

Morphological Classification of Neuronal Damage

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Summary

Objectives. To develop a morphological classification of neuronal damage for use in practical activities by researchers, pathologists, and forensic experts.

Material and methods. The neurons of the cerebral cortex of 30 experimental animals (Wistar rats) were studied. Of these: with circulatory arrest $N=10$, with clozapine poisoning in combination with alcohol $N=20$ (clozapine dose 150 mg/kg, alcohol dose 5 ml/kg); morphological material of the human cerebral cortex was studied in subarachnoid hemorrhages (SAH) $N=23$, sudden cardiac death $N=10$, coronavirus infection $N=18$. Histological preparations were stained with hematoxylin and eosin, according to Nissl, according to Feulgen (for DNA), according to Brachet (for RNA and RNP), caspase-3 was detected by immunohistochemistry.

Results. A morphological classification of neuronal damage was proposed, including: decentralization of the nucleus within a neuron, morphological changes in the nucleolus, dark neurons, chromatin remodeling, lipofuscinosis, neuronal edema, Nissl substance lysis, neuronal calcification, neuronophagia, necrosis, and neuronal apoptosis. Functional disorders that occur in the studied variants of neuronal alteration were considered. As a result of developing neuronal damage, the function of the neuronal cytoskeleton, synthesis of ribosome subunits, synthesis of ribonucleoproteins, and DNA reparation are impaired, apoptosis is activated, lysosomes are damaged, the formation of reactive oxygen species is activated, and irreversible forms of neuronal damage (neuronophagia, necrosis, apoptosis) are recorded.

Conclusion. The proposed morphological classification complements existing classifications based on the study of molecular markers of neuronal damage and can be used in experimental studies and in the practical work of pathologists and forensic experts.

Keywords: neurons, neuronal damage, morphological classification

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Introduction

Morphological studies are of great importance in understanding the processes occurring in the central nervous system, allowing for the verification of general pathological processes under the influence of damaging factors and the assessment of the nature of structural damage to neurons. In their everyday practical activities, pathologists and forensic experts solve the problems of identifying damage to neurons, their varieties, topographic localization with subsequent substantiation of their significance in the occurrence of functional disorders in various diseases of the central nervous system (CNS). Morphological classification of non-specific damage to neurons, developed at the Institute of Human Morphology [1] includes: acute swelling of nerve cells, primary irritation of neurons, hydropic changes in nerve cells, fatty degeneration, pigment dystrophies, calcification of neurons, shrinkage of nerve cells, severe changes in neurons, ischemic injury to neu-

rons, karyocytolysis, satellitosis, neuronophagia. This classification includes not only morphological signs of neuronal damage, but also etiological factors (primary irritation, ischemic injury), as well as the intensity of damage (severe changes in neurons). Over the course of the existence of this classification, new data has been found characterizing new variants of neuronal damage, clarifying the mechanisms of alteration, and variants for assessing neuronal damage based on the identification of molecular markers have been proposed. In 1990 [2], a classification of irreversible cell damage was proposed: I — apoptosis, II — autophagy, III — necrosis. In 2005 (Nomenclature Committee on Cell the National Cancer Institute (NCCD) [3] recommended a classification that characterizes other types of cell alteration. A cell can be considered nonviable if one of three morphological criteria is detected: loss of cytoplasmic membrane integrity, complete cell fragmentation (including its nucleus) with formation of apoptotic bodies,

and absorption of the dead cell (or its fragments) by another cell. Researchers came to the understanding that morphological types of cell alteration (including neurons) indicate functional, biochemical, and immunological heterogeneity of the mechanisms of their damage. Visualization of morphological changes and determination of biochemical changes in the cell are recommended to determine the type of cell death. The NCCD proposed criteria for determining morphological types of cell death and gave recommendations on the use of terminology associated with cell death [4]. At the same time, this classification does not take into account a number of morphological changes that are reversible at a certain stage, but with prolonged and intense exposure to damaging factors lead to irreversible structural changes.

In addition to existing classifications, a classification of variants of alteration of CNS neurons was proposed taking into account morphological signs of damage, including the identification of molecular markers by histochemical and immunohistochemical research methods.

The aim of the study is to develop a morphological classification of neuronal damage for use in practical activities by researchers, pathologists, and forensic experts.

Material and Methods

The neurons of the cerebral cortex of 30 experimental animals (Wistar rats) were studied. Of these, 10 of which had circulatory arrest, and 20 had poisoning by clozapine in combination with alcohol (clozapine dose 150 mg/kg, alcohol — 5 ml/kg). In addition, the morphological material of the human cerebral cortex was examined in subarachnoid hemorrhages (SAH) ($N=23$), sudden cardiac death ($N=10$), COVID-19 ($N=18$). Histological preparations were stained with hematoxylin and eosin, according to Nissl, according to Feulgen, according to Brachet, luxol blue and cresyl violet, caspase-3 was detected by the immunohistochemical method.

