CAJM

Central Asian Journal of Medicine

100 TMA anniversary

Tashkent Medical Academy Press

eISSN: 2181-1326

Scientific Journal

This journal had been publishing since 2018

8

2025

MINISTRY OF HIGHER AND SECONDARY SPECIALIZED EDUCATION OF THE REPUBLIC OF UZBEKISTAN

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Central Asian Journal of Medicine

Tashkent Medical
Academy Press

Scientific Journal
This journal had been publishing since 2018

eISSN: 2181-1326 (online)
ISSN: 2181-7812 (print)

№ 8, 2025. Vol. 1

The Central Asian Journal of Medicine is peer-reviewed scientific journal that publishes original scientific articles. The series has been founded at the Tashkent Medical Academy in 2011. The main goal of this scientific journal is to promote the development of education and research work among teachers, doctoral students and students in Medical Sciences, Medical Education, Public Health, Nursing, Rehabilitation and Therapy.

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NEUROANATOMY AND PSYCHOPATHOLOGICAL CONSEQUENCES OF MORPHOGENESIS OF THE CORPUS CALLOSUM AND CRANIAL BONES UNDER THE INFLUENCE OF PRENATAL STRESS

Gayrat K. Polvonov – researcher
Urgench State Medical Institute (Urgench, Uzbekistan)
Dilorom B. Adilbekova – D.M.Sc., professor
Tashkent State Medical University (Tashkent, Uzbekistan)
Malokhat B. Nazarova – senior lecturer
Urgench State Medical Institute (Urgench, Uzbekistan)
Abdulaziz A. Akhrorov – student
Sardorjon S. Toshpulatov – student
Farangiz O. Rustamova – student
Tashkent State Medical University (Tashkent, Uzbekistan)
ahrorovabdulaziz65@gmail.com

Abstract. *In this study, the influence of prenatal stress on the morphological and morphometric changes of the main commissural structure of the brain - the corpus callosum and skull bones during postnatal ontogenesis was studied. Rats were used as an experimental model; pregnant females were subjected to stress through immobilization, noise, and light changes. Preparations obtained on the 1st, 7th, 14th, 21st, and 30th days of postnatal development underwent morphological and morphometric analysis.*

The results showed that prenatal stress significantly disrupts the development of the corpus callosum: a decrease in the number of nerve fibers, thinning of axons, immaturity of the myelin layer, and hyperactivity of neuroglial cells were observed. Morphometrically, the thickness, length, and cross-sectional area decreased compared to the control group. These changes may be associated with a decrease in the functional integration of the central nervous system and a weakening of cognitive activity.

The study provides an important scientific basis for determining the neuroanatomical consequences of perinatal stress and understanding the pathogenesis of stress-related neuro-developmental disorders, including the autism spectrum and attention deficit syndromes.

Keywords: *prenatal stress, psychopathological consequences, morphogenesis, white laboratory rats, copus callosum, cranial bones*

Introduction.

Prenatal stress, caused by the exposure of the maternal organism to adverse factors during pregnancy, is one of the key contributors to impaired development of the offspring's nervous system. The corpus callosum, as the largest commissural tract of the brain, plays a central role in the integration of interhemispheric connections, ensuring the coordination of cognitive, emotional, and motor functions [1; 2; 3]. Disruptions in its formation associated with prenatal stress may lead to serious neuroanatomical and psychiatric consequences, including autism spectrum disorders, schizophrenia, and depressive conditions [4; 5].

The functional system "mother–fetus–offspring" represents a complex network of biological interactions that ensures the optimal development of the fetus. Prenatal stress, induced by psycho-emotional, physical, or chemical factors, disturbs the homeostatic mechanisms of this system, resulting in morphological and functional defects in the brain structures of the offspring [6; 7]. Despite numerous studies addressing the impact of prenatal stress on brain development [8; 9], many aspects related to the formation of the corpus callosum remain insufficiently elucidated.

In particular, there is a lack of comprehensive data on the mechanisms through which stress-induced alterations in the maternal organism influence the morphogenesis of the corpus callosum, as well as on the long-term psychiatric outcomes of such disturbances. Investigating these processes is

of considerable importance for developing strategies for early diagnosis, prevention, and correction of neurodevelopmental and psychiatric disorders in the offspring [10; 11].

The present study aims to examine the morphological, morphometric, and immunohistochemical characteristics of the corpus callosum in offspring exposed to prenatal stress, as well as to identify the relationships between structural changes and psychiatric manifestations.

