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MORPHOMETRIC CHANGES IN NEURONS OF THE SUPRACHIASMATIC NUCLEUS OF THE HYPOTHALAMUS RATS UNDER CONDITIONS OF LIGHT DEPRIVATION

R. Ye. Bulyk, M. I. Kryvchanska, A. I. Burachyk, K. V. Vlasova

Higher State Educational Establishment of Ukraine
"Bukovinian State Medical University", Chernivtsi, Ukraine
bulyk@bsmu.edu.ua

Abstract

The article deals with the results of studies of the morphometric state of neurons of the suprachiasmatic nuclei (SCN) of the hypothalamus of light-deprived rats. It is established that the duration of the photoperiod has a significant effect on the photoreceptor pacemakers of SCN. In the study of samples taken at 2 pm and 2 am, the light-deprived animals showed a decrease in the morphometric parameters of the pacemaker circadian periodicity in comparison with intact animals and smoothing of diurnal differences in the body area of the neuron of the rat hypothalamus SCN.

Key words: suprachiasmatic nuclei, hypothalamus, morphometric state, light deprivation.

Introduction. An integral and fundamental property of living matter are rhythmic fluctuations. Among other parameters of the medium, the photoperiod is the most reliable and stable synchronizing factor for homeothermal animals, including humans [2, 6]. The light

signal is perceived by the retina of the eye, from where it follows the retinohypothalamic pathway into the suprachiasmatic nucleus (SCN) of the hypothalamus [3]. These nuclei are assigned the role of the main driver (pacemaker) of circadian rhythms in the brain of mammals [4, 10, 11]. From the SCN information on illumination extends to the pineal gland (epiphysis of the brain) [2, 9]. This gland is a part of a system that is able to perceive changes in the level of ambient light and provide circadian rhythms of the body functioning, in particular by synthesizing its leading indole, melatonin [1, 5]. It was shown that the secretion of melatonin is subject to clear diurnal variations with a minimum value at a daytime and a maximum of about 2 am [8]. Suppression of melatonin synthesis by light is used as an experimental model of hypopinealism and is characterized primarily by melatonin deficiency [3, 7].

Despite the increased interest of scientists to studying the rhythmic activity of biological organs and systems, many questions of the morphofunctional characterization of the brain structures involved in the formation of biological rhythms remain unexplained.

Objective. To elucidate the morphometric state of the suprachiasmatic nuclei of rats' hypothalamus at different time intervals and the activity of the neurons of these nuclei under light deprivation conditions.

Materials and methods. Experiments were carried out on 48 mature male outbred white rats weighing 0.15-0.18 kg. The animals were kept in the animal house at a constant temperature, humidity and free access to water and food. The morphometric state of the suprachiasmatic nuclei of the hypothalamus at different time intervals was selected for the experimental animals in the study.

Experimental animals were divided into 2 series of studies, in each of which the sampling of the biomaterial was carried out at 2 pm and 2 am. The selected timing of the experiment was due to the different functional activity of the pineal gland during the indicated time periods of the day.

The animals of the first series (intact) were 7 days under normal light conditions - LD (light from 8 am to 8 pm, illumination by fluorescent lamps at the level of cages 500 lx). The animals of the No. 2 series were in conditions of constant darkness (light deprivation) (DD - simulation of the hyperfunction of the pineal gland) for 7 days. Experiments in the No.2 series were performed with a weak (2 lx) red light, since it practically does not affect the biosynthesis of melatonin by the pineal gland.

After the end of the 7-day experiment the following day, at 2 pm and 2 am, the animals were withdrawn from the experiment by means of a one-stage decapitation under an

ethaminal anesthetic (40.0 mg / kg intraperitoneally). The animal's brain was immediately withdrawn and placed in a 10.0% formalin solution in 0.1 M phosphate buffer (pH 7.2) for 20 hours at room temperature. After a standard procedure of dehydration and impregnation with chloroform and paraffin, the brain was embedded in paraffin. All stages of the experiment were carried out in compliance with the main provisions of the Resolution of the First National Congress on Bioethics "General Ethical Principles of Experiments on Animals" (2001), the Council of Europe Convention on the Protection of Vertebrate Animals used in experiments and other scientific purposes (18.03.1986), the Directive of the EEC No. 609 (of 24.11.1986) and the orders of the Ministry of Health of Ukraine No. 690 of September 23, 2009, No. 944 of December 14, 2009, No. 616 of 03.08.2012 and laws of Ukraine.

Morphometric analysis of the hypothalamic neurons was performed on a VIDAS-386 computer system for digital image analysis (Kontron Elektronik, Germany) in the visible spectrum. The image obtained with the AXIOSKOP microscope was entered into a VIDAS-386 digital image analysis computer system (Kontron Elektronik, Germany) using a COHU-4922 video camera (COHU Inc., USA). The image was analyzed in semi-automatic mode using the VIDAS-2.5 software package (Kontron Elektronik, Germany): the limits of the neuron body, its nucleus and nucleoli were interactively determined.