Research Results

Decentralization of the neuron nucleus. One of the most common variants of neuron damage was the displacement of the nucleus to the periphery of the cell. This was most clearly recorded in large neurons, in which a significant volume belonged to the cytoplasm, due to which the nucleus could change its position in the cell (Fig. 1, *a*). The process of migration of the neuron nucleus is regulated by complex molecular mechanisms, considered in the discussion of the results of the study.

Morphological changes in the nucleolus of the neuron nucleus. Histological examination often

revealed a shift (decentralization) of the nucleolus of neuronal nuclei, recorded in some neurons, while in other neurons the nucleolus retained a central position in the cell nucleus. In addition, neuron damage was accompanied by the disappearance of nucleoli and a decrease in the intensity of their staining. The diameter of nucleoli in cells synthesizing ribosome subunits varied from 0.5 to 7 μm . In cells where ribosome formation decreased or ceased, the size of nucleoli decreased to 0.1–0.3 μm . The shape, size, and number of nucleoli depended on the functional state of the nucleus: the larger the nucleolus, the higher its activity (Fig. 1, *b, c*).

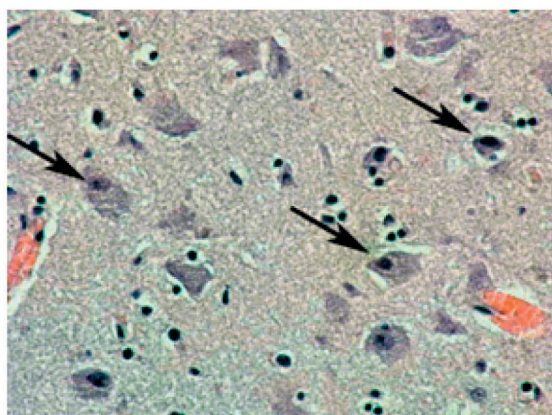
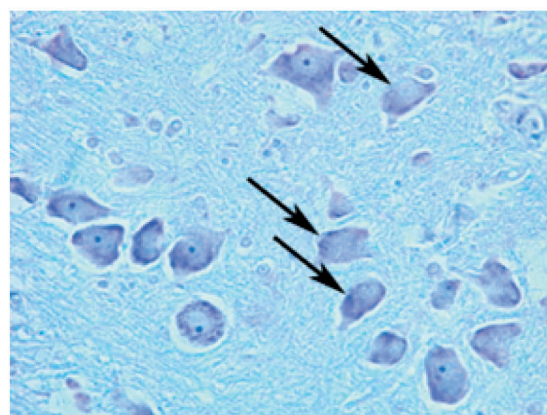
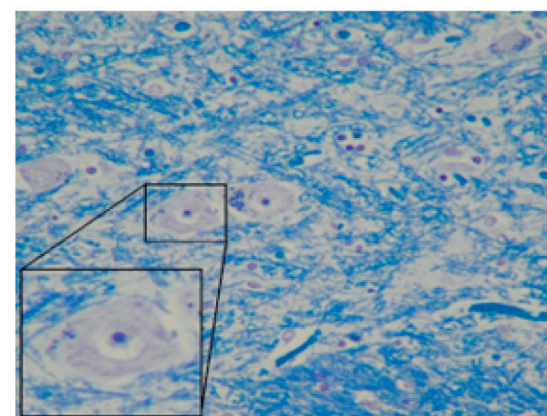
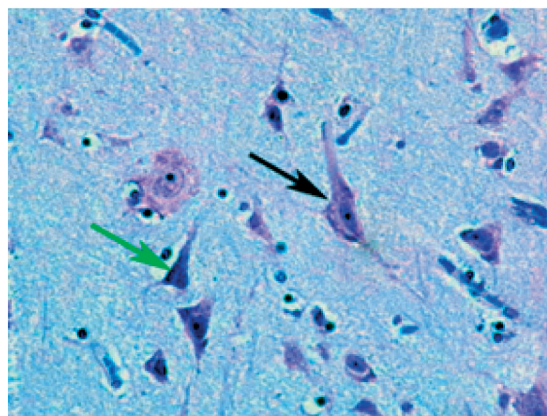
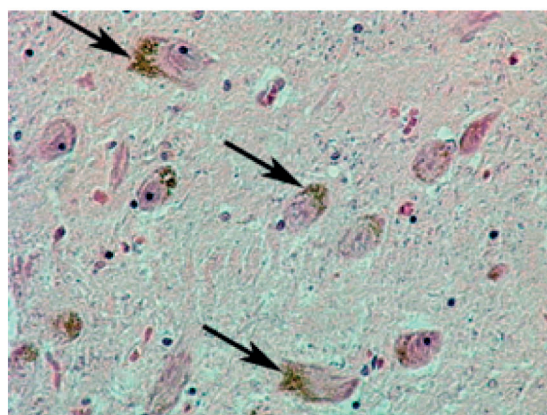
Morphological changes in Nissl substance. Basophilic Nissl substance, revealed by staining with methylene blue (Nissl method), was located in the perinuclear zone. Electron microscopic examination showed that the basophilic substance was formed by flattened cisterns of the granular endoplasmic reticulum, where ribosomes were located, providing protein synthesis in the cell. Granules of Nissl substance contained ribonucleoproteins and protein-polysaccharide complexes. Basophilia, revealed by staining histological sections using the Nissl method, was due to RNA in the ribosomes.