Prenatal stress poses a particular challenge in the context of Uzbekistan, where social, economic, and environmental factors—such as limited access to healthcare services in rural areas, high ambient temperatures, and social isolation—may exacerbate its impact on pregnant women. These factors increase the risk of neurodevelopmental disorders in the offspring, including corpus callosum abnormalities, which are associated with cognitive and psychiatric impairments [12; 14]. The development of stress models adapted to local conditions enables a deeper understanding of these influences and facilitates the creation of effective preventive strategies, making this research particularly relevant for the region [13; 15; 16].

Purpose of the research.

Study and assessment of postnatal morphogenesis of the offspring skull under conditions of prenatal stress of the maternal organism, as well as determination of neuroanatomical and psychiatric consequences.

Materials and methods.

The material for the study was 180 white laboratory rats and their offspring, examined on days 3, 7, 14, 21, and 30 of postnatal development. Prenatal stress was modeled by the influence of chronic unpredictable stress on female rats during pregnancy. Chronic unpredictable stress was modeled using a protocol that included the daily replacement of stressors for 14 days (from the 7th to the 20th day of pregnancy).

Stress factors include:

- Restriction of movement (2 hours a day in special containers);
- Noise exposure (80 dB, 1 hour per day);
- Cold stress (4°C, 30 minutes per day);
- Restriction of food and water consumption (12 hours, once every 3 days).

The control group was kept under standard conditions without stress effects.

The brain of offspring was obtained after euthanasia (using ethyl ether under anesthesia) on days 3, 7, 14, 21, and 30 of postnatal development. The samples were fixed in 10% neutral formalin, dehydrated in increasingly concentrated alcohols, and poured into paraffin. Sections with a thickness of 5 μm were stained with hematoxylin and eosin for general morphological assessment, as well as by the Nissl method for neuron detection.

The study used general morphological, morphometric, immunohistochemical, and statistical methods for a comprehensive assessment of postnatal morphogenesis of the skull bones.

Results and Discussion.

Morphological and morphometric examinations revealed pronounced alterations in the structure of the corpus callosum in the offspring of rats exposed to prenatal stress. Already at the earliest stages of postnatal development, the stress group exhibited clear signs of delayed formation and structural disorganization of corpus callosum tissues, which persisted throughout all observation periods—from postnatal day 3 to day 30.

On postnatal day 3, the stress group demonstrated perivascular space swelling, vacuolization of the neuropil, loosening of myeloarchitecture, and irregular distribution of glial cells in the corpus callosum. In contrast, the control group, whose corpus callosum structure corresponded to age norms, showed well-organized bundles of axonal fibers. In the stress group, however, fiber density and orientation were uneven, with marked signs of hypoplasia, particularly in the genu of the corpus callosum. Morphometrically, the mean thickness of the corpus callosum in this region was 210 ± 15 μm compared with 280 ± 18 μm in the control group ($p<0.01$); in the splenium, these values were 190 ± 12 μm and 260 ± 17 μm, respectively ($p<0.01$).

