



## **MORPHOLOGICAL CHANGES IN THE HIPPOCAMPUS RESULTING FROM STRESS**

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**Abstract.** This article examines the impact of chronic stress on the morphology of the hippocampus. The study compares the hippocampal structure and developmental dynamics of laboratory rat offspring born to stressed mothers with those in a control group. The results indicate that offspring exposed to stress exhibit a reduction in hippocampal neurocytes, vacuolization of the cytoplasm, changes in nuclear and cytoplasmic size, as well as neurocyte lysis. Additionally, the body weight of stressed rat offspring was found to be lower than that of the control group. The obtained data suggest that stress may have a negative impact on hippocampal structure.

**Keywords:** stress, hippocampus, neurocytes, morphology, neurogenesis, pathological changes, laboratory rats.

Currently, stress is considered a risk factor for many of the most prevalent diseases. On a global scale, stress is one of the most pressing problems of the 21st century. A person's lifestyle, work activity, social relations, and psychological state directly affect the level of stress. The impact of stress on the central nervous system, especially the hippocampus, is a very important issue in the field of neurobiology. Because the hippocampus plays a crucial role in memory, learning ability, emotional control, and mental health. Morphological changes in the hippocampus lead to a decrease in memory and learning ability. Long-term stress causes damage to neurons in the hippocampus and a decrease in neurogenesis. This indicates a weakening of cognitive functions, especially an increased risk of Alzheimer's disease and dementia with age (2,6). Stress is associated with mental illnesses such as depression and anxiety (anxiety). Reduction of the hippocampus is one of the main neurobiological changes observed in these diseases. Studies show that in individuals with depression, the volume of the hippocampus can be smaller than normal (1). This makes the issue of protecting mental health in people experiencing stress relevant. However, constant stress reduces neurogenesis and hinders the formation of new neurons (3,5). This reduces the plasticity of the brain, that is, the ability to adapt to new information



and learn new abilities. Morphological changes in the hippocampus as a result of stress are not only a scientific problem, but also have important medical and social significance. In modern society, stress is high, and mental illnesses and cognitive decline are widespread. Currently, meditation, physical activity, various therapy methods, and pharmacological agents are used to reduce stress, protect the hippocampus, and improve its function (2,4). Morphological changes in the hippocampus as a result of stress are one of the urgent problems, which can cause a decrease in cognitive functions, mental health, and the overall quality of life of a person. Therefore, it is very important to develop research and practical measures to reduce stress, protect the hippocampus, and ensure its normal functioning.

Considering the above, we set ourselves the goal of studying the structural and functional state of the nervous tissue of the hippocampus of offspring born to mothers in a state of chronic stress.

**Materials and methods of study.** To achieve the goal, 120 white laboratory rats were used. White laboratory rats were divided into 2 groups. The 1st group consisted of 20 healthy rats, which made up the control group. Female rats of the control group were given 1.0 ml of physiological saline intragastrically every morning. A subclavian catheter was used as a probe. The rat pups were neonatal on days 7, 14, 21, 30 after birth under ether anesthesia. The 2nd group was an experimental group, in which 20 white female laboratory rats were kept in specially prepared cages to induce experimental stress. Rats were exposed to various stress factors (noise and excessive light) for several weeks. This stress model was continued even after the rats became pregnant and their offspring were born. The tissues were fixed in a 10% neutral formalin solution for 24 hours, placed in paraffin blocks, and sections with a thickness of 5  $\mu$ m were prepared using a microceratome. Hematoxylin-eosin staining (assessment of the general histological structure, tissue and nuclear-cytoplasmic ratio) and Masson trichrome staining (to determine the density of connective tissue and collagen fibers) were used on the sections.

It was observed that offspring born to mothers in a state of stress had a lower body weight compared to the control group. Even after 30 days, it was found that the body weight of the rat pups in the experimental group was lower than in the control group. The average body weight of the rat pups in the control group was  $6.1 \pm 0.26$  grams, while in the experimental group it was 18% less and averaged  $5.03 \pm 0.23$  grams. In the control group, the body weight of 7-day-old rat pups reliably increased by 66.6% compared to the body weight of newborns and averaged  $18.5 \pm 0.32$  grams, while in the experimental group, a 20.5% lag in growth was



observed compared to the control group, and the body weight averaged  $14.75 \pm 0.37$  grams.

The average body weight of 14-day-old rats of the control group was  $33.25 \pm 0.44$  g, and the growth rate was 44.1%. It was established that the offspring of the experimental group during this period lagged behind the most significant (25.6%) growth in body weight and averaged  $24.7 \pm 0.23$  grams. In the control group, a significant increase in the body weight of 21-day-old rat pups by 31.4% was observed compared to the previous group of rat pups and averaged  $48.5 \pm 0.88$  grams, while in the experimental group, a lag of 17.5% was observed compared to the control group, and the average body weight was  $40 \pm 1.38$  grams.