The obtained experimental data was processed on personal computers with a VIDAS-2.5 package of applied and statistical programs (Kontron Elektronik, Germany) and EXCEL-2003 (Microsoft Corp., USA). For all indicators, the average arithmetic sample (x), its variance and the mean error (Sx) were calculated. To determine the reliability of differences in the results of studies, the Student's coefficient (t) was determined in the experimental and control groups of animals, after which the probability of sample difference (p) and the mean interval over the tables of the Student's distribution were found. Values were considered valid for which $p < 0.05$.

Results and discussion. The study of the morphometric characteristics of neurons of the hypothalamus SCN revealed the diurnal dynamics of the indices. So, in comparison with the daytime period (2 pm), at 2 am, a significant increase (by 7.8 ± 1.5%) of the body area of the SCN neurons was noted, due to the growth of the cell nucleus area. In turn, the increase in the area of the nucleus of the neuron is due to the probable increase in the area of its nucleolus, which was 5.60 ± 0.237 μm^2 (Table). At night, observations of the nuclear-cytoplasmic ratio (N/C ratio) in pacemaker neurons were 1.7 ± 0.05% and significantly more than in the daytime interval. At the same time, the specific volume of the neuron core grew by 18.2 ± 2.16%, and the cytoplasm, on the contrary, decreased by 14.2 ± 1.98%. These changes

were combined with an increase in the concentration of RNA in the nuclei by 7.3 ± 1.5%, as well as with an increase in the concentration of RNA in the nucleoli of neurons by 8.5 ± 1.7% and the area occupied by them by 26.5 ± 5.2 % in comparison with the daily period.

The values obtained indicate an increase in the functional and synthetic activity of SCN neurons in intact rats at night. In order to identify the place and role of the leading neuroendocrine transducer of circadian periodicity, the pineal gland, in the functioning of the main pacemaker of circadian rhythms, the hypothalamus SCN, we performed morphometric studies of these nuclei under conditions of constant darkness (simulation of epiphyseal hyperfunction in animals).

Modeling of increased melatonin-producing activity of the pineal gland was characterized by deeper manifestations at 2 am, compared with 2 pm. In particular, morphometrically this was manifested by a significant decrease in the area of the neuron by 21.89 ± 3.26%. This is due to a possible decrease in the areas of its nucleus by 25.67 ± 4.01% and cytoplasm - by 16.38 ± 2.12% (Table). N/C ratio was 1.52 ± 0.018 e.u., the specific volume of the nucleus of the neuron was 60.30 ± 0.679%, and the cytoplasm was 39.70 ± 0.561% of the total cell volume.

Table

Morphometric characteristics of neurons of the suprachiasmatic nucleus of the hypothalamus in rats under light deprivation (x ± Sx)

Animal series	**Neuron area, μm²**	**Nucleus area, μm²**	**Nucleolus area, μm²**	**Cytoplasm area, μm²**
Intact, 2 pm	44.28 ± 0.557	25.38 ± 0.397	4.42 ± 0.069	18.90 ± 0.336
Intact, 2 am	47.72 ± 1.262*	30.24 ± 0.897*	5.60 ± 0.237*	17.70 ± 0.658
Constant darkness, 2 pm	38.05 ± 0.730*	25.91 ± 0.610	4.51 ± 0.106	11.33 ± 0.372*
Constant darkness, 2 am	37.27 ± 0.361**	22.48 ± 0.258**	3.92 ± 0.045**	14.80 ± 0.202**

Note: significant ($p < 0.05$) changes in parameters: intact animals at 2 pm (*), at 2 am (**).

It can be assumed that light deprivation (induction of melatonin synthesis) reduces the morphometric parameters of the pacemaker of circadian periodicity. In addition, the maintenance of animals under conditions of constant darkness leads to a smoothing of the differences in the body area of the neuron of the SCN of the hypothalamus of animals in the diurnal aspect.

Conclusion. The duration of the photoperiod significantly affects the photoreceptor pacemakers of the SCN. In the study of samples taken at 2 pm and 2 am in animals under

light-induced conditions, a decrease in the morphometric parameters of the pacemaker circadian periodicity was found in comparison with intact animals and the diurnal differences in the area of the body of the neuron of the rat hypothalamus SCN were smoothed. **Prospects for further researches**. In the future, it is planned to investigate the effect of synthetic peptides of the pineal gland on the morphofunctional activity of SCN neurons for a deeper understanding of the mechanisms of participation of these structures in the regulation of circadian rhythms of rats.

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