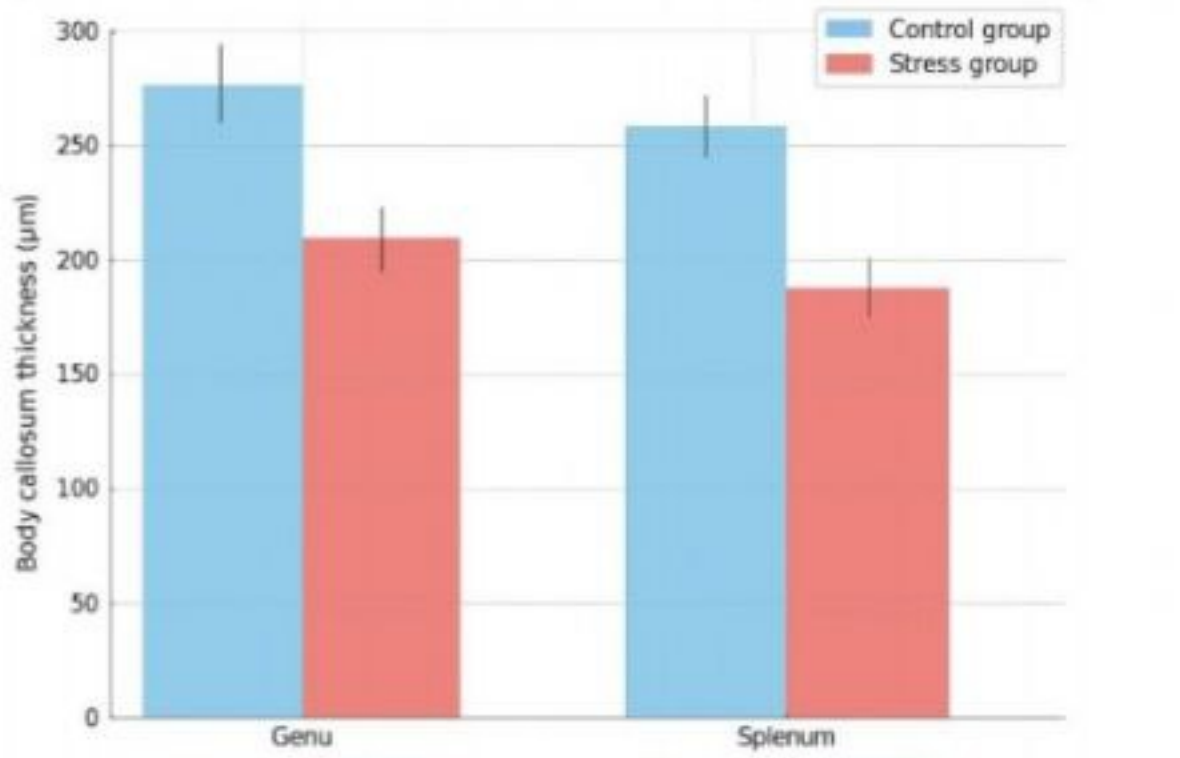

Fig. 1. Histogram illustrating corpus callosum thickness on postnatal day 3 in the offspring.
Description: The histogram compares the thickness (in μm) of the genu and splenium of the corpus callosum between control and stress groups. Animals exposed to prenatal stress demonstrated significantly reduced thickness, indicating delayed development.

By postnatal day 7, signs of impaired morphogenesis persisted in the stress group. Observations included areas of axonal degeneration, reduced intensity of basophilic staining, a decrease in the number of large neurons, and an increase in microglial proliferation, indicating activation of the neuronal inflammatory response. In the control group, the corpus callosum displayed increased thickness, consolidation of fiber bundles, and the emergence of mature myelinated axons. At the same time, the density of myelinated fibers in the genu of the corpus callosum in the stress group was 30% lower than in the control group, a finding confirmed by morphometric analysis ($p<0.01$).

Immunohistochemical examination revealed decreased levels of cell proliferation and increased apoptotic activity. On day 7, the number of Ki-67-positive cells in the stress group was 125 ± 9 cells/mm² compared with 190 ± 12 cells/mm² in controls ($p<0.01$), while the number of Caspase-3-positive cells reached 85 ± 7 cells/mm² in the stress group versus 48 ± 5 cells/mm² in the control group ($p<0.01$).

