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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии
საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებშიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman (Кириллица)**, для текста на грузинском языке следует использовать **AcadNusx**. Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи.** Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - <http://www.spinesurgery.ru/files/publish.pdf> и http://www.nlm.nih.gov/bsd/uniform_requirements.html. В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректур авторам не высылаются, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or computer-printed on a single side of standard typing paper, with the left margin of 3 centimeters width, and 1.5 spacing between the lines, typeface - **Times New Roman (Cyrillic)**, print size - 12 (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf

In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

**Articles that Fail to Meet the Aforementioned
Requirements are not Assigned to be Reviewed.**

ავტორთა საყურადღებო!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე, დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx**. შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემავსებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრაფიის ფოტოსურათები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შედეგების ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფხიხლებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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CHANGES IN THE MORPHOLOGICAL AND FUNCTIONAL STATE OF HYPOTHALAMUS NUCLEI NEURONS IN LONG-TERM CRUSHING SYNDROME

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Abstract.

Crush syndrome (CS) is a severe stress-induced condition that leads to systemic metabolic and neuroendocrine disturbances. The hypothalamus, serving as a central integrator of neuroendocrine and autonomic regulation, is particularly sensitive to prolonged stress. In this context, we examined neuronal changes in the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus in rats subjected to prolonged hind limb compression. To assess the dynamics of the morphological and functional state of hypothalamic neurons after limb compression, we employed histochemical detection of Ca²⁺-dependent acid phosphatase activity. Animals were randomly assigned to three groups: control (Co), compression for 3 hours (CS3), and compression for 6 hours (CS6). Experimental CS models were created using a specialized apparatus to apply hind limb compression for the designated durations. Brain tissue samples containing the SON and PVN were collected one hour after compression. The results demonstrated differential morphological responses in the SON and PVN following 3 hours of compression. SON neurons largely preserved their structural integrity, showing increased phosphatase activity in the neuron cytoplasm. In contrast, PVN neurons displayed pronounced cytoplasmic rarefaction and central chromatolysis. After 6 hours of compression, degenerative changes were observed in neurons of both nuclei, accompanied by a sharp rise in phosphatase activity in the neuron cytoplasm. This increase indicates enhanced metabolic processes that may support cellular regeneration and survival, reflecting the activation of neuroprotective mechanisms. Overall, these findings suggest that hypothalamic nuclei undergo distinct, time-dependent morphological and functional responses to crush syndrome, encompassing both compensatory and maladaptive processes.

Key words. Crush syndrome, prolonged compression syndrome, supraoptic nucleus, paraventricular nucleus, hypothalamus, Ca²⁺-dependent acid phosphatase.

Introduction.

Crush syndrome (CS), or prolonged compression syndrome, is a life-threatening condition with a reported mortality of 13–14% during natural disasters and technological accidents, making prompt treatment essential [1-4]. Patients with crush injuries frequently develop systemic inflammatory response syndrome and multiple organ failure, which are leading causes of death [5]. Sustained tissue compression in CS results in ischemia and degenerative–necrotic changes in the affected regions, producing severe pain and stress. Central nervous system (CNS) involvement is common, presenting as headache, anxiety, depression, cognitive impairment, and other neurological

symptoms. Furthermore, the stress associated with CS alters the function of the hypothalamic–pituitary–adrenal (HPA) axis, the central regulator of stress hormones such as cortisol, thereby contributing to systemic disturbances including CNS dysfunction [6,7].

In recent years, extensive evidence from both experimental models and clinical studies—with strong consistency across rodents, primates, and humans—has demonstrated that diverse stressors and adverse life events produce long-lasting anatomical and functional alterations in various brain structures. Particular attention has been directed to the temporal dimension of stress, namely its duration and timing, as critical determinants of the extent and persistence of neuronal changes [8,9].

The activation of neuroreflex and neurohumoral pathogenic mechanisms in the development of crush syndrome (CS) begins at the very onset of tissue compression, well before decompression occurs [7]. From the moment the compressive force is applied, the victim experiences pain, which triggers corresponding adaptive reflex mechanisms. In compression injuries, the excitatory phase is relatively prolonged, explained by the absence of blood loss and preserved cerebral circulation. Immediately after decompression, a short period of excitation is observed, followed by renewed inhibition, which manifests as more profound dysfunction of vital organs [10]. Within the compressed tissues, oxygen deficiency leads to the accumulation of partially oxidized metabolic products, the development of metabolic acidosis, and a marked increase in vasoactive substances [11]. Prolonged compression and sustained pain stimulation contribute to psychoemotional disturbances. Over time, partial adaptation to pain arises through inhibition of impulse transmission in the spinal ganglia. These processes result in altered function of internal organs and systems, weakened adaptive capacity, and disorganization of excitatory–inhibitory balance in the CNS. Furthermore, pain as a powerful afferent input activates the reticular formation, which in turn excites the cerebral cortex and transmits signals to the hypothalamus, a key center of humoral regulation [12]. Through the reticular formation, the sympathetic nervous system is stimulated, leading to adrenal medullary activation and the release of adrenaline into the bloodstream. Pituitary and adrenal hormones, via their vasopressor effects, induce spasm of peripheral blood vessels, resulting in elevated blood pressure and impaired tissue perfusion [10]. The hypothalamus is a brain region that regulates a wide range of physiological functions, including metabolism, thermoregulation, and the activity of both the nervous and circulatory systems [13]. It plays a central role in the neural regulation of endocrine functions, detecting the presence of stressors and initiating the stress response system.

