UDC: 612.826.4:612.017.2
DOI: 10.24061/2413-4260. XV.2.56.2025.22

CHANGES IN MORPHODENSITOMETRIC PARAMETERS OF THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS IN RATS UNDER STRESS OF DIFFERENT DURATIONS

I. Fedoriak, R.Bulyk

Bukovinian State Medical University
(Chernivtsi, Ukraine)

Summary

Paraventricular nuclei of the hypothalamus play the important role in both endocrine regulation of the tropic function of the adenohypophysis and the organism's neuroendocrine response to stress of various origins, particularly sustained photoperiod disturbances. However, morphodensitometric properties of the medial parvocellular subnuclei within the rat hypothalamic paraventricular nuclei across varying photoperiod durations have yet to be investigated.

Aim of the study. *To investigate the morphodensitometric parameters of the medial parvocellular subnuclei of the hypothalamic paraventricular nuclei in rats across diurnal time points under altered photoperiodic conditions.*

Materials and methods. *The study was performed on 36 sexually mature, male outbred white rats. The animals were divided into three groups, each comprising two subgroups of six rats. The rats were maintained under a different light-dark cycle for 14 days (light/dark 12:00L:12:00D, 12 hours each, LD), (dark/dark 00L:24:00D, DD), (light/ light 24:00L:00D, LL). Morphodensitometric analysis of hypothalamic neurons in rats was performed using the VIDAS-386 digital image analysis system (Kontron Elektronik, Germany) within the visible spectrum. Quantitative data on the area of neurons, their nuclei and nucleoli, the content of RNA in the cell cytoplasm, nuclei and nucleoli were obtained in a semi-automatic mode.*

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 was approved by the Biomedical Ethics Commission of [BDMU] (24.02.2019).

Statistical analysis was performed using the «STATISTICA» software (StatSoft Inc., USA, Version 10). Quantitative indicators with normal distribution were compared using Student's t-test. Differences were considered statistically significant at $p<0.05$.

The research was conducted within the framework of approved research projects of the Department of Medical Biology and Genetics: «Morphofunctional and Biochemical Basis of Dysfunction of Neurosecretory Brain Structures and Endocrine Glands and Hepatorenal System in Rats in Experimental Pathology, in Age Aspect and Ways of Its Correction» (state registration number 0119U101346, implementation period 01.2019-12.2023) and «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 and discussion. *Under the LD lighting regime, morphodensitometric analysis revealed a circadian rhythm in the morphofunctional activity of the medial parvocellular subnuclei of the paraventricular nucleus. Peak values were recorded during the daytime interval (14:00 h).*

In rats from the DD group that were decapitated at 14:00 h, the neuronal soma area was 54.77±0.605 μm², amounting to a 9.7% decrease compared to the LD group. At the same time, its cytoplasmic area measured 28.91±0.427 μm², representing a 10.4% increase relative to the LD group. A significant reduction was observed in the concentrations of RNA in the nucleus and nucleolus of the studied structures (by 14.4% and 12.1%, respectively) at 14:00 h.

Investigations conducted on samples collected at 02:00 h revealed a 10.4% increase in neuronal soma area compared to the control values, and a 10.9% increase compared to animals from the previous time interval exposed to the same experimental conditions. Nevertheless, the nucleus showed a significantly lower RNA concentration (by 31.8%), attributed to a reduction in the nucleolus (by 21.7%). Additionally, a significant decrease in RNA content was observed in the cytoplasm, where its concentration was 0.10±0.001 o.u.

In rats from the LL group at 14:00 h, no significant changes in the morphometric parameters of the mpsPN were observed. Regarding RNA concentration in the studied neuronal structures, a significant increase was detected only in the nucleolus of neurosecretory cells, where it measured 0.303±0.0023 o.u. at 14:00 h – 2.5% higher than in rats maintained under standard photoperiod conditions. At 02:00 h, no significant differences in neuron size or its structural parameters were found compared to those in the LD group. RNA concentration in the cytoplasm and nucleus of the neurosecretory cells of the mpsPN remained stable, whereas in the nucleolus it was 2.8% lower than in the LD group.

