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STUDY OF GUNSHOT INJURIES FEATURES OF PERIPHERAL NERVES BY MODERN WEAPONS IN THE EXPERIMENT

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The article shows the study results of the sciatic nerve pathomorphosis characteristics in gunshot wounds in experimental animals (pigs). Changes in the structure of nerve sheaths, its fascicles, features of nerve blood vessel injury were studied under optical magnification with digital recording of nerve histological sections. The ultrastructural changes of the sciatic nerve during the simulation of a gunshot wound of the peripheral nerve both with its anatomical section and with the preservation of anatomical integrity were studied. Histological examination of the sciatic nerve damaged by bullets of the 5.45 calibre showed significant morphological changes in the nerve structure both in case of anatomical section of the nerve and in case of its contusion. It has been proven that even if the anatomical integrity of the nerve is preserved, pronounced changes in the nervous tissue occur with a gunshot wound.

Key words: gunshot wound, nerve, pig, experiment.

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ВИВЧЕННЯ ОСОБЛИВОСТЕЙ ВОГНЕПАЛЬНИХ УШКОДЖЕНЬ ПЕРИФЕРИЧНИХ НЕРВІВ СУЧАСНИМИ ВИДАМИ ОЗБРОЄННЯ В ЕКСПЕРИМЕНТІ

У статті наведено результати дослідження особливостей патоморфозу сідничного нерва при вогнепальних пораненнях експериментальних тварин (свиней). Вивчали зміни структури оболонок нерва, його фасцикул, особливості ушкодження кровоносних судин нерва під оптичним збільшенням із записом на цифрову камеру гістологічних зрізів нерва. Досліджено ультраструктурні зміни сідничного нерву при моделюванні вогнепального поранення периферичного нерву як із його анатомічним перетином, так і зі збереженням анатомічної цілісності. При гістологічному дослідженні сідничного нерва, ушкодженого кулями калібру 5,45, виявлено значні морфологічні зміни в структурі нерва як при анатомічному перетині нерва, так і при його контузії. Доведено, що навіть за умови збереження анатомічної цілісності нерва, при вогнепальному пораненні виникають виражені зміни нервової тканини.

Ключові слова: вогнепальне ушкодження, нерв, свиня, експеримент.

The study is a fragment of the research project: "To study the clinical and neurophysiological features of the remote consequences course of gunshot injuries to peripheral nerves", state registration No. 0122U000328.

Gunshot injuries of peripheral nerves are separate from combat trauma, occupying a special place in the structure of general traumatism, as they cause high rates of disability and significantly reduce the quality of life of the wounded.

Gunshot wounds of the upper and lower extremities are often accompanied by concomitant damage to peripheral nerves (PN), which causes permanent loss of motor and sensory functions [3, 4, 13, 15]. The mechanism of nerve damage itself is multifactorial and, as is commonly believed, is caused by a combination of direct and indirect effects of traumatic factors (high temperature, shock wave). As a result, a clear choice of treatment algorithms is often impossible [14, 15]. Unfortunately, there is no reliable method of distinguishing between neuropraxia, axonotmesis, and neurotmesis on clinical examination, and although neuropraxia is thought to be the most common, a number of large published studies report a high incidence of complete disruption of the anatomical integrity of the nerve following gunshot injury [2, 14].

When considering the structure of traumatic injuries, PN injuries of the upper and lower extremities make up about 6 %, but according to indicators of permanent disability, they take the first place. In the case of damage to the limbs, severe moral and material losses occur in the daily life of patients, as the affected persons become unable to work. When studying modern research of scientists who study combat trauma of limbs [15], gunshot wounds of peripheral nerves in peacetime make up 7.1 % of the total structure of their injuries. However, at present, when there are armed conflicts in the world (including the war in Ukraine), this indicator is much higher. This index is affected by the fact that the limbs are one of the most unprotected parts of the body (taking into account the presence of body armor, helmets, etc.).