Under the influence of afferent impulses, hypothalamic neurons produce neurosecretions containing polypeptide releasing factors [14]. These releasing factors stimulate the synthesis and secretion of pituitary adrenocorticotrophic hormone, which in turn drives a cascade of activations in both the adrenal cortex and medulla. During stress, the hypothalamus is activated not only by humoral factors—such as hypoxia, hypoglycemia, and interleukins—that reach it through neurohemal pathways, but also by cholinergic and serotonergic neural inputs from the limbic system and other regions of the central nervous system. Already in the early alarm stage of stress, the hypothalamus increases production of corticotropin-releasing factor (primarily in the dorsomedial magnocellular division of the PVN) as well as nonapeptides produced in the magnocellular divisions of the SON and PVN. These nonapeptides are secreted into the systemic circulation during stress [15]. The PVN is a key structure involved in stress responses to mediate adaptive and pathological responses of the organism. During chronic stress, the PVN coordinates the responses of multiple stress effector systems and may play a role in both adaptation and pathology of chronic stress [16].

The hypothalamus is responsible for the physiological manifestations of stress, and crush syndrome (CS), caused by prolonged compression of soft tissues, represents a powerful stressor capable of disrupting neuroendocrine regulation. Although the hypothalamus and limb crush injury are not directly connected, the severe stress or shock induced by CS can indirectly affect hypothalamic function, leading to secondary disturbances of the autonomic nervous system, such as mood fluctuations and sleep disorders. Thus, the consequences of crush syndrome may influence hypothalamic activity in an indirect manner [17].

Previous studies have demonstrated neurodegenerative changes, neuronal edema, and glial activation in brain structures exposed to stress [18]. However, detailed characterization of hypothalamic nuclei in the context of crush injury remains limited. Based on these considerations, the present study aimed to investigate the morphological and functional state of neurons in the SON and PVN nuclei of the rat hypothalamus following 3 and 6 hours of hind limb compression.

Materials and Methods.

Experimental Model:

Since experiments in humans are not feasible, researchers rely on animal models to gain a deeper understanding of the pathophysiology of crush syndrome. Animal models are effectively used to induce prolonged compression syndrome, accurately replicating the pathophysiological processes observed in humans affected by crush injuries during earthquakes, landslides, or other accidents and natural disasters [7,19,20].

In this study the experimental model of CS was established using a custom-built apparatus designed to apply localized compression to one pelvic limb. This model was reproduced on a specially engineered device developed for investigating various aspects of the pathogenesis and treatment of CS (Rationalization Proposal No. 158, 10.06.1990, issued by Yerevan Medical Institute). The apparatus was designed by the staff of the YMI

Research Center [21]. It consists of two test compartments of equal volume, separated by a thin partition. One compartment contains a compression mechanism, mirrored by an identical unit in the second compartment. The compression element is a circular plate with a diameter of 2 cm. In this study, a pressure of 140 kPa was applied to an area of 3.14 cm². The system allows for controlled, uniform application and release of pressure to the limbs of small laboratory animals (in particular rats), with the applied force recorded on a dynamometer.

Two models of crush syndrome (CS) were established to represent different degrees of severity: compression of a single limb for 3 hours (CS3 group) to model a mild form of CS, and compression of a single limb for 6 hours (CS6 group) to model a moderate form. The affected area encompassed the entire inner surface of the thigh (3.14 cm²). Control animals were placed in the apparatus for the same duration but without any applied load. Following the release of compression, decompression was performed over a 1-hour period.

Animals:

Experiments were conducted on sexually mature male albino rats (mean body weight: 200 ± 30 g). The animals were obtained from the Experimental Center of the L.A. Orbeli Institute of Physiology, NAS RA, and were maintained under standard laboratory conditions: a 12-hour light/dark cycle, ambient temperature of 22–24 °C, and relative humidity of 40–60 %. All rats had free access to food and filtered tap water. Feeding was provided using standard pelleted chow (Nutrimix STD-1020). All experimental procedures were performed in accordance with the principles of laboratory animal care as outlined in the 22 September 2010 decision of the Council of European Communities [2010/63/EU] and following the ARRIVE guidelines (Animals in Research: Reporting In Vivo Experiments). All applicable international, national, and institutional regulations for the care and use of animals were strictly observed.

