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STRUCTURAL AND IMMUNOHISTOCHEMICAL FEATURES OF THE NORMAL BRAIN AND THEIR MODULATION BY EXTERNAL FACTORS.

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Abstract

This article provides a scientific analysis of the normative morphological and immunohistochemical features of the brain, as well as the factors that influence them. The normal structure of brain tissue, the distinctive morphological characteristics of neurons and glial cells, the importance of the blood–brain barrier, and their role in overall body functioning are thoroughly discussed. The article also examines the possibilities of assessing the physiological state of the brain using immunohistochemical methods that identify markers, proteins, and neurotransmitter systems. The influence of external and internal factors (genetic predisposition, toxic effects, inflammatory processes, stress, and environmental conditions) on brain morphology and immunohistochemical indicators is analyzed based on scientific literature. The results of this research serve as an important theoretical and practical foundation for the early diagnosis and prevention of neurological diseases, as well as for the development of neuroprotective therapies.

Keywords: brain, normative morphology, immunohistochemistry, neuron, glia, blood–brain barrier, markers, diagnostics, neuroprotection

Introduction

The brain is a complex organ that performs the highest integrative functions of the human body, ensuring regulation of physiological processes and adaptive responses. Structural and functional integrity of the brain depends on the balance of neurochemical signaling, vascular supply, and the plasticity of neural connections. Even minor disturbances caused by trauma, hypoxia, anesthesia, or toxic exposure can lead to profound morphological and molecular changes that affect neuronal survival, synaptic transmission, and cognitive functions.

Experimental modeling of brain injury combined with general anesthesia is an important approach to understanding the pathogenesis of traumatic encephalopathy and neurodegenerative changes that occur under clinical conditions such as surgery or accidents. In particular, nitrous oxide (N_2O), a widely used anesthetic, has been shown to influence oxidative metabolism, neurotransmitter regulation, and apoptotic pathways in neural tissues. However, the combined effects of mechanical trauma and

N_2O anesthesia on the brain's morphological and immunohistochemical structure have not been sufficiently elucidated.

This study aims to investigate the morphological, morphometric, and immunohistochemical alterations in the rat brain following experimental combined trauma under general anesthesia. The work also seeks to establish correlations between structural integrity, neuronal apoptosis, and synaptic preservation, using Caspase-3 and Synaptophysin as molecular markers. Understanding these changes at the tissue and cellular levels provides valuable insight into post-traumatic neurodegeneration and may contribute to the development of improved neuroprotective strategies during anesthesia and surgical interventions.

Materials and Methods

Experimental Design

The study was conducted in the Research Laboratory of the Bukhara State Medical Institute named after Abu Ali ibn Sina during 2024–2026. All experiments complied with national bioethical standards and were approved by the Ethics Committee of the Ministry of Health of the Republic of Uzbekistan (protocol No. 4/1439 dated September 21 and No. 4 dated August 26, 2020). The research followed international guidelines for the care and use of laboratory animals.

A total of 100 white outbred rats of both sexes, aged 3, 9, and 18 months and weighing between 110 – 350 g, were used. The animals were housed in standard plastic cages (five per cage) under controlled temperature (22–24 °C) and light–dark cycles, with free access to food and water. Before experimentation, all animals underwent veterinary examination and a 7-day quarantine period to exclude infectious diseases.The animals were randomly divided into two groups:
1. Control group (n = 30): Rats without induced trauma or anesthesia.
2. Experimental group (n = 70): Rats exposed to mechanical trauma on the right hind limb followed by general anesthesia using inhaled nitrous oxide (N_2O).

Mechanical trauma was induced using a standardized physical impact equivalent to an average human trauma force of approximately 4.85 kg, based on biomechanical modeling. The anesthetic was administered by inhalation (80% N_2O + 20% O_2 mixture). Two hours after exposure, the animals were euthanized by decapitation, and their brains were carefully dissected for further analysis.

Histological and Morphometric Analysis

Brain tissue samples (5 × 3 × 3 mm) were fixed in 10% neutral buffered formalin at room temperature. Following dehydration through ascending ethanol concentrations (40% → 96%), samples were embedded in paraffin. Sections 5–7 μm thick were cut and stained using hematoxylin–eosin and Van Gieson techniques. Morphometric evaluations were performed using an NLCD-307B light microscope equipped with an ocular micrometer.Morphometric parameters included:

Neuronal density and nuclear diameter in hippocampal CA1/CA3 and cortical pyramidal layers.Thickness of the neuronal layers and cortical laminae. Number of glial cells (astrocytes).Perivascular space diameter. Neuron cytoplasmic vacuolization and nuclear–cytoplasmic ratio.Measurements were expressed in μm or mm² using Avtandilov's morphometric standards (1990).

Immunohistochemical Studies

Immunohistochemical (IHC) analysis was carried out on paraffin-embedded brain sections (4 μm thick) using a Bond Leica Automated Immunostainer (Leica, Australia). Antigen retrieval was performed in citrate buffer (pH 6.0) at 95–98 °C for 20 min. Endogenous peroxidase was blocked with 3% hydrogen peroxide. The primary monoclonal antibodies were:Caspase-3 — to detect apoptotic activity. Synaptophysin — to assess synaptic density and neuronal connectivity.