Acute cerebrovascular events were accompanied by a significant decrease in the intensity of Nissl staining or a complete absence of staining. Spraying of Nissl substance in the cytoplasm of neurons was noted, as well as a shift to the peripheral zones of the neuron cytoplasm (Fig. 1, *d*).

Lipofuscinosis (acquired). Lipofuscin, which is formed in the cytoplasm of neurons and has autofluorescence, is one of the main markers of damage to neurons in the brain in cerebrovascular diseases. Lipofuscin exhibits variability in its properties: protein, lipid and/or carotenoid composition (Fig. 1, *e*).

Neuronal edema. Cellular (cytotoxic) edema was characterized by intracellular accumulation of fluid as a result of redistribution, rather than an increase in the fluid volume of brain tissue. Neuronal edema developed as a result of the entry of Na^+ and Cl^- , water from the interstitial spaces into the cell after damage to the central nervous system. At the light-optical level, swelling of cells and vacuolization of the cytoplasm of neurons were noted. Intracellular edema was observed in oncosis, which is one of the variants of neuronal damage. (Fig. 2, *a*).

Dark neurons. One of the histological variants of neuronal damage were the so-called dark neurons, which appeared as a result of damaging factors acting upon the central nervous system. Dark neurons are hyperchromic basophilic neurons with cytoplasm intensely stained with hematoxylin and eosin, according to Nissl and Brashe. Dark neurons are often subject to shrinkage and irreversible morphological changes (Fig. 2, *b*).

*a**b**c**d**e*

Calcification of neurons. One of the causes of damage to neurons is an increase of intracellular calcium. When staining histological sections with hematoxylin, calcification is characterized by the appearance of dark blue granules in the cytoplasm of neurons. Irreversible damage to neurons during their calcification develops through apoptosis or necrosis.

Remodeling (morphological changes) of chromatin. Chromatin in the nucleus of neurons during histological examination was represented by very small granules (euchromatin) or large aggregates (heterochromatin). The localization of chromatin in the nucleus varied. In some nuclei it was located throughout the entire area of the nucleus, while in others it was concentrated in the region of the inner nuclear membrane. When a neuron was damaged, chromatin changed intensity of staining, chromatin clumps became hypochromic, and during cell necrosis, chromatin was not stained with nuclear dyes. (Fig. 2, *c*). The chromatin of the nuclei contained despiralized forms of chromosomes responsible for the implement genetic information. Its chemical basis is deoxyribonucleoproteins — a complex of DNA with histone and non-histone proteins. Proteins make up a significant part of the chromosome substance, as well as performing a structural function, providing spatial organization of DNA in chromosomes.

Fig. 1. Damage to neurons of the human cerebral cortex in subarachnoid hemorrhages (*a, b, d, e*) and COVID-19 (*c*), magnification 400.

Note. *a* — Neurons of the 5th layer of the human cerebral cortex. Arrows show the displacement (decentralization) of neuronal nuclei. Hematoxylin and eosin staining. *b* — Arrows show that in some neurons the nucleoli are not stained. Nissl staining. *c* — Nucleoli are stained blue, indicating an acidic reaction. Staining with luxol blue and cresyl violet. *d* — Tigrolysis (black arrow), dark neurons (green arrow). Staining with Nissl. *e* — Lipofuscinosis of neurons. Staining with hematoxylin and eosin.

