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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 ВИНИТИ 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 ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

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. Корректура авторам не высылается, 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 compu-ter-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 საყურადღებოდ!

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, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის 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 ფჩხილებში 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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ELECTRIC-MAGNETIC STIMULATION PREVENTS STRESS-INDUCED DETERIORATION OF SPATIAL MEMORY

Bukia N.G., Butskhrikidze M.P., Machavariani L.P., Svanidze M.J., Nozadze T.N.

LEPL Iv. Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia.

Abstract.

In response to physical and psychological stressors, neurobiological processes are activated to maintain homeostasis. Stress alters the activity of the hypothalamic-pituitary-adrenal (HPA) axis. The mechanism for this effect is not yet clear. For the formation of moderate stress, the Chronic Immobilization Stress (CIS) model was chosen to perform the restriction of movement activity 2 hours every day for 20 days continuously.

The impacts of CIS on cognitive function (elevated multi branch maze test) and Glucocorticoid receptor (GluR) levels in the hippocampus were studied in adult rats of both sexes. Electromagnetic stimulation (EMS) is an effective and non-invasive treatment method. After the end of CIS, the parameters of EMS (10000–15000 Hz, 1.5 m/Tesla, for 20 min, 10 days) on cognitive function and GluR changes were established in intact and gonadectomized rats. The study's goals were to determine the effect of CIS on spatial learning and GluR content in the hippocampus; b. the capacity of EMS to prevent or restore disorders developed in response to stress; and c. whether emerging stress responses are gender dependent.

It has been revealed that immobilization stress increases the maze passing time and the number of errors in both male and female rats. The effect of stress was more significant in male rats than in female rats. Gonadectomy increased the maze passage time regardless of sex. EMS for ten days has a positive effect on spatial learning. In females subjected to stress, the time to complete the maze path was fully restored to the level of intact rats, although in males this time remained relatively high. The time spent to pass the trajectory was increased after ten days of stress without EMS. As a result of immobilization stress, in both intact and gonadectomized rats, the GluR content had decreased in the hippocampus but was restored after EMS.

The CIS induced a reduction of GluRs in the hippocampus that is manifested as the deterioration of spatial memory. EMS restored GluR expression and had a facilitative effect on the performance of the intended task. The effects of EMS on GluRs were minimal in absence of sex hormones.

Key words. Electromagnetic stimulation, hippocampus, glucocorticoid receptors, stress, gender.

Introduction.

The HPA-axis is one of the key systems in mammals that modifies the physiological response to psychological and physiological stimuli. At the level of the hypothalamus, neural impulses linked with a stressor are converted into an endocrine response [1,2]. The hypothalamic paraventricular nucleus is a complex integration unit that receives and integrates neuroendocrine, autonomic, cognitive, and emotional signals and is in control of triggering glucocorticoid secretion [3,4]. Corticotrophin releasing hormone (CRH) and, to a lesser extent, arginine vasopressin are then produced from the hypothalamic paraventricular nucleus into the hypophyseal portal system, where they synergistically induce the release of adrenocorticotropic hormone. The adrenocorticotropic hormone is then carried into the circulation and induces the production of glucocorticoids (cortisol in humans; corticosterone in rodents) by the adrenal cortex. The negative feedback mechanism eventually inhibits the release of glucocorticoids. Glucocorticoids are crucial mediators of the physiological stress response, influencing numerous physiological systems and allowing the body to respond to a stressor. Acute, time-limited increases in glucocorticoid levels are adaptive, but long-term elevation has been linked to ventricular dilation, cerebral atrophy, cognitive impairment, and perhaps neurotoxicity. Prolonged high cortisol levels also harm the hippocampus, attenuating negative feedback even more [5-7].

Glucocorticoid receptors (GluRs) are widely distributed in the mammalian brain, including the limbic system, hypothalamus, pituitary, cerebral cortex, and monoaminergic nuclei of the brain stem [8,9]. GluRs are activated by increased levels of glucocorticoids, either due to the natural circadian rhythm or in response to stress and transmit signals to the HPA axis. GluRs moderate glucocorticoid activity until the termination of the stress response through negative feedback inhibition, mainly at the level of the hypothalamus and pituitary [10,11].

The activity of the HPA axis is also influenced by limbic systems such as the hippocampus, the amygdala, and the prefrontal cortex [3]. The hippocampus has an abundant amount of GluRs, which play a crucial inhibitory function both in the activity of the HPA axis at baseline and in the termination of the stress response [12]. The typical reaction to stress is suppression of cortisol release by activating GR-mediated feedback mechanisms. Failure to regulate cortisol release after persistent stress indicates a breakdown in the negative feedback loop. This effect is found in the presence of a severe depressive illness.