Conclusions: *1. Morphodensitometric parameters of the medial parvocellular subnuclei of the hypothalamic paraventricular nuclei in rats exhibit fluctuations, with peak values occuring during the daytime observation period.*

2. Light deprivation induces a disruption in the rhythm of functional activity of the studied hypothalamic neurons and a shift of peak values from the daytime to the nighttime period, likely attributable to increased levels of melatonin, the primary pineal chronobiotic, in the bloodstream during this phase of the day. As a stress-limiting factor, melatonin suppresses CRH production in the mpsPN of the hypothalamus.

3. Prolonged exposure to constant illumination (light stress) did not elicit significant changes in the studied morphodensitometric parameters compared to those in rats maintained under LD and DD photoperiods. This suggests a remarkable plasticity of the mpsPN of the hypothalamus in response to experimental conditions.

Key words: *Paraventricular Nucleus; Hypothalamus; Medial Parvocellular Subnuclei; Photoperiod; Prolonged Lighting; Constant Darkness; Melatonin; Photoperiod.*

Introduction

The hypothalamus serves as the supreme subcortical center integrating autonomic, emotional, and motor components of adaptive behavior, while functioning as the primary homeostatic regulator [5, 8, 24]. Its paraventricular nuclei (PVN) have a complex cellular organization comprising magnocellular and parvocellular subnuclei, whose neurons differ not only in size and efferent innervation but also in the spectrum of synthesized neuropeptides [6, 10]. These characteristics determine the essential role of hypothalamic paraventricular nuclei in both endocrine regulation of adenohypophyseal tropic function and neuroendocrine stress response mediation [9, 19]. Of particular relevance in studying stress responses and the effects of stress-limiting factors (e.g., melatonin) are investigations of the specific subpopulations of PVN neurons involved in the synthesis of stress-releasing hormones that initiate stressor responses [1-4, 7, 11, 20]. The principal peptides jointly regulating ACTH secretion are corticotropin-releasing hormone (CRH) and vasopressin (VP). CRH-immunoreactive labeling predominantly localizes to mpsPVN, while VP-immunoreactivity appears in posterolateral magnocellular subnuclei [12, 13, 25].

Among environmental parameters, photoperiodism represents the most reliable and stable synchronizing factor for homeothermic organisms [14, 21-23].]. Light regime disruptions (prolonged illumination, constant darkness) constitute stressors inducing desynchronosis [15-18]. Despite numerous publications examining structural-functional changes in these regions during stress responses, the morphodensitometric reorganization of medial parvocellular subnuclei in rat hypothalamic paraventricular nuclei under varying photoperiods remains unexplored.

Objective. To investigate photoperiod modification effects on morphometric parameters of medial parvocellular subnuclei in rat hypothalamic paraventricular nuclei across different circadian periods.

Materials and methods

The study was conducted on 36 sexually mature male outbred white rats weighing 150-180 g. The animals were divided into three groups, each consisting of two subgroups (six animals per subgroup). Rats of the first group (intact) were maintained for 14 days under standard lighting conditions (12:00 light (L):12:00 dark (D), LD, illumination from 08:00 to 20:00, with 500 lx fluorescent lighting intensity in animal cages). Animals of the second group were kept in constant darkness for 14 days (light deprivation, DD, inducing epiphyseal hyperfunction). Rats of the third group were maintained under constant illumination of the same intensity as the first group (LL, inducing pineal gland hypofunction).

At the end of the 14-day experiment, on the following day at 14:00 and 02:00 h, the animals were anesthetized with intraperitoneal injection of etaminal sodium (40.0 mg/kg) and sacrificed by immediate decapitation. Brains were immediately extracted and fixed in 10.0% formaldehyde solution in 0.1 M phosphate buffer (pH 7.2) at room temperature for 20 hours. Following standard dehydration in chloroform and paraffin impregnation, brains were embedded in paraffin.

For hypothalamic neuron morphometric analysis, 7-μm thick histological sections were deparaffinized in xylene, rehydrated through a graded ethanol series (100%, 96%, 70%), rinsed three times in distilled water, and stained with Einarson's gallocyanin-chrome alum solution for 48 hours to visualize neuronal nucleic acids (RNA). Sections were then rinsed in distilled water, dehydrated through ascending ethanol concentrations (70%, 96%, 100%), cleared in xylene, and mounted in Canada balsam.