The effectiveness of the surgical treatment of peripheral nerves combat trauma is worse compared to the classical nerve injury, which is mainly due to the prevalence of damage to the nerve trunk, its nature, the presence of intraneural scars, disruption of vascular microcirculation in the structure of the nerve, as well as due to existing wounding of the main vessels and significant damage to the surrounding soft tissues. The presence of modern equipment and means of microsurgical treatment also significantly affect the results of surgical treatment, and the level of training of the surgeon is also important [15].

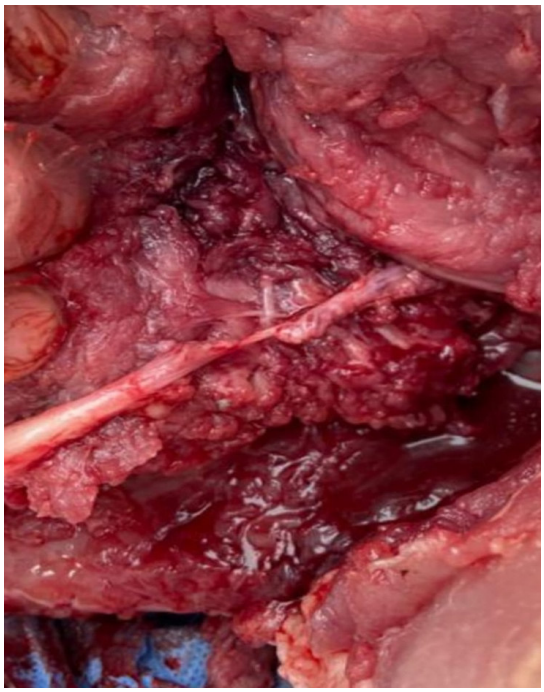
Therefore, a thorough approach is necessary for a complete understanding of the pathological processes in the nervous tissue in case of gunshot injury of peripheral nerves in order to optimize the existing and develop new treatment and diagnostic algorithms for the management of this patients' category. This can be achieved by conducting an experiment on animals using modern firearms.

Fig. 1. General appearance of the cartridge: 1 – 5.45x39 mm a cartridge with "PS" bullets with a steel core.

The purpose of the study was to establish histological results of the damaged nerve research as a result of simulating a gunshot wound to the sciatic nerve with the use of modern weapons in the experiment.

Materials and methods. The experimental study was performed on 3 healthy pigs of the Svitlogorsk breed (a series of studies). The research was conducted on a military training ground (Ministry of Defence training ground, Kyiv region) under normal environmental conditions (temperature 19°C, relative humidity 72 %, atmospheric pressure 760 mm Hg). One shot from an automatic firearm AKS-74 from a distance of 100 meters was performed to the intended colour point on the on the animal's thigh, corresponding to the anatomical projection of the sciatic nerve. A 5.45x39 mm PS cartridge was used during the experiment (fig. 1).

The acute experiment was performed under general anaesthesia in compliance with all bioethical requirements for animal experiments of the European Convention for the Protection of Vertebrate Animals for Research and Other Scientific Purposes, Strasbourg, 18 March 1986. Permission of the UVMA Bioethics Commission No. 5 dated 15.05.2021. Removal of experimental animals from the experiment was carried out by overdose (euthanasia) administration of 15 mg/kg, Sodium thiopental in a lethal dose. The dead animals were cremated after the examination. Fragments of selected damaged sciatic nerves of pigs were taken for histological examination, both in the case of simulation of the anatomical section of the nerve (fig. 2 A, fig. 2 B), and simulation of nerve contusion (without its anatomical section).



A

B

Fig. 2. Macrophotos of injured sciatic nerve: A – Visualized left sciatic nerve after a gunshot wound; B – Macropreparation: selected left sciatic nerve with gunshot injury.