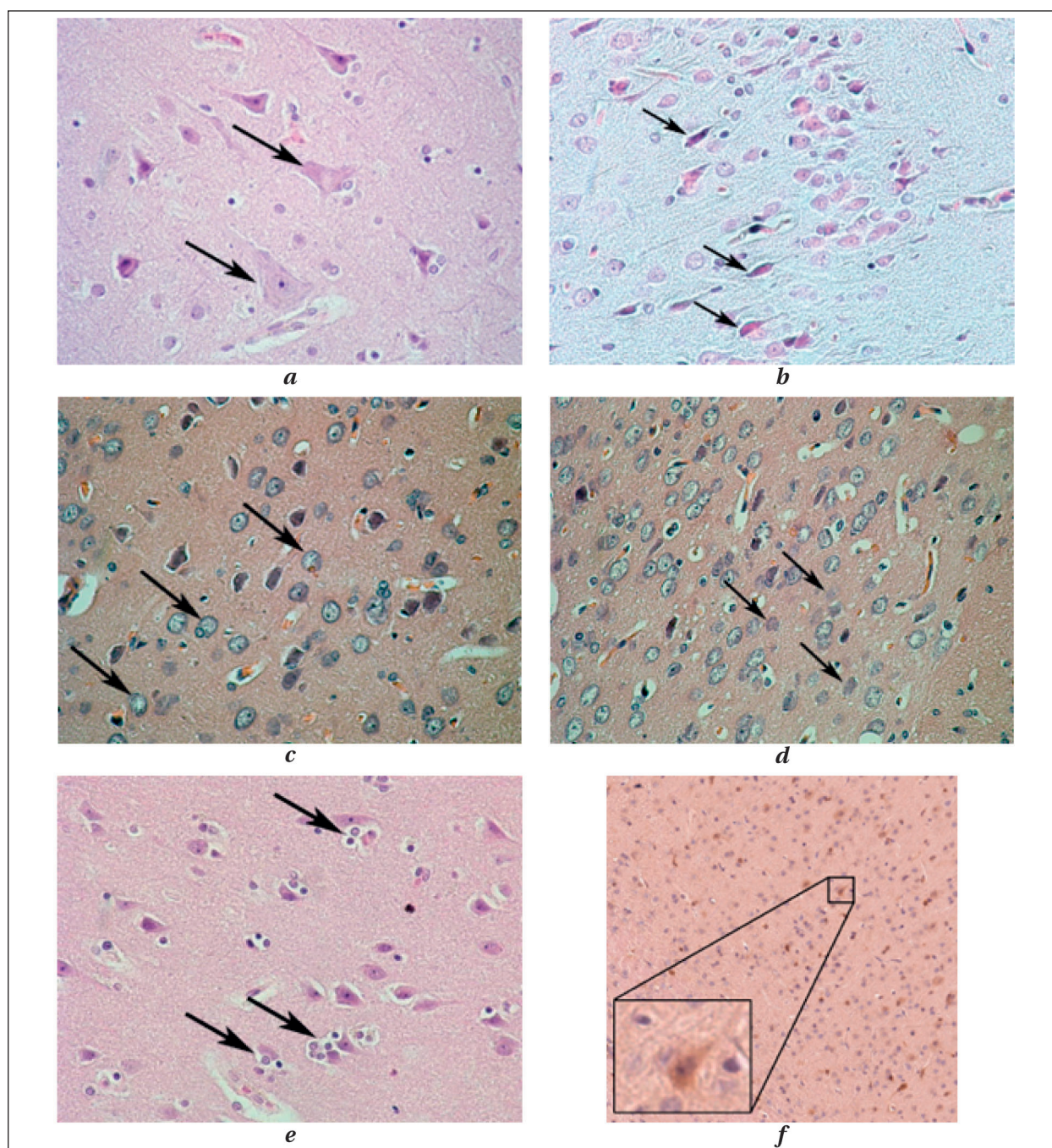


Fig. 2. Neuronal damage of the cerebral cortex of rats in cases of poisoning with clozapine in combination with alcohol (a–e) and in cases of circulatory arrest (f).

Note. a–e: hematoxylin and eosin staining. a — 5th layer of the cerebral cortex. Edema (swelling) of neurons. Magnification 400. b — 2–3rd layers of the cerebral cortex. Dark deformed neurons. Magnification 200. c — Chromatin of nuclei is not stained. Magnification 200. d — Necrosis of neurons, shadow cells. Chromatin is not stained. Magnification 200. e — Neuronophagia. Magn. 400. f — Circulatory arrest. Neurons with positive IHC reaction to Cas-3. Magn. 200

Necrosis. Necrosis is one of the irreversible types of neuronal damage. During necrosis, the integrity of the cells is preserved (unlike apoptosis, in which the cell is fragmented with the formation of apoptotic bodies). The neuron nucleus was not stained with nuclear dyes as a result of damage to nucleic acids. The cells turned into shadow cells,

which were difficult to distinguish during microscopic examination. Karyolysis characterized the transition from necrobiosis to necrosis proper and was verified by hematoxylin and eosin staining (Fig. 2, d).

Phagocytosis of neurons (neuronophagy). Neuronophagy, as a variant of lethal cell damage, was caused by contact of a neuron with microglial

cells (satellitosis). Subsequently, microglia penetrate into the cytoplasm of the neuron, completing the process of phagocytosis. During histological examination, successive stages of this process were recorded (Fig. 2, e).