Hypothalamic neuron morphometry was performed using the VIDAS-386 digital image analysis system (Kontron Elektronik, Germany) in the visible spectrum. Images acquired through an AXIOSKOP microscope with a COHU-4922 series video camera (COHU Inc., USA) were processed by the VIDAS-386 system. Semi-automated image analysis was conducted using VIDAS-2.5 software (Kontron Elektronik, Germany), with interactive delineation of neuronal soma, nucleus, and nucleolus boundaries.

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 was approved by the Biomedical Ethics Commission of [BDMU] (24.02.2019).

Statistical analysis was performed using the «STATISTICA» software (StatSoft Inc., USA, Version 10). Quantitative indicators with normal distribution were compared using Student's t-test. Differences were considered statistically significant at $p<0.05$.

The research was conducted within the framework of approved research projects of the Department of Medical Biology and Genetics: «Morphofunctional and Biochemical Basis of Dysfunction of Neurosecretory Brain Structures and Endocrine Glands and Hepatorenal System in Rats in Experimental Pathology, in Age Aspect and Ways of Its Correction» (state registration number 0119U101346, implementation period 01.2019-12.2023) and «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 and discussion

During observation periods, we identified variations in densitometric and morphometric parameters of the medial parvocellular subnucleus of the hypothalamic paraventricular nucleus (mpsPVN). In samples collected during daytime, neuronal soma area measured 60.71±0.736 μm², nucleus area 34.54±0.563 μm², nucleolus area 5.15±0.143 μm², and

cytoplasm area 26.17±0.347 μm². At 14:00 h observation, nuclear specific volume constituted 57.69±0.940% of total neuronal volume, while cytoplasmic volume was 42.31±0.564% of mpsPVN. The nucleocytoplasmic ratio of neurosecretory cells measured 1.36±0.021 AU during this period. RNA concentrations were: nucleus 0.182±0.0014 AU, nucleolus 0.296±0.0020 AU, and cytoplasm 0.131±0.0010 AU.

Analysis of morphometric parameters at 02:00 h revealed a significant reduction in neuronal soma area (by 9.3%), primarily due to decreased nuclear (–6.9%) and cytoplasmic areas (–12.5%) compared to the values observed at 14:00 h. The nucleus-to-cytoplasm ratio increased to 1.44 ± 0.018 units (6.2% higher vs 14:00 h). A statistically significant 3.1% decrease in RNA concentration was found in the cytoplasm, while nuclear and nucleolar RNA levels in mpsPVN hypothalamic neurons showed no significant changes versus intact animals from the previous time interval.

In summary, under LD conditions, morphometric and densitometric analysis demonstrated diurnal rhythms in hypothalamic neurotransducer activity – specifically in mpsPVN – with peak values occurring during daytime observation (14:00 h).

Circadian day-night oscillations represent a principal activator of diurnal rhythms in mammalian organs and systems. Melatonin, the key chronoregulatory hormone, is synthesized predominantly (>80%) by the pineal gland (epiphysis cerebri). Constant darkness (light deprivation) stimulates, while prolonged illumination suppresses pineal melatonin synthesis, consequently inducing desynchronosis of the circadian pacemaker – the hypothalamic suprachiasmatic nuclei. To elucidate the role of mpsPVN for temporal organization, we conducted combined densitometric and morphometric analyses under modified lighting conditions.

Light deprivation is known to enhance pineal gland functional activity regarding melatonin synthesis. Accordingly, one experimental rat group was maintained under such conditions (DD) for 14 days. In subgroup 1 animals decapitated at 14:00 h, neuronal soma area measured 54.77±0.605 μm² (9.7% lower vs LD group, $p<0.05$), attributable to 25.2% nuclear and 20.2% nucleolar area reductions versus LD controls. Notably, despite decreased mpsPVN area, cytoplasmic area increased significantly to 28.91±0.427 μm² (10.4% higher vs LD).

Cytoplasmic expansion correlated with increased cytoplasmic specific volume relative to soma and decreased nuclear specific volume against 29.2% reduction in nucleocytoplasmic ratio (0.92±0.014 AU vs LD at 14:00 h).