Study Design:

A total of fifteen male rats were used in this study and randomly assigned to three experimental cohorts, with five animals in each group:

1. Rats without intervention, control group - **Co group** (n = 5);
2. Rats subjected to hind limb compression for 3 hours - **CS3 group** (n = 5);
3. Rats subjected to hind limb compression for 6 hours - **CS6 group** (n = 5).

The sample size of five animals per group was selected in accordance with the 3Rs principles (Replacement, Reduction, and Refinement) while ensuring sufficient statistical power for meaningful comparative analysis.

Histochemistry study.

Experimental procedure:

For the morphohistochemical analysis, control rats and rats from the CS3 and CS6 groups were euthanized after the decompression period by intraperitoneal injection of pentobarbital (100 mg/kg). Following confirmation of death, the

rat brains were carefully removed from the cranium. The areas containing the SON and PVN were then isolated from the whole brain. They were then fixed in a 5% neutral formalin solution prepared in 0.1 M phosphate buffer (PBS, pH 7.4) for 48 h at +4 °C. Frontal-plane sections of the target brain regions were cut at a thickness of 50–60 µm using a cryostat microtome (YD-2235, Jinhua YIDI Medical Appliance Co., Ltd., China). Sections were transferred to freshly prepared incubation medium for Ca²⁺-dependent AP detection, containing 0.4% lead acetate, 1 M acetate buffer (pH 5.6), and 2% sodium glycerophosphate. Incubation was carried out in a thermostat at 37 °C for 1.5 h. After incubation, sections were rinsed in distilled water, developed in sodium sulfate solution, rinsed again, and mounted in Canada balsam (VWR Chemicals, Canada). Morphological assessment was performed under a light microscope (OPTON M-35, West Germany). Topographical identification of brain structures was guided by established atlases [22,23]. Microphotographs were acquired using with an AmScope MU800 digital camera (AmScope Inc., Irvine, USA).

In the present work, for histochemical study, the method of detecting Ca-dependent acid phosphatase (AP) developed by Meliksetyan I.B. was used [24,25]. The method provides comprehensive neuronal labeling, encompassing both the soma and cellular processes. It is suitable for assessing the viability of neural cells within damaged CNS tissue or following neurodegeneration across various animal models. This method integrates elements of traditional Nissl staining and Golgi silver impregnation, enabling simultaneous visualization of neuronal cytoarchitecture and enzyme distribution patterns. As a result, it yields not only a detailed morphological representation but also valuable insights into the metabolic status of the examined structures. The histochemical technique employed here allows for inferences regarding the functional activity of the studied neuronal populations, as it is based on the principle of detecting chemically active groups within the tissue—specifically, the activity of Ca²⁺-dependent AP. All methodological requirements necessary for this principle have been carefully fulfilled. Enzymes in living organisms act as biocatalysts, facilitating metabolic reactions through active centers that convert specific substrates [26]. A key advantage of the present method lies in its high reproducibility, a critical criterion for methodological reliability. Histochemical assays of phosphatase activity are widely utilized in diverse areas of neurobiological research. The novelty of our approach resides in its ability to resolve the Ca²⁺-dependent mechanism through the deliberate selection of substrate and reagent concentrations. This optimization overcomes the limitations of the classical Gomori method, enabling the selective visualization of not only large but also small neurons, thereby ensuring more accurate neuronal identification. The resulting morphological picture occupies an intermediate position between that obtained with Nissl staining and that achieved with Golgi silver impregnation. Our findings emphasize the pivotal role of Ca²⁺ ions in the morphological and histochemical detection of CNS neurons. In the absence of Ca²⁺ within the incubation medium, the reaction fails to proceed in a complete and reproducible manner in mammalian tissue. When acid phosphatase activity is tested, phosphate ions released

by the enzyme react with various structures in the mixture, regardless of their spatial arrangement. After incubation in a sodium sulfide solution, these ions form a visible dark brown precipitate of lead sulfide. This staining provides a clear and informative image, allowing for detailed analysis of specific metabolic pathways within the examined structures.

Results.

Analysis of the morphohistochemical characteristics of hypothalamic nuclei in native rats (Co group) revealed that hypothalamic neurons exhibit notable polymorphism, reflecting the complex morphological and functional organization of this brain region. In the anterior hypothalamus, the paired supraoptic nuclei (SON) and paired paraventricular nuclei (PVN) are composed of large cholinergic neurosecretory cells with oval or spherical somata, containing prominent clumps of tigroid substance and exhibiting a very high level of metabolic activity (Figure 1 A, B and Figure 2 A–C). Secretory granules were observed in the somata and processes of SON neurons. The magnocellular neurons of this nucleus were so intensely stained that they appeared homogeneously colored (Figure 1 a).