Apoptosis. During apoptosis, the neuron disintegrated into separate apoptotic bodies limited by the plasmalemma. Morphological changes in the early stages of apoptosis were not expressed and were associated with the activity of molecular factors — caspase-3, etc. Chromatin condensation, nuclear fragmentation, cell compaction and blebbing (the appearance of protrusions on the cell surface) were manifested in later stages of apoptosis (Fig. 2, f).

Discussion

Mechanisms of development of the considered morphological changes and their functional significance.

Decentralization of the core. Migration of nuclei in postmitotic neurons, remodeling of cell structure are caused by the interaction of the cytoskeleton and associated proteins present on the plasma membrane, which not only determine the shape of the cell, but also regulate intracellular transport [5].

Cell migration and nuclear movement within the cell are complex molecular events. In many cases, the microtubule motor proteins dynein and kinesin directly interact with the neuronal nucleus via the LINC (linker of the nucleoskeleton and cytoskeleton) complex, a protein complex associated with both the inner and outer membranes of the nucleus, and drive directional nuclear movement. Microtubule motors act on small points on the nuclear envelope through assembly with the LINC complex, directing the nucleus in a specific direction. Notably, dynein and kinesin are not simply segregated into exclusive microtubule «lanes» but can synergistically drive unidirectional nuclear movement along uniformly oriented microtubules. Such coordinated activity of opposing motors is particularly important for nuclear migration in narrow spaces. In addition, actomyosin contractility influences nuclear deformation and movement. Unlike the control forces of microtubule motors, actomyosin acts on a large area of the cell and nucleus, leading to nuclear deformation and its translocation. Actomyosin affects intracellular transport and cell shape [6].

Thus, the movement of the nucleus in the cell indicates changes in the cytoskeleton and nucleoskeleton, which underlie functional disorders of the neuron. The most important functions of cells, such as phagocytosis, pinocytosis, chemotaxis are entirely associated with damage to the cytoskeleton. Changes in the cytoskeleton of neurons disrupt the

functioning of synapses, energy metabolism and RNA transport, and also contribute to protein aggregation and irreversible alteration of nerve cells [7].

Morphological changes in the nucleolus of the neuron nucleus. In addition to the «traditional» role of rRNA synthesis and ribosome assembly, the nucleolus controls physiological processes in the cell and homeostasis. The function of the nucleolus is essential for the vital activity of neurons and its features differ between cell types. The function of the nucleolus is also critical in the presence of damaging factors, since it controls mitochondrial activity and stress signaling pathways [8].

The nucleolus is the major site of ribosomal subunit biogenesis in eukaryotic cells. Specific chromosomal structures known as nucleolar organizing zones are formed in association with ribosomal DNA genes. The presence of proteins unrelated to ribosomal subunit production suggests additional functions for the nucleolus, such as regulation of mitosis, cell cycle progression, stress response, and biogenesis of various ribonucleoprotein complexes [9].

In interphase cells, ribosomal DNA (rDNA) is localized inside and near the nucleoli, and its location indicates the transcriptional activity of ribosomal genes — inactive rDNA outside, and active inside. Moreover, the nucleolus itself acts as a spatial organizer of chromatin. Microscopic studies make it possible to study the spatially different localization of various DNA populations in relation to the nucleolar structure [10].

Inhibition of neuronal RNA polymerase-1, which transcribes rRNA (transfer of genetic information from DNA to RNA) [11], leads to structural disruption of the nucleolus and apoptosis.

Measurements of nucleolar diameter and nucleolar PARP-1 (nuclear protein (ADP-ribose) polymerase-1, which regulates gene expression) content in the hippocampus of people with cognitive impairment showed a decrease in the diameter of neuronal nucleoli and a decrease in the number of nucleoli containing PARP-1. These studies indicate that disruption of nucleolar shape and function is an early and important feature in the progression of cognitive impairment [12].

Nissl substance: morphological changes. One of the variants of neuronal damage is tigrolysis — dust-like decay of Nissl substance, detected in cortical neurons 20 minutes after a 4-minute cessation of blood flow. Disturbances in protein synthesis are of exceptional importance in the process of development of neuronal alteration in cerebrovascular diseases. At the same time, ribosome biogenesis affects the stimulation of neuronal recovery after a stroke [13].

Under stress, the basis of protein synthesis disruption is a change in rRNA processing. Stress

promotes the breakdown of tRNA (transport RNA that delivers amino acids to ribosomes that synthesize proteins, which leads to the cessation of ribosome formation). These events, developing at the molecular level, contribute to the suppression of protein translation (implementation of the amino acid sequence in the synthesized protein molecule) [14].