Simulating pineal hyperactivity, we observed significant decreases in nuclear RNA concentration (14.4%), nucleolar RNA concentration (12.1%) at 14:00 h. These findings indicate reduced functional and synthetic capacity of rat mpsPVN at 14:00 h during light deprivation.

Analysis of samples collected at 02:00 h, corresponding to peak physiological melatonin concentration, demonstrated significant morphological changes. The neuronal soma area increased by 10.4% compared to control values and by 10.9% relative to animals from the previous time interval under identical experimental conditions. In the first case, these alterations were driven by a 27.2% expansion of mpsPVN cytoplasmic area, and increases in nuclear 22.3%, r=0.88) and nucleolar 16.5%, r=0.85) areas in the second case.

Quantitative analysis revealed a nucleocytoplasmic ratio of 1.11±0.019 AU, with nuclear specific volume measuring 52.01±0.907%, representing 21.3% and 10.8% reductions respectively compared to 12L:12D controls. Quantitative analysis revealed a nucleocytoplasmic ratio of 1.11±0.019 AU, with nuclear specific volume measuring 52.01±0.907%, representing 21.3% and 10.8% reductions respectively compared to 12L:12D controls ($p<0.05$). Specific cytoplasmic volume constituted 47.93±0.839% of total cell volume, showing no significant deviation from intact animals.

Despite overall size increases in neuronal soma and neuronal components at 02:00 h versus 14:00 h observations, we detected a 31.8% decrease in nuclear RNA concentration, attributable to a 21.7% reduction in nucleolar content, along with significantly diminished cytoplasmic RNA levels (0.10±0.001 AU).

These findings indicate disrupted rhythmicity in hypothalamic mpsPVN functionality, characterized by shifted peak activity from daylight to nighttime periods under light deprivation conditions, likely mediated by elevated melatonin acting as a stress-limiting factor that suppresses CRH production in hypothalamic mpsPVN.

In order to study the effect of constant illumination on the morphological and functional organisation of the studied structures, the next stage of the experiment involved modelling light stress by keeping the experimental rats in the LL regime for 14 days. Under constant illumination (LL) conditions maintained for 14 days, experimental animals exhibited no significant morphometric alterations in mpsPVN at 14:00 h, though trends toward increased soma, nuclear and cytoplasmic dimensions were noted without reaching statistical significance versus LL controls.

The nucleocytoplasmic ratio and specific volumes of nucleus and cytoplasm remained stable, while RNA concentration showed selective elevation exclusively in nucleoli (0.303±0.0023 AU at 14:00 h), measuring 2.5% higher than LD group values (Figure).

At 02:00 h, no significant differences were observed in neuronal area or structural parameters compared to standard lighting conditions, mirroring findings from 14:00 h observations. While RNA concentrations remained stable in both cytoplasm and nuclei of mpsPVN, nucleolar RNA showed a 2.8% decrease relative to LD group values.

Prolonged light stress induced significant increases in neuronal soma area (7.4% at 14:00 h) driven by 14.1% cytoplasmic expansion relative to the figure at 14:00 h. The 02:00 h nucleocytoplasmic ratio in hypothalamic mpsPVN measured 1.46±0.030 AU, reflecting a 13.2% increase versus 14:00 h samples.

Collectively, analysis of circadian fluctuations in rat hypothalamic mpsPVNs under light stress revealed no substantial deviations from intact controls. Although chronic intense illumination represents a potent desynchronosis trigger, these conditions minimally impacted mpsPVN neurosecretory cells in our experimental paradigm.