The PVN is similar in cellular composition to the SON and is located along the third ventricle (Figure 2 a). In addition to large cholinergic magnocellular neurosecretory neurons, the PVN also contains smaller neurons, which in rats are typically positioned more medially than the magnocellular neurons (Figure 2 A–C). Enzymatic activity in these small neurons was weaker (Figure 1 A, B and Figure 2 A, C). Unlike the SON, the PVN contains triangular-shaped cells with long axons and several moderately or sparsely branched processes. Lead phosphate deposits in the form of small granules were clearly visible in both the cytoplasm and processes. The nuclei of neurons in both hypothalamic nuclei were lightly stained and centrally located within the cytoplasm (Figure 1 A, B and Figure 2 A–C).

The morphological profile of the SON following 3 hours of compression (CS3 group) was characterized by increased phosphatase activity in the cytoplasm compared to control animals (Co group; Figure 1 C, D). No significant changes in cell density or shape were observed in SON neurons. Most cells retained normal morphology, although occasionally hypertrophied neurons with extended processes were noted. In these swollen neurons, hyperchromatic nuclei were visible within the lightly stained perikaryal cytoplasm, indicating heightened cellular activity. Importantly, the nuclei in all neurons remained centrally located. This morphological pattern reflects the active functional state of SON neurons. Both long and short processes of all neurons responded, exhibiting small granules along their lengths. Long processes were traceable at considerable distances from the cell body within the plane of section, indicating preserved intercellular contacts within the SON (Figure 1 C).

After 3 hours of compression (CS3 group), neurons in the PVN exhibited diverse morphological patterns. Chromatolytic processes of varying degrees predominated, ranging from mild cytoplasmic clearing to nearly complete chromatolysis (Figure 2 D–F). Occasionally, clusters of hypertrophied neurosecretory cells were observed, in which enlarged hyperchromatic nuclei

