GENETICS

Characteristics of Interphase Chromatin in Hippocampal Neurons in Rats with Different Excitability of the Nervous System Exposed to Stress at Different Time of the Day

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The structural (number and area of chromocenters) and optical (relative optical density of chromocenters) characteristics of condensed chromatin in hippocampal CA3 neurons in rats differing by excitability of the nervous system (intact and exposed to short-term emotional painful stress) depended on the time of the day and genotypical characteristics of the experimental animals. The detected changes were independent, which attests to specificity of mechanisms determining these features and/or can be attributed to structural and functional heterogeneity of condensed chromatin (heterochromatin, euchromatin, *etc.*).

Key Words: *excitability; hippocampus; chromatin; circadian rhythm; stress*

The mechanism of adaptation to extreme environmental factors is an important problem of modern biology. The mechanisms of various biological rhythms are actively studied within the framework of this problem. It is well known that functioning of the whole organism and its systems, organs, tissues, and cells is regulated by circadian rhythms. Therefore, the effects of stress factors of different origin and intensity and the consequences of stress can differ during different time of the day, which can determine daily variations of the adaptation potential of the organism. Moreover, the reaction to stress is largely determined by the function of the nervous system and its main characteristics, which was shown for inbred rats with different excitability of the nervous system [2].

In studies of the role of biological rhythms in the organization of behavior, the hippocampus (CA3 field) attracts special attention. A specific feature of this structure is its chronotropic activity presenting as modulation of the dynamics of circadian and other behavioral rhythms [1]. Circadian periodicity of electrophysiological activity in neurons and the state of the neurotransmitter systems of the hippocampus were revealed [1]. On the other hand, the hippocampus is involved in the reaction to stress via interaction with the hypothalamic-adrenal system and centers regulating biological rhythms and shifting biorhythms during adaptation [1]. The increase of hippocampal excitability in stress is paralleled by reorganization of rhythmic processes, which can provide the basis for mental disorders.

The effect of stress can be detected at different levels of organization of living matter. This effect is associated with the mechanisms of regulation of gene expression, the realization of which depends on the structural and functional modifications of chromatin and spatial organization of the cell nucleus [10]. The organization of interphase nucleus and the effects of nuclear structures on gene activity under conditions of

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stress are extensively studied [12], but the role of neuronal chromatin in these processes is poorly understood. Circadian changes in chromatin activity and the relationship between the reaction of the neuronal gene apparatus to stress and the phase of circadian rhythms are still unknown [8].

We investigated the relationship between changes in the structural and optical characteristics of hippocampal neuronal chromatin in rats of two strains (HT1 and LT2) selected by the threshold of nervous system excitability [2] under normal conditions and after short-term emotional painful stress (EPS).

MATERIALS AND METHODS

The experiments were carried out on 5-month male rats of two strains differing by excitability the nervous system (with high (2.5 V) and low (0.7 V) excitability thresholds, HT1 and LT2, respectively), selected at Laboratory of Higher Nervous Activity Genetics (I. P. Pavlov Institute of Physiology) [2]; the animals of 44 and 43 generations were used (depending on selection program). The rats were divided into groups (5 animals each) and kept under conditions of normal day/night regimen (12/12 h) on a standard diet. Experimental animals were exposed to EPS in the morning (8.00) and evening (20.00). The device for EPS exposure consisted of an illuminated box with electrified floor. The procedure included [11] short-term (13 min) period of light signals (six 10-sec signals) and light signals associated with electrical stimulation (2.5 A, 6 series, 10-sec each, 6 sec light and 4 sec light+current), the intervals between the series 1 min [7].

The animals were decapitated immediately after the exposure. The brain was removed at low temperature, the hippocampus was isolated, the area corresponding to CA3 field was cut and placed into normal saline. Cell suspension was prepared with Pasteur pipettes and the cells were fixed in a 3:1 methanol-glacial acetic acid mixture (3 centrifugation-suspension cycles). The preparations were stained by the method of Giemza in phosphate buffer (pH 6.8) for detecting the condensed chromatin [4]. Two preparations were prepared from the brain of each animal; at least 50 nuclei per preparation were analyzed.