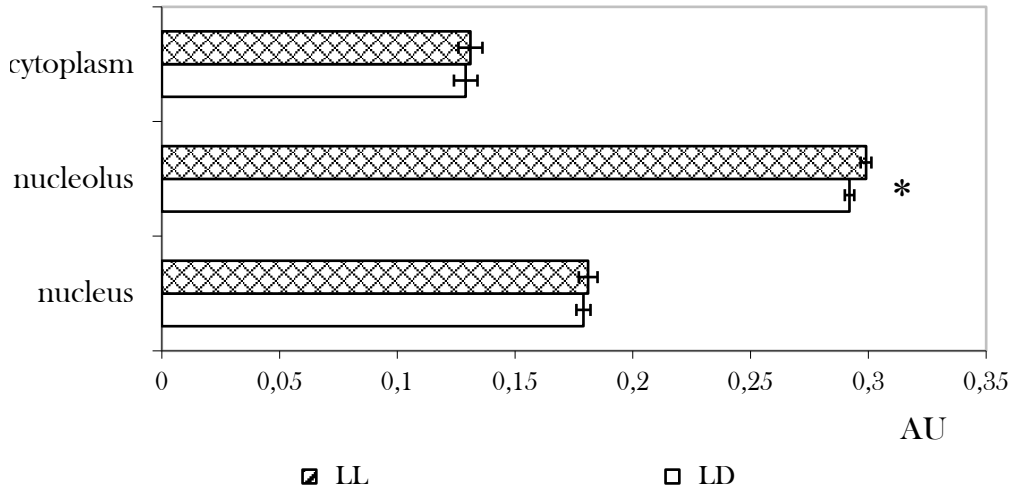

Figure. RNA concentration index (AU) in the mpsPVN neuron in rats under light stress.

Conclusions

1. Morphodensitometric parameters of hypothalamic mpsPVN in rats exhibit diurnal variations with peak values during daytime observation periods.

2. Continuous illumination (light stress) produced no significant alterations in measured parameters compared to LD and DD regimes, suggesting remarkable hypothalamic mpsPVN plasticity under experimental conditions.

3. Light deprivation disrupted hypothalamic neuron activity rhythms, shifting peak performance from daytime to nighttime periods, likely mediated by elevated melatonin levels which suppress CRH production in hypothalamic mpsPVN as a stress-limiting mechanism.

Research prospects

Future investigations will examine exogenous melatonin effects on morphofunctional activity in rat hypothalamic paraventricular nucleus neurons, aiming to elucidate deeper mechanisms of neuroendocrine regulation involving these structures across varying photoperiod durations.

References:

1. Alston M, Cain S W, Rajaratnam S M W. Advances of melatonin-based therapies in the treatment of disturbed sleep and mood. Handb Exp Pharmacol 2019; 253: 305-319. https://doi.org/10.1007/164_2018_139

2. Asefy Z, Khusro A, Mammadova S, Hoseinnejhad S, Eftekhari A, Alghamdi S, et al. Melatonin hormone as a therapeutic weapon against neurodegenerative diseases. Cell Mol Biol (Noisy-le-grand). 2021;67(3):99-106. DOI: https://doi.org/10.14715/cmb/2021.67.3.13

3. Beauchamp M T, Lundgren J D. A systematic review of bright light therapy for eating disorders. The Primary Care Companion for CNS Disorders 2016; 18: 26718. https://doi. org/10.4088/PCC.16r02008

4. Begemann K, Neumann A M, Oster H. Regulation and function of extra-SCN circadian oscillators in the brain. Acta Physiol (Oxf) 2020; 229: e13446. https://doi.org/10.1111/ apha.13446

5. Bulyk RY, Smetanyuk OV, Vlasova KV, Kryvchanska MI, Yosypenko VR, Voloshyn VL, et al. Morphohistochemical alterations of neurons of the supraoptic nucleus of the rat hypothalamus at different durations of the photoperiod and melatonin administration. J Med Life. 2021;14(6):810-5. DOI: https://doi.org/10.25122/jml-2021-0220

6. Cai ZJ. Hypothalamic aging and hormones. Vitam Horm. 2021;115:15-37. DOI: https://doi.org/10.1016/bs.vh.2020.12.002

7. Chen D, Zhang T, Lee TH. Cellular Mechanisms of Melatonin: Insight from Neurodegenerative Diseases. Biomolecules. 2020;10(8):1158. DOI: https://doi.org/10.3390/biom10081158

8. Drogovoz S M, Derymedvid' L V, Seredyns'ka N M, Luk'yanchyuk V D, Shtroblya M V, Panfilova A L, et al. Circadian Rhythms: Physiological and Pathophysiological Aspects. 2024;54:175-81. Neurophysiology. DOI: https://doi.org/10.1007/s11062-024-09949-3