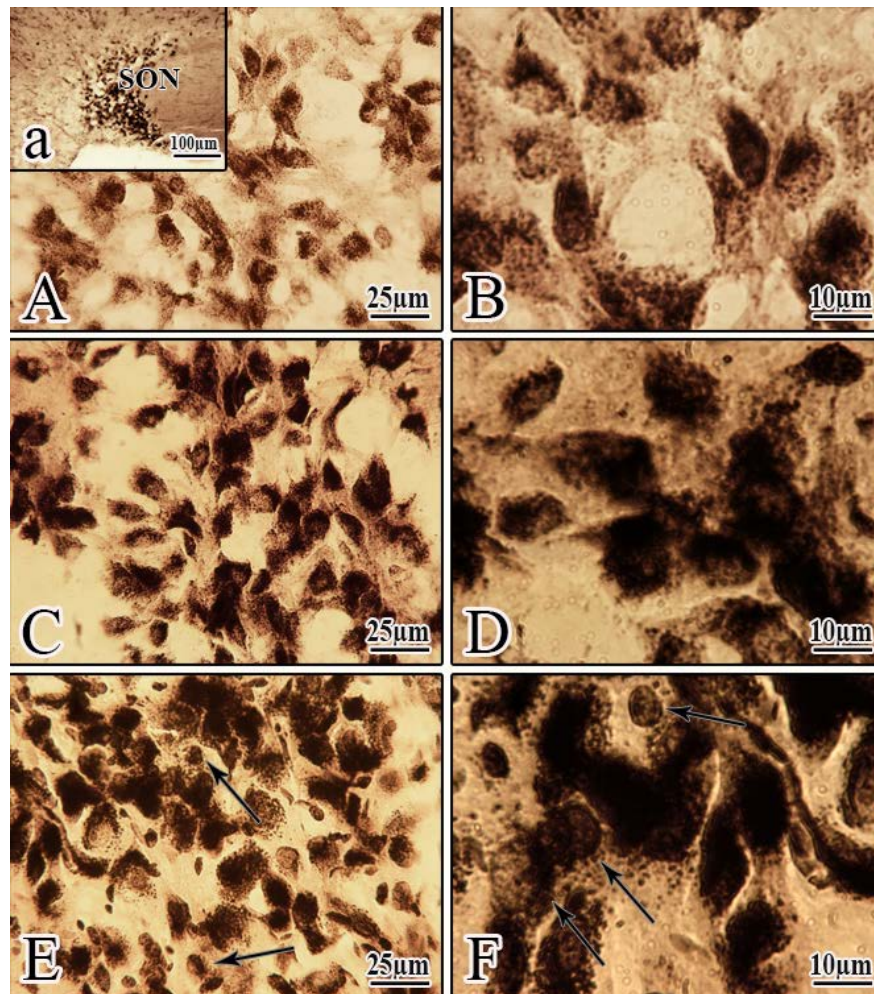


Figure 1. Micrographs of neurons in the rat supraoptic nucleus (SON) of the hypothalamus of the control group (A, B), after 3-hour (C, D), and 6-hour (E, F) hind limb compression. C and D show neurons with predominantly normal morphology, with occasional hypertrophied neurons exhibiting visible processes and hyperchromatic nuclei. E and F illustrate disrupted cell contours, neurons with such high enzymatic activity that subcellular structures are indistinguishable, thickened processes, and a tendency for neurons to cluster. Black arrows indicate central chromatolysis. Detection of Ca^{2+} -dependent acid phosphatase activity. Magnification: $\times 100$ (a); $\times 400$ (A, C, E); $\times 1000$ (B, D, F).

stood out against the lightly stained cytoplasm, indicating cellular activation and enhanced metabolic processes aimed at restoring homeostasis disrupted by stress. Notably, the nuclei in these swollen neurons remained centrally located. Processes were thickened near their points of origin from the soma or were entirely absent (Figure 2 D–F). In some magnocellular PVN neurons, changes characteristic of acute neuronal swelling were observed: cytoplasmic clearing, gradual dissolution of chromophilic substance, and enlargement of the cell body. Some cells became deformed, losing their typical oval shape, rounding up, with cytoplasmic clearing, and in certain neurons the apical process was swollen (Figure 2 E, F). Lead phosphate deposits were arranged either in a ring beneath the membrane or concentrated at one pole. Processes were poorly visible or entirely absent (Figure 2 D, E). Intensely stained, deformed neurons with indistinct nuclei and no processes were also noted (Figure 2 D). Triangular-shaped neurons underwent similar changes, with cytoplasmic clearing and somatic swelling; however, their long processes remained reactive, indicating preserved connectivity with other brain regions. Nuclei were

slightly enlarged but lightly stained and centrally positioned (Figure 2 F).

The morphological profile of the SON and PVN following 6 hours of hind limb compression in rats (CS6 group) was characterized by disrupted cell contours, with neurons losing their typical shape, becoming markedly swollen—hypertrophied—and, in some cases, showing unresponsive processes. Predominantly, swollen neurons were observed in clusters with thickened processes (Figure 1 E, F and Figure 2 G–I). The damage manifested as central chromatolysis. Compared with control animals, magnocellular SON neurons in injured rats exhibited such high phosphatase activity in the cytoplasm that the nucleus could not be distinguished from the cytoplasm, and nucleoli were difficult to identify (Figure 1 F). In the SON, against a background of intense staining characteristic of chronic hyperstimulation, granular precipitates were observed outside the cell membrane, surrounding the neuron; consequently, the cell membrane appeared prominent, with intensely stained contours and intermittently scattered granules of precipitate (Figure 1 E, F). Some neurons lost their typical