The hippocampus plays a critical role in the regulation of glucocorticoid levels due to the abundance of GRs in this structure and is involved in negative feedback loops, which eventually terminate the physiological response to stress [5,13]. According to studies in rats, prolonged exposure to corticosteroids is associated with neuronal cell death in the hippocampus, changes in cell shape, atrophy of the dendritic processes, decreased neurogenesis, and stress-induced memory impairment [3,6,14].

The HPA-axis reaction to stress is gender dependent. Females often have a more robust neuroendocrine response to acute stress, as demonstrated by higher levels of CORT and ACTH after exposure to a variety of stressors of different modalities

[15]. Sex determines differences in regulation at each level of the HPA axis and limbic regions that result in sex-dependent variations in HPA output [1,15,16].

Electric-magnetic stimulation (EMS) is a non-invasive therapeutic method for various neurodegenerative diseases. The FDA authorized repetitive TMS (transcranial magnetic stimulation) in 2008 for the treatment of mild medication-resistant depression, anxiety, and mood disorders. The electric-magnetic field (EMF) appears to be physiologically active and propagates throughout the live tissue almost without resistance [17,18]. Despite the abundance of scientific evidence, it is not yet clear how low-frequency EMS affects stress-induced responses, including impairment of cognitive function. It is also critical to investigate gender differences in the effects of EMS.

The purpose of this study was to investigate the effects of EMS on the results of spatial learning tests in the elevated multi-brunch maze and changes of GluRs content in a CIS paradigm depending on sex hormones.

Methods.

Animals: The rats were housed under standard laboratory conditions with a "12 h light–12 h dark" cycle (in order to ensure constant circadian rhythms), a constant temperature of 22°C. Water and food were available ad libitum. The number of rats in each cage was limited to 4-5 to minimize overcrowding stress that could result in an increase in corticosteroids and anxiety. All experimental procedures were conducted in accordance with the European Communities Council Directive Guidelines for the care and use of laboratory animals (2010/63/EU—European Commission) and the animal care and use committee at the Iv. Beritashvili Center of Experimental Biomedicine.

Chronic immobilization stress (CIS) is a commonly accessible model of chronic stress based on restricted movement activity. The animals were immobilized for 20 days, 2 hours per day for the induction of moderate stress.

For EMS, the device with a coil designed at Tbilisi Technical University, Georgia was used. After the immobilization procedure, experimentally established EMS settings (10000–15000 Hz, 1.5 m/Tesla, for 20 min, 10 days) were used to investigate the potential therapeutic effects of EMS.

Cognitive function: was studied using a multibranch maze test, which is highly sensitive to hippocampal dysfunction. Through the method of trial and error, rats learn to move along the optimal trajectory from the starting platform to the housing box. The process of successful learning was accompanied by food reinforcement. The method is based on rats' inherent preference for investigating a novel limb rather than a known one, which causes them to vary the choice of the target arm throughout repeated testing. To correctly determine the choice for each next trail, an animal must remember which arm was visited in the previous trial, [7,19]. Five trials (5 min each) were performed daily. The process of learning the maze was evaluated by variations in the number of errors made from trial to trial in search of the optimal path to the housing box and by the time needed to pass the maze test.

Glucocorticoid Receptors (GluRs) were identified in the hippocampus using a radioimmunoassay (Rat NR3C1/ Glucocorticoid Receptor (Sandwich ELISA) ELISA Kit LS-F4309). S-F4309 is a 96-well enzyme-linked immunosorbent assay (ELISA) for the quantitative detection of Rat NR3C1/ Glucocorticoid Receptors in samples of cell lysates and tissue homogenates. The animals were decapitated 24 hours after the final stressor. The hippocampus was dissected from the rest of the brain [20] and kept at -80°C until ELISA.

Under ether anesthesia, bilateral gonadectomy of male and female rats was performed using the standard technique [21,22]. Experiments on gonadectomized rats started 30 days after surgery.

Data analysis.

Data were statistically processed by one- and two-way factorial analysis (ANOVA) and considered significant when $P \leq 0.05$.

The experimental design.

The experiment was carried out in the following groups of female and male rats (200–250 g): intact, intact+EMS, intact stressed, intact stressed+EMS, gonadectomized, gonadectomized+EMS, gonadectomized stressed, gonadectomized stressed+EMS (n=5 in each group of rats). The spatial learning capacity of rats in the elevated multibrunch maze test and the GluRs content in the hippocampus were evaluated in all groups of rats according to gender.

Results.

CIS effects on spatial learning in an elevated maze test in female and male intact rats in the background of EMS.

Intact rats were able to pass through the multi-brunch maze trajectory and reach the nest without errors on the 6th day of training, that was reinforced by food. Female rats were able to complete this trajectory in 15±2 sec and male rats in 25±3 sec. (Figures 1 and 2). After CIS, male, and female rats moving activity were felled down. They were unable to reach the nest box by themselves. They made errors even on the sixth day of testing. Males spent 275±12 sec and females 198±16 sec to complete the trajectory (in both cases, $P \leq 0.01$).