9. Gan L, Cookson MR, Petrucelli L, La Spada AR. Converging pathways in neurodegeneration, from genetics to mechanisms. Nat Neurosci. 2018;21(10):1300-9. DOI: https://doi.org/10.1038/s41593-018-0237-7

10. Grzęda E, Ziarniak K, Sliwowska JH. The paraventricular nucleus of the hypothalamus – the concertmaster of autonomic control. Focus on blood pressure regulation. Acta Neurobiol Exp (Wars). 2023;83(1):34-44. DOI: https://doi.org/10.55782/ane-2023-004

11. Gunata M, Parlakpinar H, Acet HA. Melatonin: A review of its potential functions and effects on neurological diseases. Rev Neurol (Paris). 2020;176(3):148-65. DOI: https://doi.org/10.1016/j.neurol.2019.07.025

12. Honma S. The mammalian circadian system: a hierarchical multi-oscillator structure for generating circadian rhythm. J Physiol Sci 2018; 68: 207-219. https://doi.org/10.1007/ s12576-018-0597-5

13. Kalsbeek A, Buijs RM. Organization of the neuroendocrine and autonomic hypothalamic paraventricular nucleus. Handb Clin Neurol 2021;180:45-63. https://doi.org/10.1016/B978-0-12-820107-7.00004-5.

14. Kiessling S, Sollars PJ, Pickard GE. Light stimulates the mouse adrenal through a retinohypothalamic pathway independent of an effect on the clock in the suprachiasmatic nucleus. PLoS One. 2014;9(3): e92959. DOI: https://doi.org/10.1371/journal.pone.0092959

15. Liu C, Tang X, Gong Z, Zeng W, Hou Q, Lu R. Circadian rhythm sleep disorders: genetics, mechanisms, and adverse effects on health. Front Genet 2022; 13: 875342. https://doi. org/10.3389/fgene.2022.875342

16. Ota S M, Kong X, Hut R, Suchecki D, Meerlo P. The impact of stress and stress hormones on endogenous clocks and circadian rhythms. Front Neuroendocrinol 2021; 63: 100931. https://doi.org/10.1016/j.yfrne.2021.100931

17. Pilorz V, Helfrich-Förster C, Oster H. The role of the circadian clock system in physiology. Pflugers Arch 2018; 470: 227-239. https://doi.org/10.1007/s00424-017-2103-y

18. Qin C, Li J, Tang K. The Paraventricular Nucleus of the Hypothalamus: Development, Function, and Human Diseases. Endocrinology. 2018;159(9):3458-3472. https://doi.org/10.1210/en.2018-00453.

19. Sasaki R, Asami T, Takaishi M, Nakamura R, Roppongi T, Yoshimi A, et al. Smaller hypothalamic subregion with paraventricular nucleus in patients with panic disorder. Brain Imaging Behav. 2024;18(4):701-9. DOI: https://doi.org/10.1007/s11682-023-00834-x

20. Stanford SC. Recent developments in research of melatonin and its potential therapeutic applications. Br J Pharmacol. 2018;175(16):3187-9. DOI: https://doi.org/10.1111/bph.14371

21. Tähkämö L, Partonen T, Pesonen AK. Systematic review of light exposure impact on human circadian rhythm. Chronobiol Int. 2019;36(2):151-70. DOI: https://doi.org/10.1080/07420528.2018.1527773

22. Tan DX, Xu B, Zhou X, Reiter RJ. Pineal Calcification, Melatonin Production, Aging, Associated Health Consequences and Rejuvenation of the Pineal Gland. Molecules. 2018;23(2):301. DOI: https://doi.org/10.3390/molecules23020301

23. Vasileva Z. Melatonin and Epilepsy. Folia Med (Plovdiv). 2021;63(6):827-33. DOI: https://doi.org/10.3897/folmed.63.e58637

24. Wu H, Dunnett S, Ho YS, Chang RC. The role of sleep deprivation and circadian rhythm disruption as risk factors of Alzheimer's disease. Front Neuroendocrinol. 2019;54:100764. DOI: https://doi.org/10.1016/j.yfrne.2019.100764

25. Yan M, Lv X, Zhang S, Song Z, Hu B, Qing X, Kou H, Chen S, Shao Z, Liu H. Alleviation of inflammation in paraventricular nucleus and sympathetic outflow by melatonin efficiently repairs endplate porosities and attenuates spinal hyperalgesia. Int Immunopharmacol. 2025;149:114213.https://doi.org/10.1016/j.intimp.2025.114213.