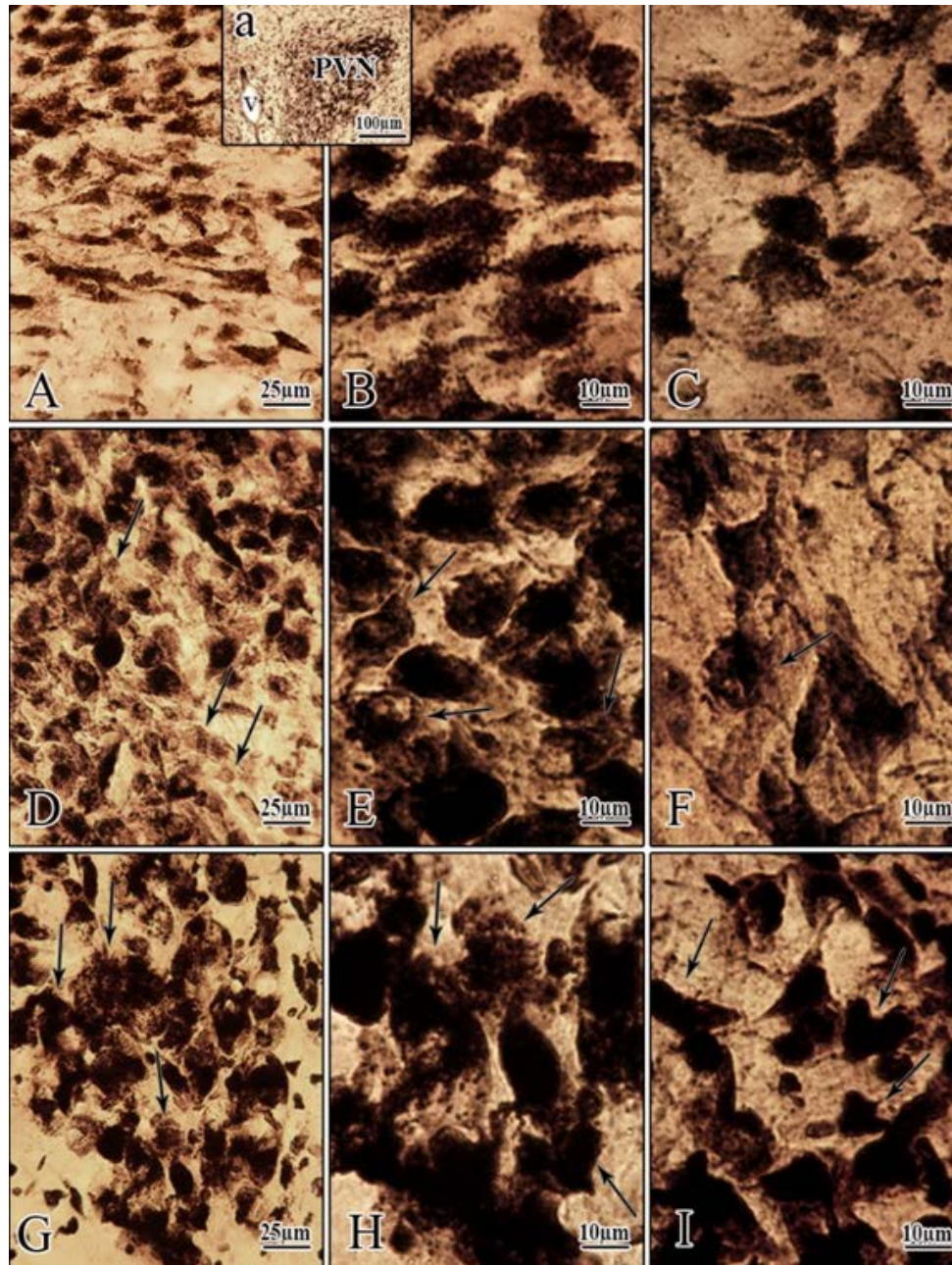


Figure 2. Micrographs of neurons in the rat paraventricular nuclei (PVN) of the hypothalamus of the control group (A–C), after 3-hour (D–F), and 6-hour (G–I) hind limb compression. D–F show deformed, hypertrophied neurons. In D and E, the neuronal nuclei exhibit high phosphatase activity, while most processes are unresponsive. In F, cytoplasmic clearing and cell body swelling are evident, with long processes still reactive. G and H illustrate swollen neurons with such elevated phosphatase activity that subcellular structures are no longer distinguishable. Cell morphology is disrupted, forming shapeless structures with indistinct, blurred contours; processes are absent, and there is a tendency for neurons to cluster. I - shows deformed neurons with increased cytoplasmic phosphatase activity and absent processes. Black arrows indicate central chromatolysis; v – third ventricle. Detection of Ca^{2+} -dependent acid phosphatase activity. Magnification: $\times 100$ (a); $\times 400$ (A, D, G); $\times 1000$ (B, C, E, F, H, I).

shape, appearing as shapeless structures with indistinct contours. Lead phosphate deposits in SON magnocellular neurons were coarse in some cells and fine-granular in others (Figure 1 F). These changes are characteristic of SON neurosecretory cells with high functional activity. Among the large neurons, smaller, rounded neurons were also observed. Their cytoplasm was lightly stained and acquired a light-brown hue, swollen nuclei were lightly colored with sharply defined nuclear envelopes due to peripheral accumulation of small phosphatase precipitate granules, indicating increased metabolism, and their dark-

stained nucleoli were often displaced (Figure 1 F). Such features are typically associated with primary neuronal excitation.

After 6 hours of compression (CS6 group), swollen PVN neurons exhibited such high phosphatase activity that subcellular structures were obscured by dark, diffuse staining (Figure 2 G, H). The cytoplasm was intensely stained, and lead phosphate deposits appeared as large aggregates. Nuclei and nucleoli were completely indistinguishable. The characteristic oval shape of the cells was lost, and the cell membranes appeared with indistinct contours. Neurons showed a tendency to cluster.

Severely hypertrophied and deformed PVN neurons often formed aggregations, around which foci of cellular depletion were observed (Figure 2 H). In brain sections, alongside the large neurons, numerous small cells were reactive, likely representing interneurons. Triangular-shaped neurons in the PVN were also affected, frequently losing their typical form and appearing as shapeless structures. Occasionally, shrunken, hyperchromatic neurons with no visible processes were observed. In most deformed neurons, the cytoplasm was intensely stained, and lead phosphate deposits formed large aggregates. Even in triangular neurons that retained their characteristic shape and size, phosphatase activity was extremely high, giving a homogeneous staining appearance, and long processes remained reactive (Figure 2 I).