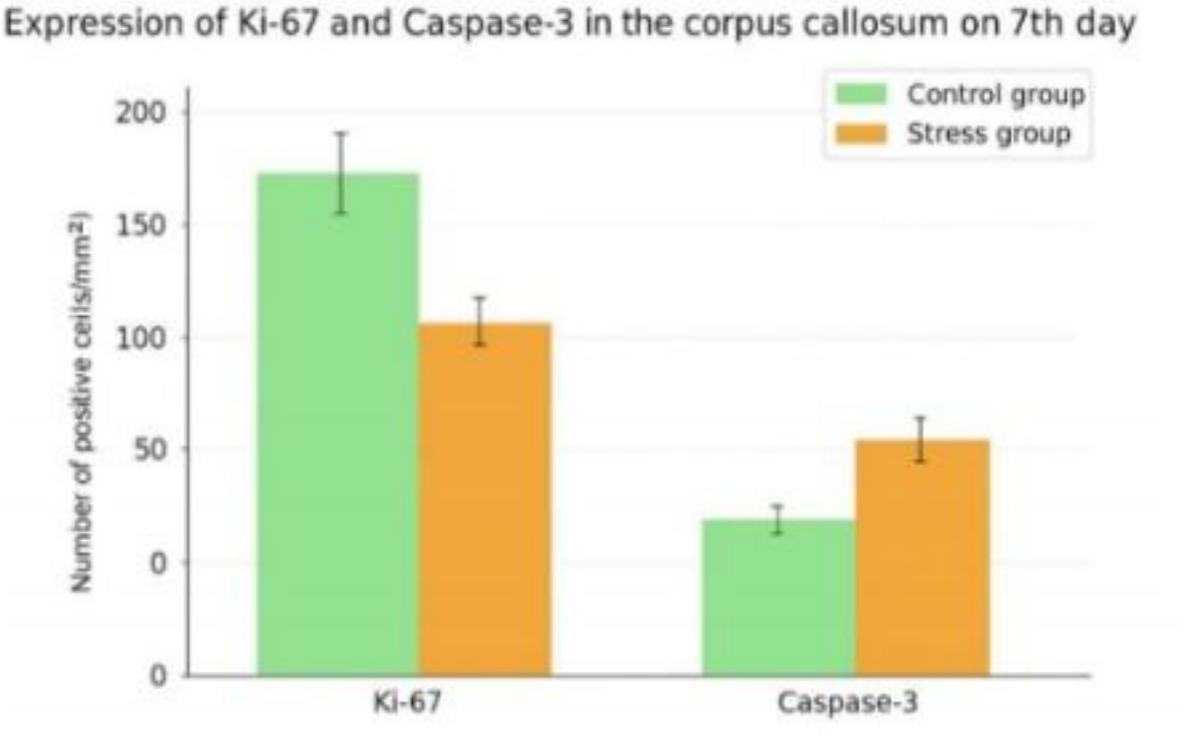

Fig. 2. Histogram showing Ki-67 and Caspase-3 expression in the corpus callosum on postnatal day 7.
Description: The histogram illustrates the relative numbers of Ki-67- and Caspase-3-positive cells in the corpus callosum of control and stress-exposed offspring. Prenatal stress results in reduced proliferative activity and increased apoptosis.

By postnatal day 14, the stress group exhibited pronounced disorganization of the white matter of the corpus callosum. The cross-sectional area of the structure was reduced, and the density of myelinated fibers had not reached normative values. Histological preparations revealed numerous areas with hypocellularity, enlarged inter-fiber spaces, and reduced staining intensity with the Luxol Fast Blue method. The number of glial elements remained elevated, corresponding to previously observed microglial activation in response to neurotoxic conditions. In some samples, astrocytic edema and pericellular swelling were also detected. Visual inspection of sections showed poorly defined boundaries between the genu and the body of the corpus callosum in the stress group, further confirming the immaturity of commissural system structural demarcations.

On postnatal day 21, the differences between control and stress groups reached their peak for several parameters. The mean thickness of the genu of the corpus callosum was 400 ± 22 μm in controls and 295 ± 19 μm in the stress group ($p<0.001$). Myelinated fiber density was 5600 ± 320 vol./mm² in controls versus 4100 ± 280 vol./mm² in the stress group ($p<0.001$). Ki-67 levels decreased to 110 ± 10 cells/mm² in the stress group compared with 170 ± 13 in controls, whereas Caspase-3 expression increased to 105 ± 9 cells/mm² in the stress group versus 55 ± 6 in controls.

Table 1.

Morphometric parameters of the corpus callosum on postnatal day 21.

Parameters	Controll group	Stress group
Thickness (μm)	400 ± 22	295 ± 19
Myelin density vol./mm²	5600 ± 320	4100 ± 280
Ki-67 (cell/mm²)	170 ± 13	110 ± 10
Caspase-3 (cell/mm²)	55 ± 6	105 ± 9

By postnatal day 30, the control group displayed a fully matured structural organization of the corpus callosum: clear lamination, high density of myelinated axons, orderly fiber orientation, and uniform glial scaffolding. Mature oligodendrocytes were observed, indicating stable myelination activity. In contrast, the stress group retained signs of morphological immaturity: areas of reduced optical staining density, disrupted fibers, enlarged inter-fiber spaces, and persistent moderate vacuolization.

In certain samples, diffuse infiltration by microglial elements persisted, interpreted as a consequence of chronic stress-induced inflammation. Additionally, focal astrocytic changes with signs of reactive gliosis were identified, reflecting compensatory-protective activation of glial cells. Furthermore, in the transition zone from the body of the corpus callosum to the splenium, localized regions with markedly reduced myelin density—confirmed by decreased staining intensity with the Luxol method—were detected.