Neuronal edema. A decrease in plasma osmolarity causes rapid water uptake by astrocytes but not neurons. Astrocytes osmotically swell because they express functional water channels (aquaporins), whereas neurons lack functional aquaporins. However, neuronal edema develops when blood flow to the brain is disrupted (cytotoxic edema), such as in strokes, sudden cardiac arrest, or traumatic brain injury. Ischemic neuronal edema is not osmotic but results from depolarization due to dysfunction of the ATP-dependent sodium/potassium ATPase (Na^+/K^+ pump). In addition, ion/water cotransporters and the amino acid water pump are involved in ischemia [15].

The resistance of pyramidal neurons to osmotic swelling is explained by the absence of functional AQP4 water channels. Neurons are not osmoresistant, and their swelling is controlled by an AQP4-independent mechanism [16].

Cerebral edema impairs cerebral perfusion and may lead to transtentorial herniation. The blood-brain barrier plays an important role in maintaining a stable microenvironment of the central nervous system. In ischemic stroke, disruption of the blood-brain barrier structures leads to increased paracellular permeability, which promotes extravasation of blood components and causes vasogenic cerebral edema. The glymphatic system and meningeal lymphatic vessels provide a channel for the penetration of cerebrospinal fluid into the brain [17].

Following acute CNS injury, cells of the neurovascular unit undergo pre- and posttranscriptional changes in the activity of ion channels and transporters. These changes result in inadequate ion transport and the generation of abnormal osmotic forces, which ultimately manifest as cerebral edema [18].

Dark neurons. The hyperchromic dark neuron is a cell with active protein synthesis, in which irreversible alteration processes develop under prolonged and intense exposure to unfavorable factors [19].

Dark neurons resulting from alteration must be differentiated from artificial (artifactual) dark neurons damaged during fixation, wiring and staining, in which there were no damage processes during life. It has been shown that the number of artificial neurons increases with non-compliance with the methods of fixation of preparations: namely, replacing 4% buffered paraformaldehyde solution

with immersion. An important feature of artifactual neurons is isomorphism [20].

Most of the altered neurons are at the stage of reversible changes. The structure of some dark neurons in the cerebral cortex of rats was restored within 2 days as a result of hypoglycemic seizures. Non-restoring dark neurons were removed from the cortex in two different ways. If neurons were present in an undamaged environment, they were removed by apoptosis, and neurons present in a damaged environment were removed by necrosis [21].

One hypothesis explains the formation of dark neurons by the sol-gel transition of the cytoplasm of compacted neurons. Changes in the molecular composition of the glycocalyx lead to a decrease in the volume of the neuron as a result of the effect on the cytoskeleton, the nucleus, the membrane of which is associated with the cytoskeleton, and actin. As a result, contraction of the cytoskeleton and increase in intracellular pressure leads to loss of water and shrinkage of neurons. Another hypothesis explains the phenomenon of spreading dark neurons through dendritic connections. Research provides evidence indicating the presence of physical contact at the synaptic zone. The presynaptic and postsynaptic membranes of dendrites are linked by β neurexin and neuroligin. The intercellular regions of these adhesive proteins provide a connection between the presynaptic zone and the postsynaptic densification. This type of connection provides for rapid intercellular interaction as a result of actin polymerization of two closely located neurons. It is suggested that the role of β neurexin-neuroligin is to polymerize actin and transmit information from one neuron to neighboring neurons, which forms new populations of dark neurons [22].

Calcification of neurons. In a cohort of 1130 patients with acute ischemic stroke, hippocampal calcification was detected on CT scans. Hippocampal calcification was detected in 381 (34%) patients. The prevalence increased with age from 8% at age ≤ 40 years to 45% at age ≥ 80 years [23].

Photodynamic effects on neurons and glial cells, causing oxidative stress and ischemic damage, are accompanied by a violation of calcium homeostasis. Oxidative stress and ischemia-induced processes in nervous tissue lead to an apoptotic or necrotic scenario of cell death [24].

Reperfusion carries a risk of acute calcium-dependent damage to the blood-brain barrier, however, its mechanism is unknown. Measurement of the activity of NADPH-oxidase type 5 (NOX5), a calcium-activated enzyme that generates ROS, showed that reoxygenation or calcium overload increases ROS levels in the brain in a NOX5-dependent manner *in vivo*, post-ischemic ROS generation, infarct volume, and functional outcomes were wors-

ened in mice by NOX5-KI activation. Pharmacological inhibition of NOX5 prevented acute reoxygenation-induced injury [25].

Remodeling (morphological changes) of chromatin. Chromatin is formed by despiralized forms of chromosomes in a non-dividing nucleus, implementing genetic information. The chemical basis of chromatin is deoxyribonucleoprotein — a complex of DNA with histone and non-histone proteins. Chromosome proteins account for about 65% of the mass of these structures. The content of non-histone proteins in chromosomes is significantly less than histones, but they are extremely diverse (more than 100 fractions). Morphologically, heterochromatin and euchromatin (open chromatin) are distinguished, differing in functional properties. Based on the morphological features of chromatin (its shape and location), various types of neurons can be categorized [26].