Discussion.

In our study using a rat model of prolonged hind limb compression for 3 hours, both hypothalamic nuclei examined contained numerous closely apposed neurons, likely reflecting the activation of compensatory and adaptive properties. Morphological responses differed between the SON and PVN. Neuronal damage in these hypothalamic nuclei primarily manifested as central chromatolysis. Analysis of the magnocellular hypothalamic nuclei revealed both stereotypical and specific structural changes. In the SON, no significant alterations in cellular architecture were observed, aside from increased phosphatase activity in the cytoplasm and nuclei of hypertrophied neurosecretory cells, indicating enhanced metabolic activity. In PVN neurosecretory cells, changes characteristic of acute neuronal swelling were evident. Most PVN neurons were less intensely stained, reflecting slowed metabolic processes, although clusters of large neurons with intensely stained nuclei—indicative of heightened metabolic activity—were observed in certain regions. Following 3 hours of compression, PVN neurons likely experienced disrupted cellular respiration due to widespread metabolic activation in the perikarya, leading to reduced activity of several enzymes. Overall, a decrease in metabolic processes was noted. Cytoplasmic hypochromia of these cells likely reflects a decline in phosphatase activity, serving as morphological evidence of impaired metabolism [27].

Thus, prolonged limb compression for three hours induces more pronounced reactive changes in the PVN compared to the SON. This difference is likely attributable to the distinct functional roles of these hypothalamic nuclei within the HPA axis and fluid homeostasis, their receptor expression profiles, or the specific signaling pathways engaged during CS. While both the SON and PVN participate in stress responses, their contributions are not identical: the SON predominantly produces vasopressin, whereas the PVN synthesizes both vasopressin and oxytocin. Moreover, owing to the presence of additional neuronal populations, the PVN plays a more complex and integrative role in stress regulation, including control over ACTH secretion and modulation of other stress-related factors. Unlike the SON, which primarily contains magnocellular neurons responsible for vasopressin and oxytocin production, the PVN also harbors smaller neurons with diverse functional properties. As a central hub of stress regulation, the PVN is

critically involved in the activation of the HPA axis. Its neurons promote the release of corticotropin-releasing hormone, which subsequently stimulates ACTH secretion and drives cortisol production [6].

After 6 hours of hind limb compression, neurons in both hypothalamic nuclei of rats exhibited a pronounced increase in acid phosphatase activity compared to controls, indicating activation of metabolic processes and enhanced cellular metabolism, which support regeneration and promote cell survival. In the SON and PVN, predominantly large, hypertrophied, and deformed magnocellular neurons were observed, densely clustered. These changes reflect compensatory and adaptive processes occurring in the SON and PVN under prolonged compression. High phosphatase activity in the cytoplasm of hypothalamic neurons appears directed toward maintaining cell viability and suggests the involvement of phosphorylation as one of the transcriptional pathways preceding the production of stress-dependent peptides [15,28]. Morphologically, these neurons exhibited intense enzymatic activity in both the cytoplasm and nuclei, with lead phosphate granules markedly enlarged into clumped formations, characteristic of acutely stimulated neurons. The observed changes in SON and PVN neurons after prolonged compression resemble acute swelling, recognized as reversible cellular alterations.

Although the morphological findings of this study are unequivocal, future investigations will extend beyond qualitative assessment. Specifically, changes in the neuronal morphology of hypothalamic nuclei following limb compression will be evaluated through quantitative analyses in rats using larger sample sizes, thereby ensuring the statistical robustness and reliability of the results.

The increased phosphatase content in neurosecretory cells likely reflects modifications in neurosecretory processes in response to stress [29,30]. Overall, the morphological features of damage in these brain regions following prolonged hind limb compression represent a nonspecific neuronal response to diverse pathological exogenous and endogenous stressors. The primary mechanism of neuronal injury and death is often attributed to excessive corticosteroids, particularly cortisol, characteristic of stress states [31]. During stress, adaptive mechanisms are engaged across multiple systems—including the hypothalamus, anterior pituitary, adrenal cortex, tissues, and organs [6]. Prolonged activation of these systems leads to increased secretion of glucocorticoids and catecholamines, which, through changes in gene expression, can result in dysregulation of immune responses [32]. Thus, crush syndrome (CS) may induce pathological alterations within hypothalamic nuclei, particularly manifesting as degenerative changes in neurosecretory cells. These effects are likely attributable to impaired microcirculation and the influence of toxic metabolites released from the compressed tissues [33].

Study Limitations.

