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AGE CHANGES IN THE TIGROID SUBSTANCE OF NEURONS OF THE LATERAL PREOPTIC NUCLEUS OF HYPOTHALAMUS UNDER DIFFERENT LIGHT MODES

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Abstract

The article presents analysis of the results of the original histochemical studies of tigroid substance of neurons of the lateral preoptic nucleus of hypothalamus in mature and old rats under the influence of different light modes. In all observations, the tigroid substance was located in the cytoplasm of neurons of the lateral preoptic nucleus of hypothalamus in the form of individual granular formations of different sizes and shapes. The amount of tigroid substance of neurons of the lateral preoptic nucleus of hypothalamus in mature rats is greater than in older rats. At the same time, it should be noted that different experimental conditions significantly affected the amount of tigroid substance in neurons of the lateral preoptic

nucleus of hypothalamus in old rats. In particular, under conditions of light deprivation, the optical density of specific histochemical staining for tigroid substance in neurons of the lateral preoptic nucleus of hypothalamus increased significantly ($p < 0,001$), and under conditions of light stimulation, on the contrary, probably decreased ($p < 0,001$).

Key words: sleep-wake cycle; lateral preoptic nucleus of hypothalamus; circadian rhythms; photoperiod.

Introduction. Good sleep is one of the "three whales" of health, along with proper nutrition and physical activity [1]. People spend about a third of their lives asleep, and its quality determines their overall health [2].

Sleep is a complex physiological process regulated by homeostatic and circadian processes that involve various neural structures, and among them hypothalamic regulation is extremely important. Today it has been proven that there are several neuropeptide-producing neurons in hypothalamus that are involved in regulation of the sleep-wake cycle. The lateral preoptic nucleus of hypothalamus plays a key role in initiating and maintaining sleep [3]. About 85% of neurons in this nucleus contain inhibitory neurotransmitters galanin and gamma-aminobutyric acid and provide innervation of the main monoamine systems of brain, which determine the period of wakefulness [4]. Excessive light stimulation and human activity at night are the most common causes of sleep-wake disorders [5], leading to mental and neurodegenerative diseases [6], increased risk of cancer, metabolic, cardiovascular disease [7] and premature death [8]. The density of the tigroid substance allows to determine the functional activity of the neuron, when scattering and reduction of this substance reflects profound dystrophic changes in neurons [9].

The objective of the research was to determine the effect of different lighting patterns on the quantitative characteristics of tigroid substance of neurons of the lateral preoptic nucleus of hypothalamus in mature and old rats.

Materials and methods. The experiments were performed on 36 adult and 36 old white nonlinear male rats. All experimental animals were divided into six series, each of them, in turn, consisted of two groups (six animals).

Series №1 - mature rats that stayed under standard lighting conditions (light from 8 a.m. to 8 p.m.) for seven days. Series №2 - mature rats that spent seven days in constant darkness (light deprivation). Series №3 - mature rats that were in constant light (light stimulation) for seven days. Series №4 - old rats, which were kept for seven days under standard lighting conditions, those identical to mature rats. Series №5 - old rats kept for seven

days in constant darkness. Series №6 - old rats, which stayed in constant light for seven days. In order to identify circadian differences, the material was taken at 12-hour intervals (at 2 p.m. and at 2 a.m.), performing a one-time decapitation under etaminal anesthesia (40.0 mg/kg, intraperitoneally). All stages of the experiment were carried out in compliance with the basic requirements of the European Convention for the Humane Treatment of Animals.

The removed animal brain was fixed for 22-24 hours in the neutral buffered 10% formalin solution. Next, standard plates of brain tissue about 1 mm thick were cut out and dehydrated in an ascending battery of spirits. They were then embedded in paraffin at 58⁰C and serial histological sections 5 μm thick were made using a sled microtome. After deparaffinization of serial histological sections, staining was performed by the histochemical method according to Nisl with neutral red (modification) for the tigroid substance of neurons (Nisl substance).

Staining intensity was assessed by computer microdensitometry on digital copies of histochemically stained images (Delta Optical Evolution 100 microscope and Olympus SP550UZ digital camera). First we obtained a computer value of the brightness of the color in an 8-bit analysis system using microprobe method with a licensed copy of the computer program ImageJ v1.48, and then these values were translated into the value of relative optical density (in opt. density un.) by logarithmic conversion. This value is easy to interpret because it ranges from 0 (absolute transparency) to 1 (absolute opacity).