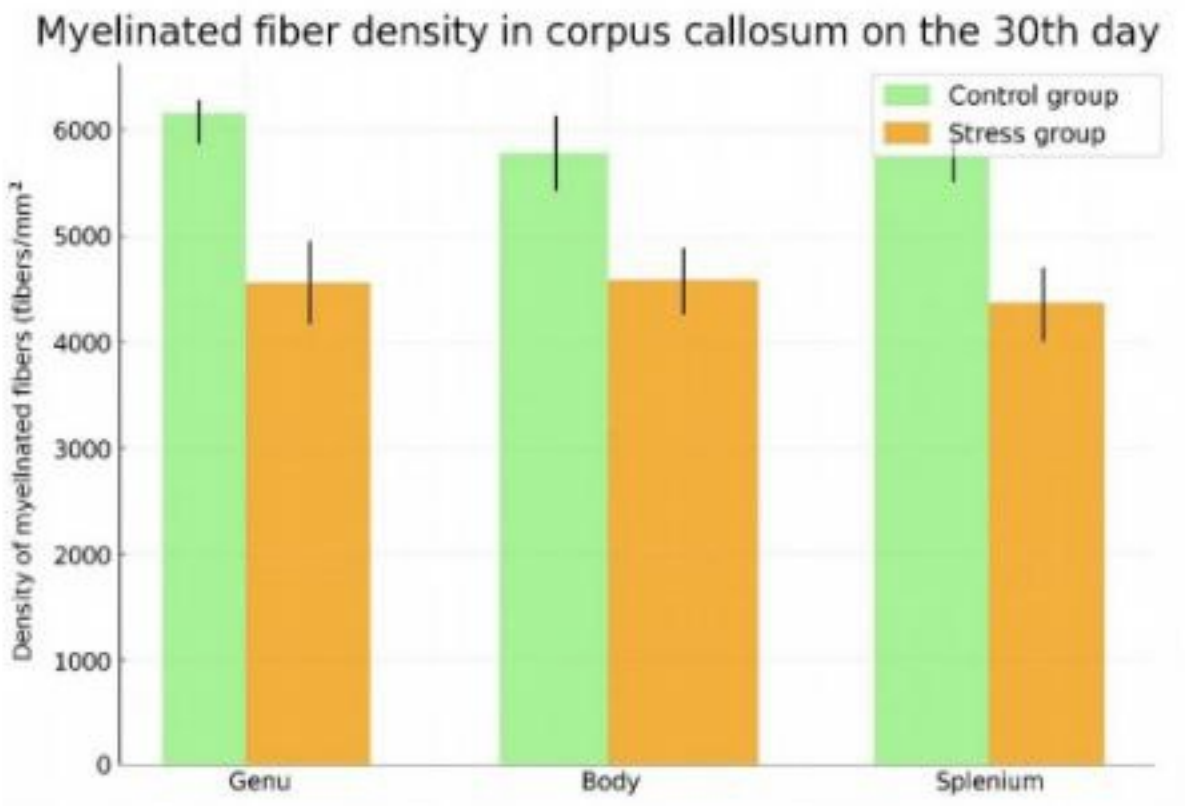

Fig. 3. Diagram showing the density of myelinated fibers in the corpus callosum on postnatal day 30.

Description: The diagram compares the density of myelinated fibers in the corpus callosum of control and stress groups in the late postnatal period. In the stress group, density is significantly reduced, particularly in the splenium region.

During the morphological analysis of the heads of the control and stress group offspring, significant differences were revealed in both the macro- and microstructure of the bones of the skull dome and base. Even in the late stages of intrauterine development, when analyzing fetal samples (18-20 days of pregnancy), a significant slowdown in ossification was observed in the stress group. In particular, the areas of the frontal and parietal bones were characterized by a lower density of staining during histochemical staining with alcian blue and a weak activity of alkaline phosphatase - a marker of osteoblastic activity. The appearance of bone trabeculae in these zones occurred later than in the control group, indicating a shift in the timing of osteogenesis onset.