A histological examination of 2 nerve trunks of a pig, which were damaged by 5.45 bullets, was carried out. The material was collected after the death of the animals. The first sciatic nerve from the samples had a thickness of 5 mm and a marginal defect for ¾ of the thickness of the nerve within 1 cm. Another sample had a thickness of 4 mm without visible damage from the outside. The material was fixed in 10 % buffered formalin (pH 7.4) for 48 hours, dehydrated and sealed in paraplast [1]. Histological sections 4 μm thick stained with hematoxylin and eosin were made from the obtained blocks.

Histological sections were examined visually and photographed using an Olympus B53 microscope with an SP180 digital camera (12214x920 RGB pixels).

Limitations of the study: in general, the small number of experimental animals during the study, which led to the lack of opportunity to distribute the participants and form groups based on similar characteristics, makes it impossible to conduct any reliable statistical analysis.

Results of the study and their discussion. In general, the investigated nerves had a normal structure. They consisted of bundles of nerve fibers surrounded by endoneurium. They were complexed into bundles of the second order, which were complexed into the nerve trunk itself. The latter was surrounded by epineurium.

In the case of partial destruction of the nerve trunk by a bullet at the site of the injury, preservation of a small peripheral part of bundles of nerve fibers adjacent to the epineurium was observed (fig. 3 A). The edges of the destructive bundles of nerve fibers (fig. 3 A, 3 B) had unclear borders, fragments of torn nerve bundles. Signs of minor hemorrhages were observed. Directly near the edge of the torn bundles of nerve fibers, venous blood is observed, while the capillaries and arterial-type vessels appear empty (fig. 3 C). Phenomena of diapedesis of erythrocytes were observed (fig. 3 D).