Information system based on the model of visual processes of adaptive image segmentation was used for cytogenetic data processing, analysis, and accumulation [3]. This system singles out the neuron nuclei from the totality of nerve and glial cell nuclei at the initial image, measures their parameters, distinguishes and measures the areas of condensed chromatin with different optical density. The system forms related tables of data including the results of segmentation and measurements (graphic and text objects) and ensures the export of measurement matrices into multiparametrical statistical analysis software.

RESULTS

The areas of hippocampal neurons in control rats and animals exposed to EPS at different time of the day were virtually the same.

The areas of chromocenters (CC) in intact LT2 rats were larger than in HT1 animals; no circadian changes were detected (Table 1). Similar differences in the areas of C-heterochromatin area in the rat hippocampal neurons were observed previously in animals of different strains [4]. The number of CC in LT2 animals was higher than in HT1 ones only during the morning hours (Table 1). No differences in the optical density were detected between the two strains; this parameter decreased during the evening hours.

The relative optical density of CC in rats of both strains and number of CC (only in LT2 animals) varied over 24 h: these parameters in the evening were

TABLE 1. Characteristics of Chromatin of Hippocampal Neuron in Control Rats (Nominator) and Rats Exposed to EPS (Denominator) at Different Time of the Day ($X \pm m$)

Rat strain		Number of CC	Area of CC, μ^2	Relative optical density of condensed chromatin
HT1	morning	10.52±0.49	2.37±0.11	0.195±0.003
		14.47±0.84*	1.47±0.06*	0.151±0.003
	evening	9.74±0.40	2.35±0.09	0.116±0.003+
		15.39±1.02*	2.33±0.07	0.140±0.003*
LT2	morning	14.46±0.51**	2.88±0.11**	0.220±0.003
		11.16±1.12*	2.18±0.09*	0.178±0.004*
	evening	9.92±0.45	2.91±0.10**	0.114±0.003+
		18.05±1.08*	2.04±0.08*	0.135±0.004*

Note. $p<0.05$: *compared to the control; **compared to other strain; +compared to morning hours.

lower than in the morning hours. These changes can be due to evening and night activities of rats, associated with the corresponding intensity of the matrix processes during the dark time and, hence, decondensation of certain chromatin sites [8]. It is also possible that these processes are associated with selective activation of certain gene groups in the brain. For example, circadian changes in the expression of Fos proteins [13] and BDNF mRNA [9] were detected in rat hippocampus.

The present study showed that decrease of condensed chromatin areas is a universal reaction to stress. It is noteworthy that this reaction does not depend on the time of the day in LT2 rats, while in HT1 animals it manifests only in the morning, while in the evening no deviations from the control was observed.

The decrease in the size of C-heterochromatin sites in response to short-term and long EPS was previously demonstrated on other models [4].

Changes the number of CC under conditions of EPS were different: the increase in this parameter did not depend on the time of the day in HT1 rats, while in LT2 animals the number of CC decreased in the morning and increased in the evening.

These data suggest that the sensitivity of the genetic system to external factors in animals differing by excitability of the nervous system (extreme variants of the normal) is characterized by different circadian rhythms. This assumption is confirmed by other facts obtained previously on HT1 and LT2 rats. For example, the rhythms of sensitivity to phenyl thiocarbamide differ in rats with low and high excitability thresholds of the nervous system [6]. It was found that the differences in emotional reactivity of different rat strains characterized by different functional state of the nervous system depend on the phase of circadian rhythms [7]. Circadian rhythm of changes in behavioral parameters, no doubt, correlates with activity of neuronal genome primarily in brain structures involved in the realization of behavior. Studies on *Drosophila* strains differing by the level of functional activity of the nervous systemproved the effect of circadian rhythm on the relationship between manifestation of behavioral signs (motor activity) and genetic system activity (incidence of crossovers) [5].

Changes in the optical density after stress exposure were not specific for each strain, which was previously demonstrated on rat embryos of the same strains. However, the study of the effect of circadian rhythm on optical density showed the direction of changes in this parameter. In the morning optical density reduced and in the evening increased in response to EPS in animals of both strains. These changes reflect the universal mechanisms of circadian regulation of genome activity during exposure to stress factors.

Changes in the studied parameters (number and area of regions of condensed chromatin, their relative optical density in intact and stressed rats) are independent, which indicates that these signs are regulated by specific mechanisms and/or depend on structural and functional heterogeneity of condensed chromatin (heterochromatin, euchromatin, *etc.*) sites.

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