nucleolus (r=0.94). A direct correlation has been also found between the area of the neuron and its cytoplasm (r=0.68). The nuclear-cytoplasmic ratio (NCR) was within 2.65±0.023 units, the specific volume of the neuron nucleus constituted 73.37±0.562%, and the cytoplasm was 26.62±0.561%.

Table 1

Morphometric changes in neurons of the hypothalamic supraoptical nucleus in old rats with melatonin administration against a background of immobilization stress (x±Sx)

Series of experimental animals		Neuron area, μm²	Area of the neuron nucleus, μm²	Area of the neuron nucleoli, μm²	Area of the neuron cytoplasm, μm²
1.	Control, 14.00	277,72±4,358	75,51±1,280	31,49±4,510	202,21±4,230
	Control, 02.00	309,95± 8,050 $p_1<0,01$	88,93 ± 5,086 $p_1<0,05$	37,19± 8,927	221,03±6,012 $p_1<0,05$
2.	Immobilization stress, 14.00	305,41±8,085 $p<0,05$	68,800±2,728 $p<0,05$	31,85±5,657	236,61±7,933 $p<0,01$
	Immobilization stress, 02.00	265,61±6,327 $p<0,001$ $p_1<0,01$	97,96±3,778 $p_1<0,001$	27,19±4,350	167,64±4,707 $p<0,001$ $p_1<0,001$
3.	IImmobilization stress+melatonin, 14.00	314,01±12,288 $p<0,05$	111,79±7,095 $p<0,001$ $p_2<0,001$	84,71±8,577 $p<0,001$ $p_2<0,001$	202,11±7,205 $p_2<0,01$
	Iммоbilization stress+ melatonin, 02.00	287,45±12,272	108,72±6,758 $p<0,05$	78,61±8,857 $p<0,001$ $p_2<0,001$	178,73±7,472 $p<0,05$ $p_1<0,005$

Note: p – probable changes concerning the parameters of the control group of animals of the same time interval; p_1 – probability of difference in the parameters of animals of the previous time interval within the series; p_2 – probability of difference concerning the parameters of the same time interval in animals that were exposed to immobilization stress. Each series consisted of 20 animals

The study of morphometric characteristics of neurons of the hypothalamic supraoptic nucleus revealed daily dynamics of indices. Thus, compared with the daytime period (14.00), by 02.00 there was a probable increase 11.5% in the body area of neurons of the hypothalamic supraoptic nucleus, caused by an increase in the area of the cell nucleus 17.7% (Table 1), that is confirmed by a direct correlation (r=0.33). In its turn, the increase in the area of the neuron nucleus is caused by the increase in the area of its nucleolus, which constituted 37.19± 8.927 μm² (Table 1), the correlation coefficient between the indices under study was 0.59. In addition to that, during the night period of observation, the NCR in vasopressin-synthesizing structures was 2.95±0.019% and probably higher (11.2%) than during the daytime. At the same time, the specific volume of the neuron cytoplasm increased 2.9%, and the nucleus, on the contrary, decreased 7.2%.

These changes were combined with an increase in the concentration of RNA in the nuclei themselves 13.2% compared to the daytime period. In addition to that, we did not register any significant changes in the concentration of RNA in the cytoplasm of the neuron of the hypothalamic supraoptical nucleus.

The values obtained indicate an increase in the functional and synthetic activity of neurons of the supraoptical nucleus in the control group of rats during the night period.

Immobilization stress is a traditional model of an acute stress situation, in which, in addition to movement restriction, there is also a pronounced emotional component associated with the inability to avoid a threatening situation. When animals were kept under immobilization stress conditions at14:00, the area of the neuron of the supraoptical nuclei of the hypothalamus reached 305.41±8.085 μm² and was probably larger (10.0%) concerning the similar value in the control group of rats. At the same time, we found an increase in the size of its cytoplasm 16.9% (r=0.78). The probable increase in the NCR 41.0% concerning the control group of animals, which constituted 3.74±0.018 units, also attracted attention. At the same time, the specific volume of the cytoplasm probably increased against a background of a decrease in the specific volume of the nucleus of the neurons under study. In addition to that, the specific volume of the nucleolus was within 10.84±1.929 units and was probably larger concerning the volume of the studied structure in the neurons of the control group of animals during the daytime observation period.

Immobilization stress resulted in a probable decrease in the concentration of RNA in the nucleus 33.0% at 14.00. Similar changes were registered in the nucleolus and cytoplasm of the studied hypothalamic neurons, in which the amount of RNA was probably lower than the values in the control group of animals (Table 2).

Keeping animals under immobilization stress conditions caused more pronounced changes in the morphofunctional state of neurons of the hypothalamic supraoptical nuclei at 02.00 hour than at 14.00 hour. Thus, the area of the neuron nucleus constituted 265.61±6.327 μm² and was probably lower (14.0%) than that in the control group of animals. The mentioned changes were accompanied by a decrease in the area of the nucleolus and the area of the neuron cytoplasm, which constituted 167.64±4.707 μm2 and was probably lower (24.0%) than in animals, kept under standard lighting conditions. It should be noted that staying of animals under immobilization stress conditions disturbed the daily rhythm of the morphofunctional activity of neurons of the hypothalamic supraoptical nuclei. Their greater activity, in contrast to rats kept under normal lighting, was recorded during the daytime observation period (Table 1). The NCR in the neurons of the hypothalamic supraoptical nuclei at 02.00 was probably lower than that in the control group of animals 39.0% due to a decrease in the specific volume of the neuron cytoplasm of the hypothalamic structure under study in experimental animals.