Secondary antibodies conjugated with horseradish peroxidase (HRP) were applied, and immunoreactivity was visualized using DAB (diaminobenzidine) chromogen, counterstained with Mayer's hematoxylin. Expression intensity was evaluated semiquantitatively on a 0–3 scale across 10 high-power fields (×400), and the Expression Coefficient (EC) was calculated as: $EC = \Sigma(B \times P)/100$, where B = staining intensity (0–3) and P = percentage of positively stained cells.

Statistical Analysis

All quantitative data were processed using IBM SPSS Statistics v.23 and Microsoft Excel 2010. Normality was assessed via the Shapiro–Wilk and Kolmogorov–Smirnov tests. Parametric data were compared using Student's t-test, while nonparametric data were analyzed by the Mann–Whitney U-test. Results were expressed as mean ± SD, and differences were considered statistically significant at $p < 0.05$.

Results

Histological analysis of brain sections from the control group showed normal cytoarchitecture of the cerebral cortex and hippocampal regions. Neurons exhibited clear nuclear and cytoplasmic boundaries, with well-preserved pyramidal layers, minimal perivascular spaces, and an intact neuroglial network. Synaptic structures appeared compact and continuous.In the experimental group subjected to combined trauma and nitrous oxide (N_2O) anesthesia, significant structural alterations were observed. The cerebral cortex demonstrated neuronal shrinkage, vacuolization of the cytoplasm, and irregular nuclear morphology. Degenerative changes were most prominent in the hippocampal CA1 and CA3 regions, characterized by neuronal pyknosis, loss of nucleoli, and fragmentation of chromatin. Perivascular edema and widening of pericellular spaces were also noted, indicating vascular compromise and blood–brain barrier disturbance.

The pyramidal layer exhibited partial disorganization, and glial cell proliferation (reactive gliosis) was visible around degenerated neurons. Morphometric measurements confirmed a statistically significant reduction in neuronal density ($p < 0.05$) and layer thickness, particularly in the hippocampus and prefrontal cortex. The neuroglial index was markedly increased, reflecting compensatory astrocytic activation.

Quantitative morphometric assessment revealed the following trends: Neuronal density decreased by approximately 25–30% in the experimental group compared with controls.Neuronal nuclear diameter reduced by 15–20%, indicating nuclear condensation during apoptosis.Perivascular space diameter increased nearly 2-fold ($p < 0.01$), signifying edema and hypoxic effects.Gliocyte count elevated by 35–40%, showing astroglial activation and possible inflammatory response. Neuron cytoplasmic vacuolization significantly higher in the experimental group, especially in older (18-month-old) rats, suggesting enhanced vulnerability of aged brain tissue.

Immunohistochemical staining for Caspase-3 revealed minimal or no reactivity in control rats, indicating physiological cell turnover. In contrast, the experimental group displayed strong cytoplasmic and nuclear positivity in hippocampal and cortical neurons. The expression coefficient (EC) for Caspase-3 increased by 2.5–3 times relative to controls ($p < 0.01$), demonstrating activation of apoptotic pathways.Synaptophysin, a presynaptic vesicle marker, exhibited uniform and intense staining in the control group, confirming intact synaptic connectivity. In the experimental group, a marked reduction in staining intensity and density of Synaptophysin-positive terminals was observed, particularly in hippocampal CA3 and cortical layers. The EC value for Synaptophysin decreased by 30–40% ($p < 0.05$), indicating synaptic loss and impaired neurotransmission.

Conclusion

The present experimental study demonstrated that combined mechanical trauma and nitrous oxide (N_2O) anesthesia induce distinct morphological, morphometric, and immunohistochemical changes in the rat brain. The following conclusions were drawn:Structural Impact: General anesthesia with N_2O following trauma caused significant cortical and hippocampal neuronal degeneration, characterized by cytoplasmic vacuolization, nuclear condensation, and disorganization of neuronal layers.Morphometric Changes: There was a reduction in neuronal density and nuclear diameter with concurrent enlargement of perivascular spaces, indicating hypoxic–ischemic damage. Glial Activation: Reactive astrocytosis was evident, reflecting neuroinflammatory responses aimed at limiting secondary neuronal injury.Apoptotic and Synaptic Alterations: Increased Caspase-3 expression confirmed enhanced apoptosis, whereas decreased Synaptophysin expression signified synaptic

loss and reduced neural communication.Age-Related Susceptibility: Older animals (18 months) exhibited more pronounced degenerative changes, indicating that aging intensifies vulnerability to anesthetic and traumatic effects. Overall, these results highlight that nitrous oxide anesthesia, when combined with trauma, may exacerbate neurodegenerative and apoptotic processes, thereby compromising neuronal integrity and synaptic function. The findings emphasize the importance of optimizing anesthetic management and exploring neuroprotective interventions to mitigate anesthesia-induced neural injury in clinical settings.

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