Exposure to EMS significantly reduced maze passing time in intact stressed animals, from 275±12 to 75±7 sec in males and from 198±16 to 29±8 sec in females (in both cases, $P \leq 0.01$) on the 6th day of training. However, in intact stressed males, the error-free running time through the maze remained elevated (75±7 sec) compared to unstressed rats, in the presence of EMS (17±7 sec) or without (25±3 sec) (in both cases, $P \leq 0.01$). In stressed female rats, the time to pass through the maze was reduced due to exposure to EMS (29±8) ($P \leq 0.01$) and was as in intact unstressed rats (Figures 1-3).

CIS effects on spatial learning in an elevated multi-brunch maze test in female and male gonadectomized rats in the background of EMS.

On the 6th day of testing, gonadectomized rats spent longer time to complete the elevated multi-brunch maze test compared to intact rats (males 55±5 sec and females 45±6 sec) ($P \leq 0.01$). In castrated stressed male rats subjected to EMS, the completion time decreased from 263±9 sec to 134±8 sec ($P \leq 0.01$) however it was significantly longer than in castrated unstressed rats (41±6 sec) ($P \leq 0.01$). In ovariectomized female rats, the time dynamic changed the same way as in male rats. In more detail, in rats exposed to EMS, the passage time decreased from 220±9

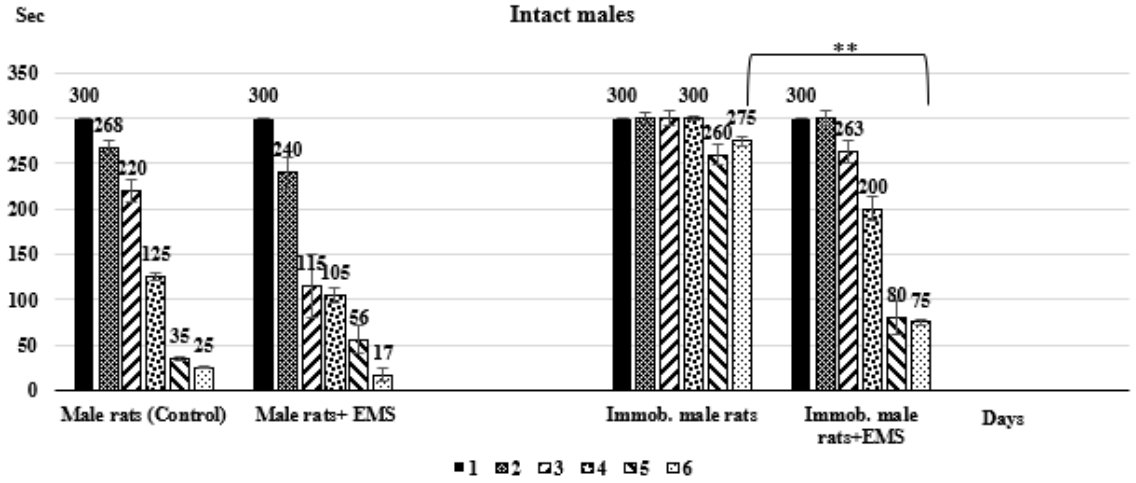

Figure 1. *Learning dynamics in intact stressed and unstressed male rats with and without EMS in the multi-brunch maze test.*

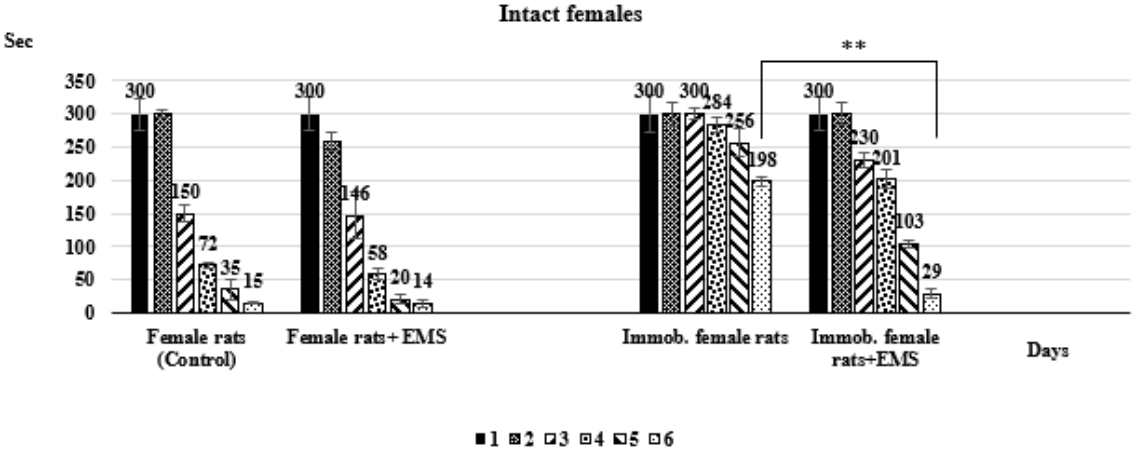

Figure 2. *Learning dynamics in intact stressed and unstressed female rats with and without EMS in the multi- brunch maze test.*