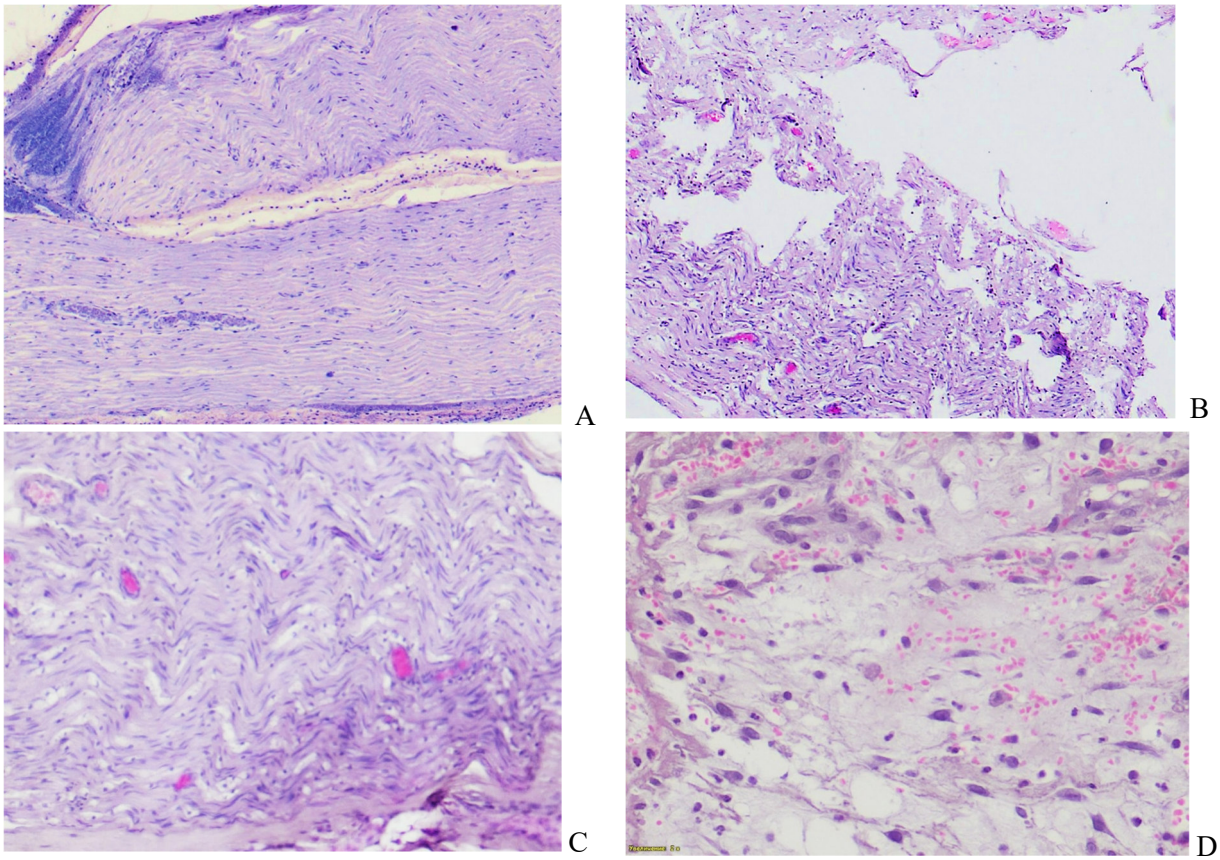


Fig. 3. Microphotos of the partially destroyed nerve section: A. The central part. Moderate swelling of the perineurium is noted. Expansion and overflow of blood veins. Staining with hematoxylin and eosin. Microphoto, 100 x; B – Peripheral part of the partially destroyed nerve section. Ruptures of bundles of nerve fibers, their fragmentation. Expansion and overflow of small veins with blood. Staining with hematoxylin and eosin. Microphotograph, 100x; C – Venous full blood, empty capillaries and arterial type vessels. Phenomena of diapedesis of erythrocytes were observed. Staining with hematoxylin and eosin. Microphoto, 200 x; D – Phenomena of diapedesis of erythrocytes near the ends of torn bundles of nerve fibers. Staining with hematoxylin and eosin. Microphoto, 400x.

Under conditions of the nerve trunk contusion, continuity of its constituent components along the length is observed. There are separate hemorrhages in the epineurium (fig. 4 A). In the thickness of the nerve, slight disorganization of nerve fibers with areas of their enlightenment was observed (fig. 4 B). Small veins were filled with blood. Capillaries and small intratruncal arteries appeared empty. On one part of the nerve, a transverse rupture of a bundle of nerve fibers was found (fig. 4 C), which looked like a gap

with uneven edges and small tissue fragments. At the edges of the defect, it was possible to observe moderately expressed phenomena of diapedesis of erythrocytes. Small hemorrhages were also found in the thickness of the nerve (fig. 4 D). Individual or groups of tissue basophils were found in distant parts of the nerve, part of which was destroyed, and in the nerve that had suffered a contusion. Most of them showed minimal signs of degranulation (fig. 4 E). This was manifested by the detection of a small number, or even single granules, outside the cells of this type.