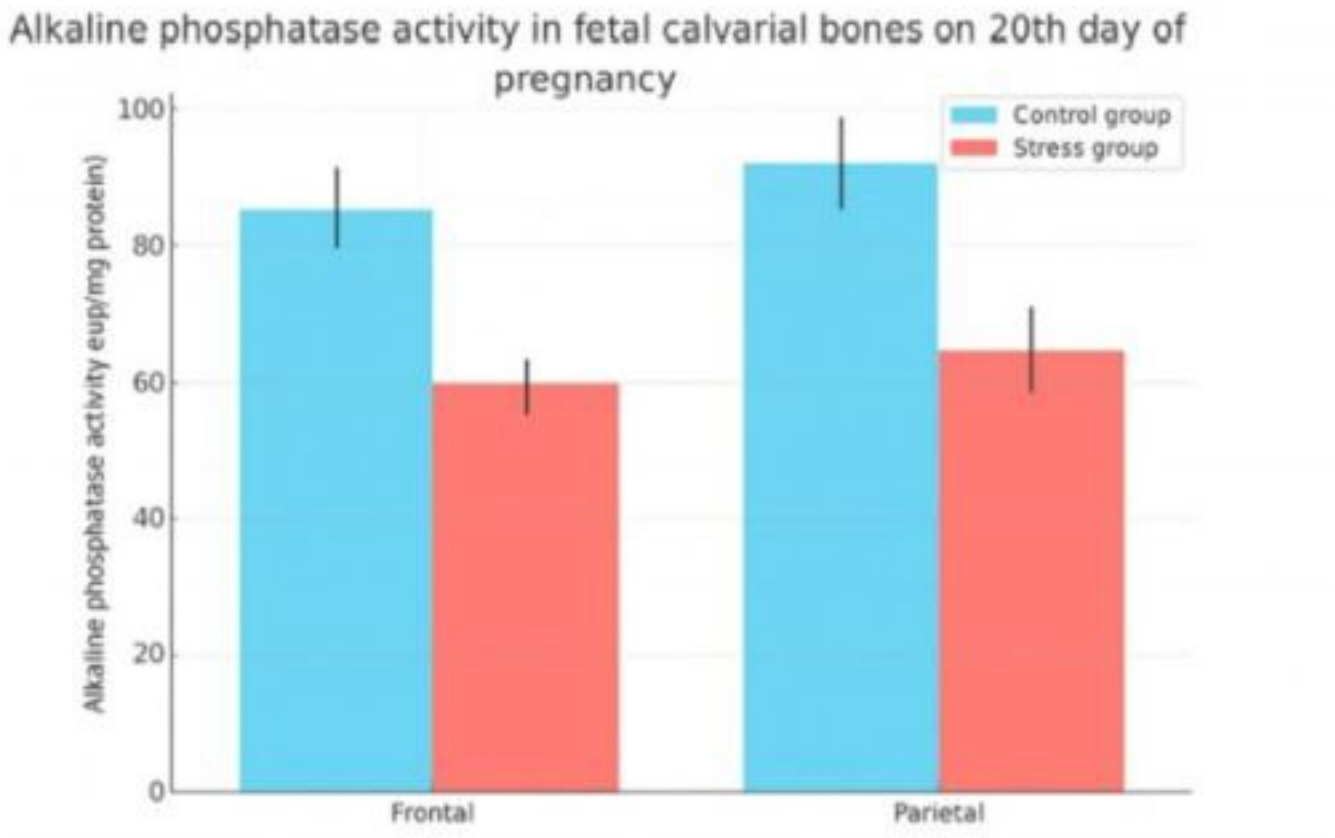

Figure 4. Histogram demonstrating the activity of alkaline phosphatase in the bones of the cranial vault.

Description: The histogram shows the quantitative activity of alkaline phosphatase (unit/mg protein) in the frontal and parietal bones in the fetuses of the control and stress group on the 20th day of pregnancy. In the stress group, activity is significantly reduced, reflecting the suppression of osteogenesis.

One of the main manifestations of the negative impact of prenatal stress on the offspring's body is persistent behavioral changes observed at various stages of postnatal ontogenesis. These behavioral phenomena, formed against the backdrop of disorders in the development of the central nervous system, particularly the мозole body and prefrontal cortex, reflect profound neurobiological restructuring affecting the emotional sphere, cognitive functions, and interhemispheric regulation. Evaluation of offspring behavior was carried out using validated behavioral tests: open field, Barnes labyrinth, and positive labyrinth.

In the "open field" test, active research behavior was observed in the control group animals at 30 days of age: high motor activity, frequent crossing of central zones, normal frequency of vertical stations, and moderate number of corner care activations. The average number of intersections of squares was 82 ± 6, vertical stands - 21 ± 2, and the number of grinding acts - 4 ± 1. At the same time, the offspring of the stress group exhibited signs of pronounced behavioral inhibition: reduced overall motor activity (48 ± 5 intersections), rare vertical postures (9 ± 2), high anxiety, expressed in the predominance of time spent in corner sectors, and an increase in grumming acts (7 ± 1, $p<0.01$).