ЗМІНИ МОРФОДЕНСИТОМЕТРИЧНИХ ПОКАЗНИКІВ ПРИШЛУНОЧКОВОГО ЯДРА ГІПОТАЛАМУСА ЩУРІВ ЗА УМОВ СТРЕСУ РІЗНОЇ ТРИВАЛОСТІ

І. В. Федоряк, Р. Є. Булик

Буковинський державний медичний університет
(м. Чернівці, Україна)

Резюме.

Пришлуночкові ядра гіпоталамуса відіграють важливу роль не тільки в ендокринній регуляції тропної функції аденогіпофіза, а й у реалізації нейроендокринної відповіді організму на стрес різного генезу, зокрема тривале порушення фотоперіоду. Нез'ясованими залишаються морфоденситометричні параметри присередніх дрібноклітинних суб'ядер пришлуночкових ядер гіпоталамуса щурів за різної тривалості фотоперіоду.

Мета дослідження. З'ясувати морфоденситометричні показники присередніх дрібноклітинних суб'ядер пришлуночкових ядер гіпоталамуса щурів у різні періоди доби за модифікацій фотоперіоду.

Матеріал та методи дослідження. Роботу виконано на 36 статевозрілих самцях безпородних білих щурів. Тварин поділено на три групи, кожна з яких складалася з двох підгруп (у кожній по шість тварин). Щури перебували 14 діб в умовах різних світлових режимів (12:00C: 12:00T, LD), (00C:24:00T, DD), (24:00C:00T, LL). Морфоденситометричний аналіз нейронів гіпоталамуса щурів проводили з використанням комп'ютерної системи цифрового аналізу зображення серії VIDAS-386 (Kontron Elektronik, Німеччина) у видимому спектрі. Кількісні параметри площі нейронів, їхніх ядер та ядерець, вмісту РНК у цитоплазмі клітин, їхніх ядрах і ядерцях отримували в напівавтоматичному режимі за допомогою ліцензованого програмного забезпечення.

Наукові 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 наукового дослідження затверджений Комісією з питань біомедичної етики БДМУ від 24.02.2019 року.

Статистична обробка результатів здійснювалася з використанням програмного забезпечення «STATISTICA» (StatSoft Inc., USA, Version 10). Порівняння кількісних показників з нормальним розподілом проведено за допомогою t-критерію Стьюдента, вірогідність відмінностей вважали статистично значущою при $p<0,05$.

Дослідження виконували в рамках затверджених тем науково-дослідних робіт кафедри медичної біології та генетики «Морфофункціональне і біохімічне обґрунтування дисфункції нейросекреторних структур головного мозку й ендокринних залоз та гепаторенальної системи щурів при експериментальній патології, у віковому аспекті та шляхи її корекції» (державний реєстраційний номер 0119U101346, термін виконання 01.2019 р.–12.2023 р.) та «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 та їх обговорення. За режиму освітлення LD виявлено добовий ритм морфофункціональної активності присередніх дрібноклітинних суб'ядер пришлуночкового ядра (пдсПЯ) гіпоталамуса з найбільшими показниками у денний період спостереження (14.00 год).

У щурів групи DD, яким проводили декапітацію о 14.00 год, площа соми нейрона становила 54,77±0,605 мкм^2 і була вірогідно нижчою на 9,7% щодо такої в тварин LD. Водночас, площа його цитоплазми знаходилася у межах 28,91±0,427 мкм^2 і була на 10,4% більшою відносно тварин групи LD. Нами відмічено вірогідне зменшення таких параметрів, як концентрації РНК в ядрі та ядерці досліджуваних структур (на 14,4 і 12,1% відповідно) о 14.00 год. Дослідження, проведені на зразках, відібраних о 02.00 год, виявили зростання площі тіла нейрона на 10,4% щодо контрольних величин, а також на 10,9% відносно тварин попереднього часового проміжку, які перебували за аналогічних умов експерименту. Не зважаючи на це, в ядрі виявлено вірогідно нижчу концентрацію РНК (на 31,8%), що викликано її зменшенням у ядерці (на 21,7%). Водночас встановлено вірогідне зменшення вмісту РНК і в цитоплазмі, де її концентрація становила 0,10± 0,001 о.о.щ.