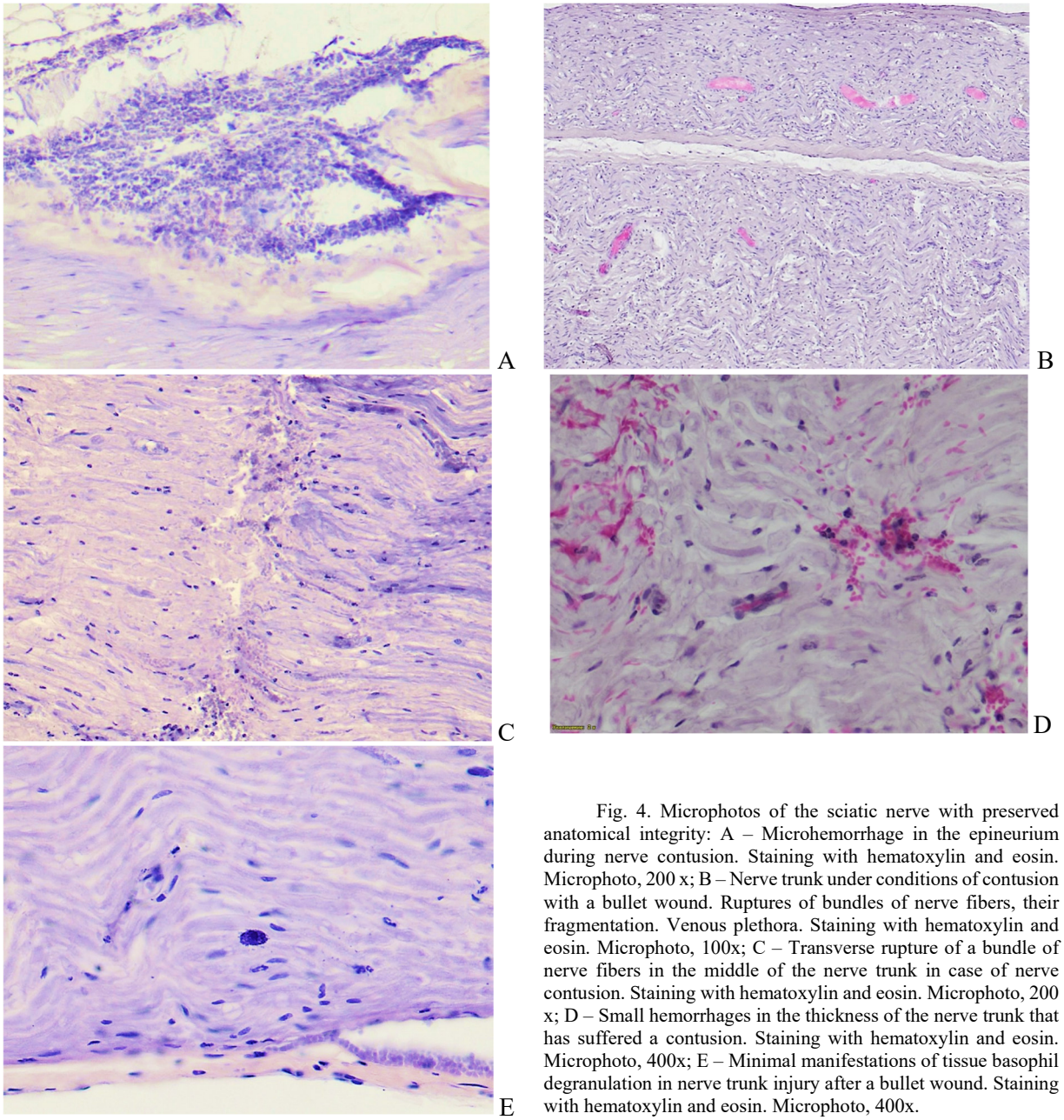


Fig. 4. Microphotos of the sciatic nerve with preserved anatomical integrity: A – Microhemorrhage in the epineurium during nerve contusion. Staining with hematoxylin and eosin. Microphoto, 200 x; B – Nerve trunk under conditions of contusion with a bullet wound. Ruptures of bundles of nerve fibers, their fragmentation. Venous plethora. Staining with hematoxylin and eosin. Microphoto, 100x; C – Transverse rupture of a bundle of nerve fibers in the middle of the nerve trunk in case of nerve contusion. Staining with hematoxylin and eosin. Microphoto, 200 x; D – Small hemorrhages in the thickness of the nerve trunk that has suffered a contusion. Staining with hematoxylin and eosin. Microphoto, 400x; E – Minimal manifestations of tissue basophil degranulation in nerve trunk injury after a bullet wound. Staining with hematoxylin and eosin. Microphoto, 400x.