The average body weight of 30-day-old rat pups born to control rats was  $71.4 \pm 0.97$  g, with a growth rate of 32%. The body weight of the offspring in the experimental group during this period was  $60.5 \pm 0.63$  grams, which is 15.2% less than in the control group. By the 60-day period, the growth rate of the body weight of rats in the control group was 29% compared to the previous period, where the body weight was equal to  $100.7 \pm 2.4$  grams. The body weight of the rats in the experimental group was  $83.7 \pm 1.0$  grams, which is 16% less than in the control group. When comparing the histological structure of the brain of offspring born to mothers in the experimental and control groups, it was established that the cortical part is fully formed by day 14. In rat pups of the experimental group, as in the control group, bipolar cells with 2-4 nuclei were detected in the cerebral cortex. The 2nd and 3rd layers of the cerebral cortex are closely packed together. During this period, the difference in the brain of rat pups in the control and experimental groups is insignificant. The cortical neurocytes in the experimental group are relatively round and smaller than in the control group. In these neurocytes, there is a small margin in the cytoplasm, the nucleus is brightly colored and relatively large in size.

In control animals, the basophilic substance is formed in large cells in the form of lobules in the apical and basal zones of the cytoplasm, while in experimental animals, the cytoplasm of such cells is diffusely stained with thionine, and the Nissl substance is located in the perinuclear region of the cytoplasm.

The brain of the offspring of the control group of rats was fully formed by 21 days, and the morphological structure of the neurocytes was fully formed. In most cells of varying sizes, both large and small, in different layers of the cortex, basal processes are clearly visible, and the nucleus is mainly rounded.

Neurocytes in the brain of 21-day-old offspring in the experimental group are light-colored, their cytoplasm is poorly developed, rounded, the neurocytes are often



located perinuclearly, and in some neurocytes they are located in the basal zone. Neurocytes have a rough structure, and the boundaries of their nuclei are clearly defined. By this time of the experiment, a decrease in neurocytes is observed. It was established that the size of neurocytes on this day of the experiment was smaller than in the control group. This was especially pronounced in the cytoplasm. The boundaries of the cytoplasm and nucleus are unclear compared to the control group. The location of basophilic substance in the cytoplasm of neurocytes located in the hippocampus differed from the control group. It was found that the basophilic substance is located in the center of the cytoplasm, and the surroundings are whiter. By day 21 of the experiment, pathologically altered cells were also detected in the hippocampus. Here, solitary cells with a whitish cytoplasm and a swollen nucleus can be found.

By the first days of postnatal ontogenesis, it was established that the majority of large and small neurocytes in the experimental offspring were pronounced vacuolated. In this case, perinuclear edema, large and small vacuoles located on the periphery, or neurocytes filling the entire cytoplasm can be found in the cytoplasm. The neurocytes of the experimental group have a rounded shape and a reticular cytoplasm. The nuclei of these neurocytes are surrounded by a border of reticular substance. The nuclei of these cells were often found to be swollen. It was noted that the nuclei are whitish, in some cases basophilic. During this period of the experiment, the number of glial cells was 22% less than in the control group. It was noted that the number of perineural satellites was 30% less than in the control group.

By the 30th day of the experiment, it was established that large and medium-sized neurocytes of the hippocampus were vacuolated, and there was perinuclear edema in its cytoplasm. The nuclei of these cells have clear boundaries, and the nucleolus appears as a small black dot. During this period, shadow cells of neurocytes are detected. Focal lysis of neurocytes is detected during this period.

By day 60 of the experiment, the structure of neurocytes in the experimental group differs from that in the control group. By this time, cells with vacuolated cytoplasm are found individually, and the cytoplasm of mainly neurocytes has a reticular structure. The basophilic substance is located around the nucleus. The nuclei of these cells were found to be whitish.

Their size was increased compared to the control group. It was established that the chromatin glomeruli in most cases are located on the periphery of the nucleus.

Thus, the results of this study showed that offspring born to mothers in a state of chronic stress are subjected to morphological changes in the hippocampus. When



comparing the body weight, the structure of neurocytes, and pathomorphological changes in the hippocampus of rat pups in the experimental and control groups, negative effects were observed in offspring exposed to stress. It was established that the body weight of the rat pups in the experimental group was lower than in the control group, and their growth rate was lower. Also, a decrease in neurocytes in their hippocampal tissue, vacuolization in the cytoplasm, perinuclear edema, and nuclear changes were noted. The lysis of neurocytes and the appearance of pathologically altered cells indicate that neurons in the hippocampus can be damaged as a result of stress. All this confirms that stress has a serious impact not only on mental health, but also on the structure of the brain and cognitive functions. Therefore, it is important to develop special measures to prevent chronic stress and reduce its impact, to continue research on the protection of the hippocampus and the support of cognitive functions.

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