У щурів групи LL о 14.00 год вірогідних змін морфометричних параметрів пдсПЯ нами не виявлено. Щодо концентрації РНК у досліджуваних структурах нейрона, необхідно вказати на її вірогідне зростання тільки в ядерці нейросекреторної клітини,

ISSN 2226-1230 (PRINT) ISSN 2413-4260 (ONLINE)
KEY TITLE: NEONATOLOGÌÂ, HÌRURGÌÂ TA PERINATAL'NA MEDICINA (ONLINE)
ABBREVIATED KEY TITLE: NEONATOL. HÌR. PERINAT. MED. (ONLINE)

яка о 14.00 год становила 0,303±0,0023 о.о.щ. та на 2,5% була більшою за показники щурів, які перебували за стандартного фотоперіоду. О 02.00 год вірогідних відмінностей між площею нейрона та його структур щодо величин у тварин групи LD, не встановлено. У цитоплазмі та ядрі нейросекреторних клітин пдсПЯ гіпоталамуса показник концентрації РНК не зазнавав істотних змін, водночас у ядерці вона була меншою на 2,8% щодо величин у щурів групи LD.

Висновки: 1. Морфоденситометричні параметри присередніх дрібноклітинних суб'ядер пришлуночкових ядер гіпоталамуса щурів зазнають коливань з найбільшими показниками у денний період спостереження. 2. За умов світлової депривації спостерігається порушення ритму функціональної активності досліджуваних нейронів гіпоталамуса та зміщення найбільших показників з денного на нічний період спостереження, що, ймовірно, викликане зростанням в цьому проміжку доби кількості в крові провідного епіфізарного хронобіотика – мелатоніну, що як стрес-лімітувальний чинник пригнічує продукцію КРГ пдсПЯ гіпоталамуса. 3. Утримування тварин за умов тривалого освітлення (світловий стрес) не призводило до вірогідних змін досліджуваних морфоденситометричних параметрів порівняно з такими у щурів, які перебували за світлових режимів LD та DD. Це дає підстави зробити припущення про широкі межі пластичності пдсПЯ гіпоталамуса за таких умов експерименту.

Ключові слова: пришлуночкове ядро, гіпоталамус, присередні дрібноклітинні суб'ядра, фотоперіод, тривале освітлення, постійна темрява, мелатонін, фотоперіод.

Contact Information:
Ihor Fedoriak – Postgraduate Student, Department of Medical Biology and Genetic of Bukovinian State Medical University (Chernivtsi, Ukraine).
e-mail: www.igorfed1987@gmail.com
ORCID ID: https://orcid.org/0009-0007-2462-8264

Roman Bulyk – MD, Doctor of Medicine, Professor, Head of the Department of Medical Biology and Genetic of Bukovinian State Medical University (Chernivtsi, Ukraine).
e-mail: bulyk@bsmu.edu.ua
ORCID ID: https://orcid.org/0000-0003-0651-534X
Scopus Author ID: https://www.scopus.com/authid/detail.uri?authorId=57509776400
Researcher ID: http://www.researcherid.com/rid/D-4122-2017

Контактна інформація:
Федоряк Ігор Вікторович – аспірант кафедри медичної біології та генетики Буковинського державного медичного університету (м. Чернівці, Україна).
e-mail: www.igorfed1987@gmail.com
ORCID ID: https://orcid.org/0009-0007-2462-8264

Булик Роман Євгенович – 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
Scopus Author ID: https://www.scopus.com/authid/detail.uri?authorId=57509776400
Researcher ID: http://www.researcherid.com/rid/D-4122-2017

Received for editorial office on 25/04/2025
Signed for printing on 20/06/2025