Thus, the conducted observations showed that a gunshot wound with a 5.45 bullet can lead both to a direct violation of the integrity of the nerve trunks and to their contusion. The degree of mechanical damage to the nerve trunk directly depends on the contact with the bullet and the hydrodynamic impact. The above data coincide with the experience of [2, 6, 5, 9] publications. In general, the changes in the nerves immediately after the injury can be described as “shock nerve”. This is manifested by a sharp anemia of the intra-truncal arterial vessels and capillaries, and a sharp expansion and overflow of blood in the veins. Gunshot injuries are accompanied by minor hemorrhages, with spasm of blood vessels in the area of the injury, occurring within a few minutes after the trauma. Despite a very short period of time after the injury, a reaction of tissue basophils was detected, which was presented by the initial manifestations of their degranulation.

When conducting the experiment, we proved that there is an unevenness of nerve tissue damage taking into account the so-called distant factors. We could observe changes in the nerve fascicles structure

depending on the distance of the damaging factor direct impact (1 mm, 2 mm, 3 mm, etc.). Accordingly, the farther from the nerve tissue is the zone of the factors action affecting the firearm projectile, the less pronounced are the changes. Although, as stated above, it was possible to observe structural changes in the nervous tissue in more distant areas from the injury zone. In the case of preserving the anatomical integrity of the nerve, but in the presence of a contusion, attention is paid to the length and severity of the changes. Parabiotic and necrotic changes occur in the tissues around the nerve, which negatively affect the efficiency of reinnervation and, accordingly, the final result of surgical treatment.

An important role in the recovery of the peripheral nerve is played by the condition of the nerve bed (mesoneurium). Irreversible damage occurs in the bed around the nerve, which creates unfavorable conditions for regeneration, therefore, to stimulate the recovery processes, it is recommended to use a set of measures aimed at accelerating the recovery of the nerve. Therefore, interventions directly on the nerve itself should also include the reconstruction of the mesoneurium through the use of regenerative technologies (lipofilling and bone marrow aspirate), or transplantation of fat, skin-muscle, or skin-fascial flaps. Given the irreversible changes and microcirculation disorders, in this category of patients, the delayed method of neurorrhaphy should be considered as an option, so that later, as a second stage, other surgical methods can also be used (including the method of primary nerve grafting).

The data we received coincide with the experience of scientists [7, 8, 9, 11, 12] when conducting similar experimental studies and comparing them with clinical data.

It is necessary to take into account all the features of the pathomorphosis of a gunshot wound to nerves, which will determine and personalize the tactics of intervention for each patient for obtaining better recovery rates.

Conclusions

1. During the histological examination of gunshot wounds to the nerves, phenomena of severe irreversible damage were observed in the nerve tissues: ruptures of bundles of nerve fibers and their fragmentation, areas of venous full blood and empty capillaries and vessels of the arterial type, phenomena of erythrocyte diapedesis;

2. Phenomena of the so-called “shock nerve” in case of a gunshot injury - sharp anemia of intra-truncal arterial vessels and capillaries, overflowing of veins with blood had a spread of at least 8-12 mm from the place of direct injury. Such changes in the nerve trunk confirm the need to use delayed reinnervation.

3. The conducted experiment confirmed the severity of changes in the structure of the nerve trunk and paraneural tissues in case of a gunshot wound, especially pronounced changes in microcirculation, even with the preservation of the anatomical integrity of the injured nerve.