Regulation of chromatin by epigenetic mechanisms plays a central role in gene expression. Aberrant chromatin regulation observed in many diseases is due to defects in epigenetic gene regulation, which leads to abnormal gene expression programs. These defects are caused by mutations in genes encoding enzymes that modify DNA, histones, and shape chromatin architecture [27].

Chromatin is an active participant in the DNA repair process. Changing the patterns of histone modifications formed by numerous histone-modifying enzymes and chromatin remodeling are key to high-quality DNA repair [28].

Regulation of open chromatin formation is an important mechanism for controlling gene expression patterns. Genome-wide studies of various cell tissues, including cells of the nervous system, have revealed tissue- and cell-type-specific landscapes of chromatin accessibility [29].

Hypoxia, inadequate nutrient supply cause chromatin compaction associated with ATP depletion. The mobility of the linker histone H1 is significantly reduced. Studies illustrate the ability of chromatin architecture to physically respond to environmental conditions, directly link cellular energy status to chromatin compaction, and provide insight into the effects of ischemia on cellular nuclear architecture [30].

Chromatin structure varies across cell types, with neuronal chromatin showing greater regional variability than other cells [31].

Enzymes and metabolites can modulate chromatin by regulating the activity of chromatin proteins, including histone-modifying enzymes. Dysregulation of this metabolic activity has been implicated in cerebrovascular diseases, among others [32].

In the mammalian cell nucleus, a clear spatial separation of active euchromatic and inactive heterochromatic genomic regions is observed. In normal

nuclei, as shown by microscopy, euchromatin is localized inside the nucleus, and heterochromatin is located at the periphery of the nucleus. Interactions with the nuclear lamina are necessary for constructing an architecture from separated chromatin phases [33].

Euchromatin spatially organized into transcriptionally inactive domains interspersed with foci of transcriptional activity. RNA induces the formation of transcriptional pockets that displace transcriptionally inactive chromatin [34].

Lipofuscinosis (acquired). Lipofuscin formation is promoted by oxidative stress, aging and other factors. Accumulation of oxidized protein aggregates and highly cross-linked materials such as lipofuscin affects cell viability. Postmitotic neurons cannot remove lipofuscin via phagocytosis and autophagy, which accumulates as endocytosomal granules in the cell cytoplasm, impairing neuronal function. In addition to being a potent source of oxidants, lipofuscin induces apoptosis. The cytotoxicity of lipofuscin is closely related to the presence of iron in the material. As with the formation of other large protein aggregates, the process responsible for their engulfment and degradation is macroautophagy. Autophagosomes and lysosomes are the storage sites for this type of biomolecular aggregate, contributing to the mitigation of their cytotoxicity [35].

The presence of lipofuscin in neurons coexists with its localization in microglia. Glial cells deposit lipofuscin clusters in pericapillary areas, which has a negative impact on the homeostasis of neurons and glia [36].

An increase in the lipofuscin content in neurons is associated with the aging process. At the same time, pigment formation has been demonstrated not only in old but also in young animals, as well as in individuals subjected to stress, dietary and environmental interventions. Electron microscopic studies on animals have shown significant variability in the structure of individual lipofuscin granules, but the presence of «transparent vacuoles» surrounded by a single membrane is one of the characteristic features of neuronal lipofuscin in older people [37].

Lipofuscin, being a photosensitizer, potentiates intracellular dyshomeostasis, playing a decisive role in inhibiting the function of the proteasome (a multiprotein complex that carries out proteolysis of defective proteins), activating mitophagy, autophagy, disrupting lysosomal stability and producing reactive oxygen species [38].

Isolated lipofuscin aggregates containing elevated concentrations of proline, calcium, and iron cause cellular damage via a pyroptosis-like mechanism. Lipofuscin activates mitochondrial ROS production and induces lysosomal dysfunction via alterations in the lysosomal membrane, resulting in

decreased lysosome numbers and impaired cathepsin D activity. CTSD gene deficiency is considered to be the main cause of neuronal lipofuscinosis [39].

Necrosis. It was believed that necrosis in stroke, traumatic brain injury, has no genetic regulation. Modeling of neuronal injury by calcium overload revealed that JIL-1/mitogen — and stress-activated protein kinase-1, 2 are regulators of neuronal necrosis through phosphorylation of serine 28 of histone H3 (H3S28ph). In addition, activation of Trithorax (Trx) was identified. To test the role of the JIL-1/PRC1/Trx cascade in mammals, glutamate-induced necrosis was studied in rat cortical neuron cultures and in models of cerebral ischemia. It was found that the cascade is activated under these conditions, and inhibition of the cascade suppresses necrosis both *in vitro* and *in vivo* [40].