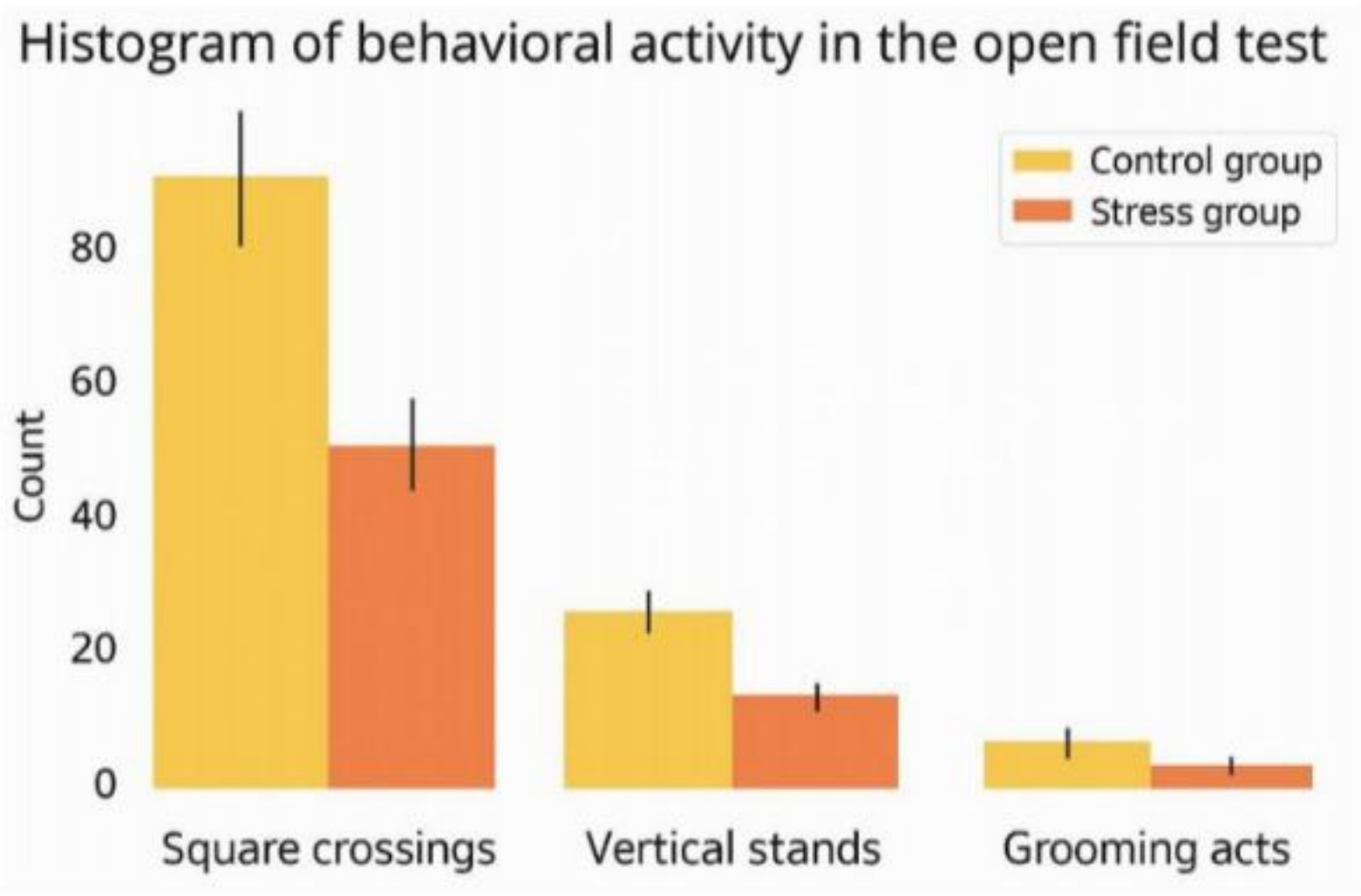

Fig. 5. Behavioral activity histogram in the "open field" test.

Description: The histogram compares motor and anxiety behavioral indicators between the control and stress group: the number of intersections, vertical stations, and grumming acts. In the offspring of the stress group, there is a decrease in motor activity and increased anxiety.

Morphometric analysis showed that a decrease in the thickness of the corpus callosum, a decrease in the density of myelinated fibers, and a disruption of the spatial architectonics of the white matter directly correlated with the degree of motor activity, anxiety, and cognitive impairment in animals. The most pronounced positive correlation was found between the thickness of the мозoled body (in the knee area) and the number of intersections in the "open field" test ($r = 0.79$, $p<0.01$). Animals with a thinner calloused body exhibited less mobility, avoiding behavior and stiffness. The negative correlation between the thickness of the мозoled body and the number of grumming acts was $r = -0.63$ ($p<0.05$), which reflects the connection between the deficiency of interhemispheric connections and increased anxiety.

