Long-Term Effects of Prenatal Stress on the Characteristics of Hippocampal Neurons in Rats with Different Excitability of the Nervous Systems

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We studied the effects of prenatal emotional painful stress on numerical density of neurons and characteristics of heterochromatin in developing and mature hippocampus of rats with different excitability of the nervous systems. It was shown that prenatal stress reduces the numerical density of neurons in hippocampal CA3 field in 24-day-old and adult (3 months) low excitable animals and chromocenter area in cells of developing hippocampus in embryos of both strains. The difference in chromocenter areas in offspring of stressed females was retained on postnatal day 24.

Key Words: *prenatal stress; hippocampus; density of neurons; heterochromatin; highly excitable and low excitable rat strains*

Exposure of pregnant females to stress factors of different nature and duration affects some forms of adaptive behavior in the offspring [6,7]. The nature and extent of the post-stress response depend of individual characteristics, in particular, on excitability of the central nervous system (CNS) [4]. Mechanisms underlying the effects of prenatal stress on the offspring are poorly understood.

Here we studied numerical density of neurons in the hippocampus of 17-day embryos, 24-day-old rats, and adult animals (3 months) and quantitative characteristics of their heterochromatin (HC) in rats of strains dramatically differing by excitability of the nervous system.

MATERIALS AND METHODS

The experiments were performed on rats of two strains selected by high (HT) and low (LT) thresholds of CNS excitation. Prenatal emotional painful stress (PEPS) was applied on day 16 of pregnancy (early third trimester). Females of both strains (experimental groups) were placed into a transparent chamber with grid floors and subjected to a single stress session by random combination of electrical pulses and flashes over 13 min [1]. After 24 h some females were decapitated, uterine horns were dissected, the embryos isolated and fixed. Offsprings (males) of the remaining animals were used in the experiment 24 days and 3 months after birth. Embryos and offspring of intact females in the studied age groups served as controls. In each group, 10 animals were examined.

At certain time points, the rats of each strain from the corresponding age groups (control and experimental) were decapitated, the brain was isolated, fixed in Carnoy fluid, dehydrated, and embedded in paraffin. The whole embryos were fixed and then processed. Frontal sections of the forebrain (7 μ) were prepared at the level of developing hippocampus in embryos and hippocampal CA3 field in remaining rats [10]. The sections were stained with hematoxylin by Boehmer, embedded in Canada balm and examined under a light microscope. Sections were assessed qualitatively and then numerical density of neurons (number of cells

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TABLE 1. Effects of PEPS on Relative Number of Hippocampal Cells (per 1 mm^2 Nerve Tissue) in Embryos and Young and Adult Rats of HT and LT Strains ($M\pm m$)

Strain		Embryos	Young rats	Adult rats
HT	control	118.00±2.83$^+$	18.65±0.80$^+$	12.45±0.50$^+$
	experiment	114.20±3.56	15.30±0.63*	10.86±0.51*
LT	control	89.42±1.96	8.71±0.40	6.61±0.17
	experiment	93.70±2.44	7.25±0.75	6.80±0.22

Note. *p<0.05 in comparison with controls; ^+p<0.01 in comparison with LT controls.

TABLE 2. Effects of PEPS on C-HC Area (μ^2) in Neuronal Nuclei in Developing Hippocampus of Embryos and Mature Hippocampus of Young Male HT and LT Rats ($M\pm m$)

Strain		Embryos	Young rats
HT	control	6.98±0.24$^+$	2.91±0.08$^+$
	experiment	5.42±0.20*	2.61±0.08*
LT	control	4.36±0.15	2.32±0.04
	experiment	3.59±0.10*	2.14±0.04*

Note. p<0.05 in comparison with: *correponding controls; $^+$LT controls.

per 1 mm^2 of the nervous tissue) was counted [5]. To analyze quantitatively HC in neurons of the developing and mature brain, the area of developing hippocampus from the head of the extracted embryos and the area of hippocampal CA3 field in 24-day-old males were isolated [10] and placed in saline. The cell suspension was obtained, and the cells were fixed in methanol–glacial acetic acid mixture (3:1, fixation was repeated 3 times) and air-dried. To reveal C-HC, differential staining by modified Sumner's method was performed [2]. TV image analyzer was used to analyze the samples and estimate of the area of chromocenters. At least 5 samples from 5 animals were analyzed in each case; not less than 50 nuclei with similar area were selected in each sample.