It has been established that responses to damage, including DNA repair, DNA methylation and autonomous mechanisms of neuronal necrosis, are different in humans and experimental animals [41].

RNA plays an important role in the mechanisms of neuronal damage. RNA-binding proteins are crucial for the regulation of RNA processing and transport. RNA processing defects are increasingly recognized as critical determinants of neurological diseases. These mechanisms underlie changes in neuronal function, increased susceptibility of neurons to the influence of damaging factors, changes in RNA expression, where RNA-binding proteins play a central role in maintaining neuronal function and morphology [42].

One of the variants of necrotic damage to neurons is ferroptosis, which develops when the balance of free iron and lipid peroxidation is disrupted. This process leads to the disruption of the structure and functions of plasma membranes. Morphological manifestations of ferroptosis in nervous tissue are a decrease in the number of mitochondria, compaction, and (or) disorganization of the cristae mitochondria, rupture of their outer membrane, and an increase in the density of internal membranes. The etiological factor of ferroptosis is a deficiency of GPx4 (Glutathione peroxidase 4, glutathione peroxidase 4), which is necessary for the reduction of toxic lipid peroxides. In ischemic stroke, when blood flow to the brain is limited, there is increased iron absorption, oxidative stress, and disruption of the integrity of the blood-brain barrier. An imbalance of proteins involved in the transport and storage of iron increases lipid oxidation and contributes to neuronal damage, indicating the possibility of brain cell death, especially neurons, as a result of ferroptosis [43].

Phagocytosis of neurons (neuronophagy). Following stroke, neurons undergo damage due to ongoing ischemia and excitotoxicity. They release «find me» (ATP), «eat me» (phosphatidylserine) sig-

nals that bind to the opsonins complement components C1q and C3b, inducing microglia to phagocytose damaged neurons. Blocking these neuronal factors or microglial phagocytic receptors prevents delayed neuronal loss and behavioral impairment in rodent models of ischemic stroke [44].

Microglia engulf and remove opsonized and non-opsonized targets such as pathogens, apoptotic cells, and cellular debris. Phagocytosis is also critical in neural development, homeostasis, and repair mechanisms [45]. In addition to the microglia, astrocytes have been shown to respond to neuronal injury [46].

Apoptosis. There are two types of apoptosis: extrinsic (caspase-dependent) and intrinsic (non-caspase-related). The intrinsic pathway is triggered by intracellular factors that detect DNA damage, the presence of viral pathogens, or respond to the absence of external signals from other cells. In contrast, the extrinsic pathway of apoptosis is triggered by an external signal, most often from natural killer (NK) cells or CD8-positive cytotoxic T lymphocytes (CTLs). The extrinsic pathway of apoptosis induction begins with the binding of specific ligands (TNF α , FASL, etc.) to plasma membrane receptors whose cytoplasmic regions contain death domains. The resulting complex of molecules is called the Death Inducing Signaling Complex (DISC). Subsequent activation of caspase-8 leads to activation of effector caspases 3 and 7 and subsequent irreversible cell damage [47].

The internal triggering of apoptosis is associated with mitochondria, changes in their membrane potential and the release of pro-apoptotic proteins of the Bcl-2 family. It depends on the activation and release of cytochrome C, flavoprotein AIF (apoptosis-inducing factor), and procaspase-2 into the cytoplasm. The causes of the release of intracellular apoptotic signals by a damaged cell are varied: hypoxia, radiation, viral infection, increased intracellular calcium concentration, etc. Mitochondria also play an important role in other forms of cell damage: pyroptosis, ferroptosis and necroptosis [48].

Conclusion

The proposed morphological classification of neuronal damage is based on the structural changes in nerve cells detected by histological, histochemical and immunohistochemical methods.

Taking into account the unity of structure and function, we examined functional disorders that arise during neuron alteration. In particular, the displacement of the nucleus is caused by damage to the cytoskeleton of neurons. Morphological changes in the nucleoli are associated with a violation of the synthesis of ribosome subunits. A decrease or absence of staining of the Nissl substance indicates a violation of the synthesis of ribonucleoproteins.

Dark neurons indicate the accumulation of protein products in the cytoplasm of cells and a violation of their utilization. Chromatin remodeling leads to violations of DNA repair, synthesis processes, etc. Lipofuscin is a marker of neuronal damage, activates apoptosis, damages lysosomes. Calcinosis promotes the formation of active oxygen species. Neuronopha-

gia, necrosis, and apoptosis are irreversible forms of neuronal damage.

The proposed morphological classification of neuronal damage complements existing classifications that primarily take into account molecular mechanisms of damage.

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