References

1. Hrabovyi OM, Prosha MV. Izopropanol-tseloyidno-parafinovyy metod rozlyvu materialu dlya histolohichnykh doslidzhen. Ukrainian Journal of Medical Engineering and Technology, 1994; (1-2): 44–47. [in Ukrainian]

2. Mikhaylusov R, Negoduyko V, Nevzorov V, Nevzorova O, Denysiuk T. Dinamika izmeneniya makrostruktury makrofagalnykh kletok ranevogo kanala posle ognestrelnogo raneniya. Georgian Med News. 2019 Sep;(294):136–141. PMID: 31687966. [in Russian]

3. Tsymbaliuk VI, Gayko GV, Strafun SS, Lyabakh AP, Grytsai MP, Buriianov OA, et al. Likuvannia poranenykh z boiovymy travmamy kintsivok (za dosvidom ATO/OOS): monografiia. Kyiv: PAT “Desna”, 2020. 196 p. [in Ukrainian]

4. Berg RJ, Okoye O, Inaba K, Konstantinidis A, Branco B, Meisel E, et al. Extremity firearm trauma: the impact of injury pattern on clinical outcomes. Am Surg. 2012 Dec;78(12):1383–7. PMID: 23265128.

5. Humphrey C, Henneberg M, Wachsberger C, Maiden N, Kumaratilake J. Effects of Re-heating Tissue Samples to Core Body Temperature on High-Velocity Ballistic Projectile-tissue Interactions. J Forensic Sci. 2017 Nov;62(6):1466–1471. doi: 10.1111/1556-4029.13473. Epub 2017 Feb 23. PMID: 28230904.

6. Jin Y, Haitao L, Cheng W, Wang X, Han R, Li R, Dong D. The experimental and numerical investigation on the ballistic limit of BB-Gun pellet versus skin simulant. Forensic Sci Int. 2019 May;298:393–397. doi: 10.1016/j.forsciint.2019.02.033. Epub 2019 Mar 15. PMID: 30947143.

7. Jones PE, Meyer RM, Faillace WJ, Landau ME, Smith JK, McKay PL, Nesti LJ. Combat Injury of the Sciatic Nerve - An Institutional Experience. Mil Med. 2018 Sep 1;183(9-10):e434–e441. doi: 10.1093/milmed/usy030. Erratum in: Mil Med. 2018 Sep 1;183(9-10):246. PMID: 29590419.

8. Kerkhoff W, Mattijssen EJAT, Zwanenburg EA, Oostra RJ. Relationship between bullet diameter and bullet defect diameter in human calvariums. Int J Legal Med. 2020 Jan;134(1):267–272. doi: 10.1007/s00414-019-02197-9. Epub 2019 Nov 16. PMID: 31734727; PMCID: PMC6949314.

9. Khomenko IP, Tertyshnyi SV, Vastyanov RS, Talalayev KO, Turchin MI, Panasenko SI. Experimental background of thermometry using with diagnostic purposes in soft tissue gunshot damages. World of Medicine and Biology. 2022;1(79):237–243. doi: 10.26724/2079-8334-2022-1-79-237-243

10. Mahoney PF, Carr DJ, Miller D, Teagle M. The effect of helmet materials and simulated bone and tissue layers on bullet behaviour in a gelatine model of overmatch penetrating head injury. Int J Legal Med. 2017 Nov;131(6):1765–1776. doi: 10.1007/s00414-017-1665-8.

11. Mathieu L, Alqassab S, Fakhi RM. Sniper-induced sciatic nerve injury. BMJ Mil Health. 2020 Jun;166(3):210. doi: 10.1136/jramc-2019-001269.
12. Mikhaylusov R, Negoduyko V, Pavlov S, Oklei D, Svyrydenko L. Dynamics of ultrastructural rearrangements of skeletal muscle fibroblasts after simulated gunshot shrapnel wounds. Georgian Med News. 2021 Nov;(320):157–161.
13. Pannell WC, Heckmann N, Alluri RK, Sivasundaram L, Stevanovic M, Ghiassi A. Predictors of Nerve Injury After Gunshot Wounds to the Upper Extremity. Hand (N Y). 2017 Sep; 12(5): 501–506. doi: 10.1177/1558944716675294.
14. Secer H, Daneyemez M, Tehli O, Gonul E, Izci Y. The clinical, electrophysiological, and surgical characteristics of peripheral nerve injuries caused by gunshot wounds in adults: a 40-year experience. Surg Neurol. 2008; 69: 143–152.
15. Tsymbaliuk VI, Strafun SS, Tretyak IB, Tsymbaliuk IV, Gatskiy AA, Tsymbaliuk YV, et al. Surgical treatment of peripheral nerves combat wounds of the extremities. Wiad Lek. 2021; 74 (3 cz 2): 619–624. doi: 10.36740/WLek202103210