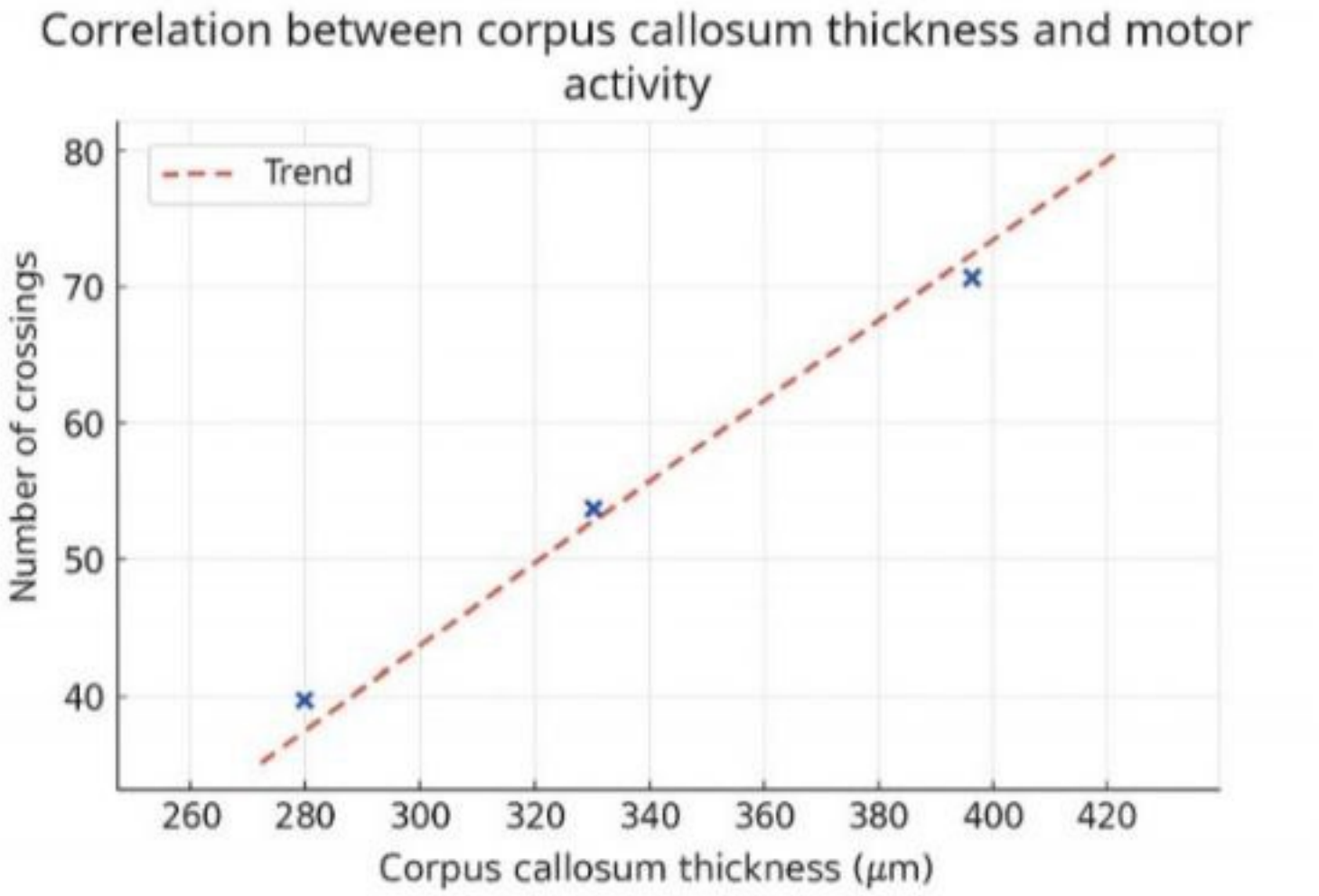

Fig. 6. Correlation diagram between callous body thickness and motor activity.
Description: The diagram shows the relationship between the thickness of the corpus callosum and the number of intersections in the "open field" test. The thickness of the structure correlates positively with the level of research activity.

The obtained results emphasize the importance of a comprehensive analysis of the state of brain structures in assessing the functional state of the body. The decrease in osteogenic potential and cranial growth retardation, occurring in parallel with the developmental disorder of the мозоle body, reflect the general suppression of growth and maturation processes of the CNS and musculoskeletal system under prenatal stress conditions.

Gender differences also maintained statistical significance in the analyzed parameters. In males of the stress group, as in the previous sections, the severity of both morphological and behavioral disorders was higher. During the correlation analysis of these data, the correlation coefficients reached higher values: for example, in male's $r = 0.82$ (thickness of the callus body and mobility), while in females - $r = 0.65$. This may indicate a greater sensitivity of the male body to the destabilization of the prenatal environment.

Conclusion.

Analysis of neurovascular alterations in the corpus callosum demonstrates that prenatal stress induces pronounced disruptions in the development of the vascular network, including reduced capillary density, destabilization of the blood–brain barrier, and activation of inflammatory processes within the vessel walls. These pathological changes in the vascular component create an additional morphological substrate for delayed myelination, hypoxia of neuroglial cells, and the initiation of neuroinflammatory cascades, all of which must be taken into account when designing therapeutic strategies aimed at preventing cognitive impairments associated with prenatal stress. Furthermore, statistically significant associations have been identified between the neuroanatomical characteristics of the corpus callosum, the morphometric parameters of cranial bones, and the behavioral responses of the offspring. These correlations support the hypothesis that prenatal stress disrupts the coordinated development of the central nervous and skeletal systems, leading to functional and behavioral disorders. The establishment of such interrelations is of considerable importance both for fundamental neuroembryology and for the development of clinical approaches to the prevention of neuropsychiatric disorders arising from harmful exposures during the antenatal period.

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