Table 2

Diurnal fluctuations in the RNA concentration in neurons of the supraoptical hypothalamic nucleus of rats exposed to immobilization stress and melatonin administration (x±Sx)

Series of experimental animals		RNA concentration in the nucleus, con.un.opt.den.	RNA concentration in the nucleoli. cond.un. opt.den.	RNA concentration in the cytoplasm, cond.un.opt.den.
1.	Control, 14.00	0,144±0,0025	0,338±0,0029	0,072±0,0021
	Control, 02.00	0,190±0,0078 p_1<0,001	0,308±0,0121 p_1<0,05	0,071±0,0038
2.	Immobilization stress, 14.00	0,096±0,0031 p<0,001	0,182±0,0112 p<0,001	0,039±0,0025 p<0,001
	Immobilization stress, 02.00	0,111±0,0021 p<0,001 p_1<0,01	0,257±0,0069 p<0,01 p_1<0,001	0,065±0,0021 p_1<0,001
3	Immobilization stress+melatonin, 14.00	0,134±0,0039 p_3<0,001	0,197±0,0093	0,095±0,0036 p_3<0,001
	Immobilization stress+melatonin, 02.00	0,136±0,0040 p_2<0,001	0,211±0,0103 p_2<0,01	0,105±0,0043 p_3<0,001

Note: p – probable changes concerning the parameters of the control group of animals of the same time interval; p_1 – probability of difference in relation to the parameters of animals of the previous time interval within the series; p_2 – probability of difference in relation to the parameters of the same time interval in animals exposed to immobilization stress. 20 animals were in each series

Analyzing the night stage of the experiment, we note that, as in rats kept under habitual photoperiod, a higher RNA concentration in the nucleus of the neuron of the hypothalamic supraoptical nuclei was also recorded at 02.00 at the level of 0.111±0.0021 con. units of optical density. In this daily interval, the indices of the concentration of nucleic acid in the nucleolus of the structures under study were lower in comparison with the values of the control group of animals of the same time interval (Table 2).

In comparison with the daytime period (14.00), a decrease in the body area of the neurons of the hypothalamic supraoptical nuclei was detected by 02.00, stipulated by a probable decrease in the area of the cell cytoplasm (r=0.83). This became the reason for the decrease of the NCR in the night-time period of observation in the neurons under study, which constituted 1.79±0.031 units.

For the purpose to correct pharmacologically the detected disorders caused by immobilization stress, the animals were intraperitoneally administered melatonin. In addition to that, at 14.00 we registered a probable increase in the areas of the nucleus and nucleolus of the neuron of the hypothalamic supraoptical nucleus, which were 111.79±7.095 μm² and 84.71±8.577 μm², respectively, against a background of a decrease in the area of its cytoplasm, concerning animals that were exposed to immobilization stress without melatonin administration. During this time period, the concentration of RNA in the structures of neurons under study increased accordingly. At the same time, melatonin did not correct the activity rhythm of neurons of the supraoptic nucleus, which remained the same as in animals exposed to immobilization stress and inversely related to animals under normal light conditions. At 02.00 hour, the areas of the nucleus, nucleolus, and cytoplasm of the neuron of the hypothalamic supervisory nucleus, as well as the level of RNA concentration in the nucleus and cytoplasm of the neuron were probably greater than in rats exposed to immobilization stress without melatonin administration. This is evidence of a positive effect of melatonin, which contributes to the increase in the functional activity and correction of deviations in the morphodensitometric parameters of the neurons under study of the hypothalamic supraoptical nucleus of rats caused by immobilization stress during the specified period, when the body normally produces the most melatonin.

Conclusions

1. Under standard light conditions, rats have a daily rhythm of morphofunctional activity of neurons of the hypothalamic supraoptical nuclei with maximum activity at night.
2. Keeping animals under immobilization stress conditions provoked a rhythm disturbance of the morphofunctional activity of the studied neurons and caused more pronounced changes in the morphodensitometric parameters of their structural components at 02:00 than at 14:00 hour, which was accompanied by a probable decrease in the area of the nucleus, nucleolus and cytoplasm of the neuron compared to similar values in the control group of animals.
3. Melatonin administration to rats, exposed to immobilization stress, had a positive effect and contributed

to an increase of the functional activity and correction of deviations in the morphodensitometric parameters of the neurons under study of the hypothalamic supraoptical nucleus of rats, mainly at 02:00, when the body normally produces the most melatonin.

Prospects of scientific research

The influence of immobilization stress and melatonin on the morphofunctional activity of neurons of the hypothalamic ventricular nuclei for a deeper understanding of the mechanisms of participation of these structures in the regulation of neuroendocrine processes during stress and stress reactivity of the organism is planned to be investigated in the future.

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Відомості про автора:
Agranov O. – Post-graduate, Department of Medical Biology and Genetics, Bukovinian State Medical University, Chernivtsi, Ukraine.
e-mail: sagranov03@gmail.com
ORCID: https://orcid.org/0009-0008-7627-2359
Bulyk R. – Doctor of Medical Sciences, Professor, Department of Medical Biology and Genetics, Bukovinian State Medical University, Chernivtsi Ukraine
E-mail: bulyk@bsmu.edu.ua
ORCID ID: https://orcid/org/0000-0003-0651-534X

Відомості про авторів
Агранов О.С. – аспірант кафедри медичної біології та генетики Буковинського державного медичного університету, м. Чернівці, Україна.
E-mail: sagranov03@gmail.com
ORCID ID: https://orcid.org/0009-0008-7627-2359
Булик Р.Є. – д.мед.н., професор, завідувач кафедри медичної біології та генетики Буковинського державного медичного університету, м. Чернівці, Україна.
E-mail: bulyk@bsmu.edu.ua
ORCID ID: https://orcid.org/0000-0003-0651-534X

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