Statistical analysis of the data included assessing normality of the distribution, comparison of variances and arithmetic means using Student's t test.

RESULTS

At all time points, the studied parameters largely depended on rat strain (Tables 1, 2). Thus, normal rats of HT strain had significantly higher density of both neuroblasts in the developing brain and neurons in the hippocampal CA3 field of mature brain. Larger HC area was typical of this strain. PEPS significantly reduced the number of neurons in CA3 field of young HT rats; this effect persisted in 3-month-old animals. Embryos and young LT rats did not respond to PEPS, which suggests that hippocampal stress reactivity and morphogenesis depend on genetically determined level of CNS excitability.

PEPS experienced by pregnant females decreased the area of chromocenters in cells of developing brain of LT and HT embryos in comparison with controls. In the offsprings of stressed females of both strains chromocenter areas remained reduced on day 24 of postnatal life.

Thus, PEPS induces long-term changes in the hippocampus at the cellular and tissue levels, which may be either dependent (numerical density of neurons) or independent (C-HC area) on the constitutional characteristics of the nervous system. It is probably linked with ontogenetic heterochrony of structural and functional characteristics of the nervous system associated with excitability. Based on these results we can assume that long-term effects of PEPS on the behavior of offsprings of stressed mothers may be related with both impaired neurogenesis and alterations in chromosome structure in neurons inducing changes in gene expression (reduced HC area→despiralization of chromatin→ncreased access of transcription factors to gene promoters→enhanced gene expression) and as a result, to modification of neuronal characteristics. And if the first suggestion has scarce evidence [3,8,9], the second one is a priority and its further development will provide the basis for genetic engineering correction of PEPS pathological effects.

REFERENCES

1. A. I. Vaido, N. V. Shiryaeva, and V. V. Vshivtseva, *Byull. Eksp. Biol. Med*, **129**, No. 4, 452-456 (2000).
2. N. A. Dyuzhikova, Y. N. Savenko, K. N. Dudkin, *et al.*, *Morfologiya*, **131**, No. 2, 43-50 (2007).
3. V. A. Otellin, L. I. Khozhai, and N. E. Ordyan, *Prenatal Stressors and Developing Brain. Adaptive Mechanisms, Direct and Delayed Effects* [in Russian], St.-Petersburg (2007).

4. N. V. Shiryaeva, A. I. Vaido, and N. G. Lopatina, *Zh. Vyssh. Nervn. Dyeyat*., **46**, Issue 1, 157-162 (1996).
5. N. V. Shiryaeva, V. V. Vshivtseva, N. A. Maltsev, *et al*., *Morfologiya*, **131**, No. 2, 46-52 (2007).
6. H. Abe, N. Hidaka, C. Kawagoe, *et al*., *Neurosci. Res*., **59**, No. 2, 145-151 (2007).
7. P. J. Brunton and J. A. Russell, *J. Neuroendocrinol*., **22**, No. 4, 258-271 (2010).
8. N. Jia, K. Yang, Q. Sun, *et al*., *Dev. Neurobiol*., **70**, No. 2, 114-125 (2010).
9. K. Odagiri, H. Abe, C. Kawagoe, *et al*., *Neurosci. Lett*., **446**, No. 1, 25-29 (2008).
10. G. Paxinos and C. Watson, *The Rat Brain in Stereotaxic Coordinates*, Amsterdam (2007).