The primary aim of our study was to qualitatively characterize the morphological and functional changes in hypothalamic neuronal structures at different time points following limb compression in rats. Given that we examined a large number of samples from the affected brain regions and employed a reliable and reproducible

histochemical method, we consider our qualitative assessment valid for evaluating these cellular structures. Nevertheless, the absence of quantitative and morphometric analyses of neuronal changes represents a limitation of the present study. We plan to incorporate quantitative assessments of cellular alterations in future investigations. Additionally, since our study did not include evaluation of animals during the recovery phase after hind limb compression, we cannot determine whether the observed cellular changes are reversible. The morphological features observed resemble acute swelling, which is typically recognized as a reversible change; this should be acknowledged as a limitation of the current study. The number of animals in each group ($n = 5$) was chosen in accordance with the 3R principle (Replacement, Reduction, Refinement), which may be insufficient to provide statistical power for qualitative analysis and constitutes an additional limitation of this study.

Conclusion.

This study demonstrates that crush syndrome (CS) affects the morphological and functional state of neurons in the SON and PVN of the rat hypothalamus. CS, accompanied by pain and stress, induces alterations in the activity and function of various brain regions. Neuronal damage in the SON and PVN following moderate (3 hours) and severe (6 hours) hind limb compression primarily manifests as central chromatolysis, with the SON showing greater resilience to the indirect effects of prolonged compression compared to the PVN. The observed morphological features resemble acute swelling, which is typically recognized as a reversible change provided timely assistance is provided. Because degenerative alterations in hypothalamic neurosecretory cells during CS are accompanied by functional impairments in the hypothalamus, these changes may exacerbate the overall physiological condition and contribute to the development of multiple organ dysfunction during the decompression period.

Author contributions.

Study Concept and Design: IAS and DMH. Acquisition, Analysis, and Interpretation of the Data: IAS, AIV, ANH, MAA, KKV, NKA and DMH. All of the authors have contributed substantially to the manuscript.

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Ethical approval and consent to participate.

The experimental protocol corresponded to the conditions of the European Communities Council Directive (2010/63/UE) and the "ARRIVE" guidelines (Animals in Research: Reporting In Vivo Experiments). The protocol was approved by the Institutional Review Board of the L. A. Orbeli Institute of Physiology (protocol code: N4, approval date: July 22, 2021).

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ИЗМЕНЕНИЯ МОРФОФУНКЦИОНАЛЬНОГО СОСТОЯНИЯ НЕЙРОНОВ ЯДЕР ГИПОТАЛАМУСА ПРИ СИНДРОМЕ ДЛИТЕЛЬНОГО РАЗДАВЛИВАНИЯ

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Абстракт

Синдром длительного сдавления (crush syndrome, CS) – это тяжёлая реакция на стресс, приводящая к системным метаболическим и нейроэндокринным нарушениям. Гипоталамус, как центральный интегратор нейроэндокринной и автономной регуляции, высокочувствителен к длительному стрессу. В данном исследовании изучались морфологические изменения в нейронах супраоптического (SON) и паравентрикулярного ядер (PVN) гипоталамуса у крыс, подвергнутых длительной компрессии задней конечности. С целью изучения динамики морфофункционального состояния клеточных структур ядер гипоталамуса крыс после раздавливания задней конечности был применён гистохимический метод выявления активности Ca^{2+} -зависимой кислой фосфатазы. Животных случайным образом разделили на три группы: контрольную (Co), группу с раздавливанием в течение 3-х часов (CS3) и группу с раздавливанием в течение 6-и часов (CS6). Экспериментальные модели CS были созданы на специальной установке в условиях компрессии задней конечности крыс в течение заданного времени. Образцы головного мозга, содержащие SON и PVN, были взяты через 1 час после снятия давления. Результаты исследования показали, что после 3-х часа компрессии в SON и PVN наблюдается различная направленность морфологических реакций. Нейроны SON в основном сохраняют морфологическую картину с повышением активности фосфатазы в цитоплазме нейронов, тогда как в нейронах PVN отмечается выраженное просветление цитоплазмы, центральный хроматолиз. Через 6 часов в нейронах обоих ядер гипоталамуса наблюдаются дегенеративные процессы, резкое повышение активности КФ в цитоплазме нейронов, что говорит об активации обменных процессов, повышении метаболизма, которые определяют оптимизацию процесса регенерации и клеточное выживание, что свидетельствовало об активации нейропротекторных механизмов. Эти результаты свидетельствуют о том, что ядра гипоталамуса подвергаются различным, зависящим от времени раздавливания морфофункциональным реакциям при CS, отражающим как компенсаторные, так и дезадаптивные процессы.

Ключевые слова: краш-синдром, синдром длительного сдавления, супраоптическое ядро, паравентрикулярное ядро, гипоталамус, Ca^{2+} -зависимая кислотная фосфатаза.