Statistical processing of the obtained results was performed using a licensed copy of the computer program PAST. The Wilki-Khan-Shapiro test was used to check the normality of distribution. Since the hypothesis about the normality of distribution was not rejected, the difference in mean trends between the study groups was evaluated by parametric methods of statistical analysis: calculation of arithmetic mean and its error, unpaired two-sample Student's criterion. At the same time, the Mann-Whitney test was additionally used in order to increase the reliability of the results of testing the differences between the study groups in the average trends.

Results. In all observations, the tigroid substance was located in the cytoplasm of neurons of the lateral preoptic nucleus of hypothalamus in the form of individual granular formations of different sizes and shapes. The location of the substance in the cytoplasm could be both uniform and with a more asymmetric concentration in certain spots, sometimes around the cell nucleus. The color intensity of both individual granules and the cytoplasm as a whole varied considerably.

The results for experimental animals kept under normal lighting are presented in table 1.

Table 1

Optical density of a specific coloring for tigroid substance in neurons of the lateral preoptic nucleus of hypothalamus of rats

Time of the day	Optical density of histochemical staining for tigroid substance (in un. of opt. density)	
	Mature rats	Old rats
2 p.m.	0,258±0,0019	0,214±0,0017 (p ₂ <0,001)
2 a.m.	0,263±0,0017 (p ₁ <0,05)	0,216±0,0018 (p ₁ <0,05) (p ₂ <0,001)

Note: p₁ - probability of difference compared to the previous time interval within one series; p₂ is the probability of difference compared to older rats.

These data suggest that the amount of tigroid substance of neurons of the lateral preoptic nucleus of hypothalamus in mature rats is greater (p<0,001) than in older rats, whereas in both mature and old rats, the amount of this substance does not depend on the time of observation.

Table 2

Optical density of a specific coloring for tigroid substance in neurons of the lateral preoptic nucleus of hypothalamus of rats under light deprivation conditions

Time of the day	Optical density of histochemical staining for tigroid substance (in un. of opt. density)	
	Mature rats	Old rats
2 p.m.	0,257±0,0022	0,234±0,0021 (p ₂ <0,001)
2 a.m.	0,264±0,0021 (p ₁ <0,05)	0,253±0,0024 (p ₁ <0,05) (p ₂ <0,001)

Note: p₁ - probability of difference compared to the previous time interval within one series; p₂ is the probability of difference compared to older rats.

In fact, this tendency (less tigroid substance of neurons of the lateral preoptic nucleus of hypothalamus and lack of significant response to the period of the day) was observed under light deprivation and light stimulation. However, it should be noted that different experimental conditions significantly affected the amount of tigroid substance of neurons of the lateral preoptic nucleus of hypothalamus in old rats. In particular, under conditions of light deprivation (table 2), the optical density of specific histochemical staining for the tigroid

substance in neurons of the lateral precautionary nucleus of hypothalamus increased significantly, and under conditions of light stimulation (table 3), on the contrary, significantly decreased. At high magnification (100x lens, oil immersion) it was seen that under the conditions of light stimulation the granules of the tiger substance became smaller, when under the conditions of light deprivation their size did not noticeably change.

Table 3

Optical density of a specific coloring for tigroid substance in neurons of the lateral preoptic nucleus of hypothalamus of rats under light stimulation conditions

Time of the day	Optical density of histochemical staining for tigroid substance (in un. of opt. density)	
	Mature rats	Old rats
2 p.m.	0,252±0,0020	0,183±0,0018 (p ₂ <0,001)
2 a.m.	0,259±0,0024 (p ₁ <0,05)	0,192±0,0019 (p ₁ <0,05) (p ₂ <0,001)

Note: p₁ - probability of difference compared to the previous time interval within one series; p₂ is the probability of difference compared to older rats.

Conclusions. The tigroid substance of neurons of the lateral preoptic nucleus of hypothalamus in mature rats does not respond to changes in light mode, but in older rats it is smaller and changes characteristically. Light stimulation leads to a decrease (p<0,001) in the optical density of immunohistochemical staining for the tigroid substance in both mature and old rats, in the latter the reduction was more significant. At the same time, light deprivation leads to a significant increase (p<0,001) in the optical density of immunohistochemical staining for the tigroid substance in neurons of the lateral preoptic nucleus of hypothalamus.

Prospects. In future it is planned to investigate the effect of exogenous melatonin as an experimental therapy for the correction of deviations in the quantitative characteristics of tigroid substance of neurons of the lateral preoptic nucleus of hypothalamus in mature and old rats caused by changes in light mode.

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