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ULTRASTRUCTURAL CHANGES OF PINEALOCYTES OF THE PINEAL GLAND UNDER THE ACTION OF METHYLENE BLUE IN RATS OF DIFFERENT AGES

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The purpose of the study was to investigate the ultrastructural changes of the pineal cells of the pineal gland in normal conditions and under the conditions of long-term exposure to methylene blue. In 14-day-old intact rats, the pineal gland is represented as a formed organ. With age, in 45- and 90-day-old animals, the ultrastructural state of the cytoplasm and nucleus of pinealocytes corresponded to increased activity, but was less pronounced than in 14-day-old animals. Under the effect of methylene blue, in the pineal gland of 14- and 45-day-old rats, structural signs of weakly expressed enhancement of the function of light and dark pinealocytes were noted. In 90-day-old animals, under the effect of methylene blue in the pineal gland, along with the enhancement of the function of light pineal cells, signs of normalization of structure and function were noted in dark ones, which is obviously connected with the inclusion of adaptive mechanisms to the effect of methylene blue. The obtained results open the prospect for further study of the patterns of structural and functional changes of the endocrine glands under the conditions of nitrate intoxication and represent the basis for the development of effective measures for the correction of endocrine system lesions that occur in people as a result of professional activity.

Key words: gland, endocrine system, intoxication, nitrate poisoning, correction.

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УЛЬТРАСТРУКТУРНІ ЗМІНИ ПІНЕАЛОЦИТІВ ЕПІФІЗА ЗА УМОВ ДІЇ МЕТИЛЕНОВОГО СИНЬОГО У ЩУРІВ РІЗНОГО ВІКУ

Метою дослідження було вивчення ультраструктурних змін пінеальних клітин епіфіза в нормі та за умов тривалого впливу метиленового синього. У 14-добових інтактних щурів епіфіз представлений як сформований орган. З віком у 45- і 90-добових тварин ультраструктурний стан цитоплазми і ядра пінеалоцитів відповідав підвищеній активності, однак менш виражено, ніж у 14-добових тварин. За умов дії метиленового синього в епіфізі 14-, 45-добових щурів відмічались структурні ознаки слабо вираженого посилення функції світлих і темних пінеалоцитів. У 90-добових тварин при дії метиленового синього в епіфізі поряд з посиленням функції світлих пінеальних клітин, в темних відмічались ознаки нормалізації структури і функції, що очевидно пов'язано з включенням адаптаційно-пристосувальних механізмів до дії метиленової сині. Отримані результати відкривають перспективу подальшого вивчення закономірностей структурно-функціональних змін залоз внутрішньої секреції в умовах дії нітратної інтоксикації та представляють підґрунтя для розробки ефективних заходів корекції уражень ендокринної системи, що виникають у людей у результаті професійної діяльності.

Ключові слова: залоза, ендокринна система, інтоксикація, отруєння нітратами, корекція.

The study is a fragment of the reseach project "Histophysiological state of the endocrine system under the effect of adverse environmental factors", state registration No. 0120U002026.

It is known that the pineal gland, or epiphysis (pineal gland or upper brain appendage) is a small oval glandular formation that belongs to the diencephalon and is located in a shallow sulcus between the upper tubercles of the midbrain above the thalamus. According to data [1], the pineal gland closely cooperates with all diencephalic elements, implementing its humoral connections not only through blood, but also through cerebrospinal fluid. The ventricular system of the brain permits the humoral signals of the