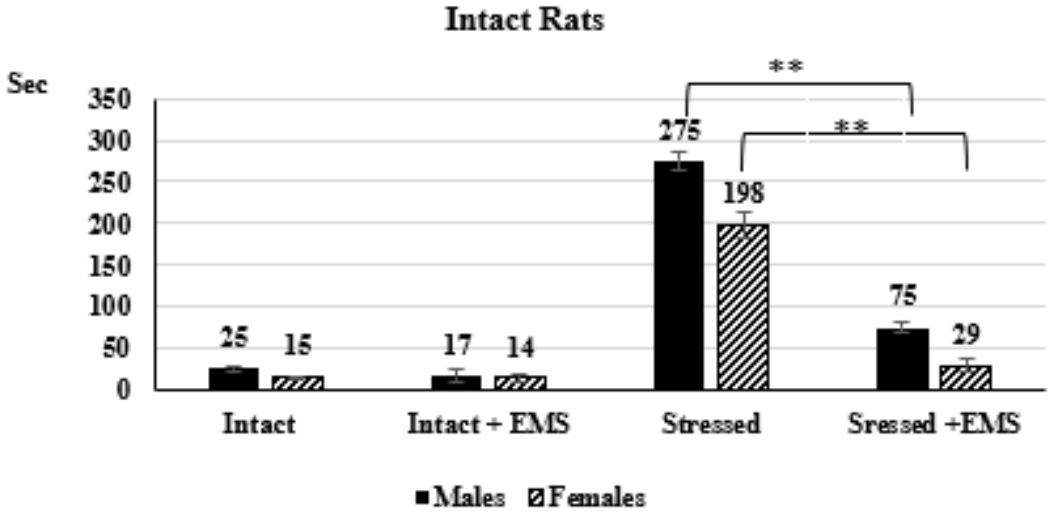

Figure 3. *The effect of CIS on the time to complete the maze test in intact rats of both sexes exposed to EMS.*

sec to 132±5 sec (P≤0.01), while ovariectomized unstressed rats passed the same trajectory in 38±4 sec (P≤0.01). (Figures 4-6).

It might be assumed that the effect of EMS on spatial learning in the elevated multibranch maze is mediated by sex hormones. Without sex hormones, the effect of EMS is minimal.

Analysis of NR3C1 gene expression in intact and gonadectomized rats.

GluRs are key mediators of the neuroendocrine response to stress. We have determined the effects of CIS on the regulation of GluRs in the Hippocampus of male and female rats. In intact female and male rats GluR levels was in males 5.5±0.09 and females 5.2±0.07 ng/ml. Therefore, predominate amount was in males compared to females (P≤0.05). Gonadectomy decreased GluR levels in both sexes - males 5.1± 0.08, females 4.65± 0.22 (P≤0.05) (see Figures 7 and 8).

Analysis of hippocampal NR3C1 gene expression in intact and gonadectomized rats both sexes after CIS in the background of EMS.

CIS decreased GluR levels in rats of both sexes, but more significant depression of GluR levels was detected in stressed male rats compared to females. After CIS GluR levels were decreased in intact male rats from 5.5±0.09 to 2.21±0.35 ng/ml and in intact females from 5.2±0.07 to 3.45±0.39 (in both cases, P≤0.01) ng/ml. Therefore, our data revealed stressor-specific alterations in GluR levels, which were more pronounced in males compared to females. Presumably, females, compared to males, have strongly expressed compensatory reactions, which prevent the process of GluRs reduction as a result of stress. As mentioned above, more CIS-induced deterioration of spatial learning was detected in males (Figures 7 and 8).

CIS also decreased GluR levels in gonadectomized rats in the same manner as in intact males, from 5.1±0.08 to 2,55±0.56 ng/ml (P≤0.01) in males and from 4.65±0.22 to 2.45±0.41 ng/ml (P≤0.01) in females. However, there was a significant difference (P≤0.01) between reduction levels in intact stressed (3.45±0.39) and ovariectomized (2.45±0.41) female rats, but no such changes in male rats.

EMS upregulated GluRs in intact female and stressed male rats, but the increase was greater in females (from 3.45±0.39 to 4.7±0.42 ng/ml) then in males (from 2,21±0.35 to 4.2±0.37 ng/ml) (both cases, P≤0.01). At the same time, EMS improved the

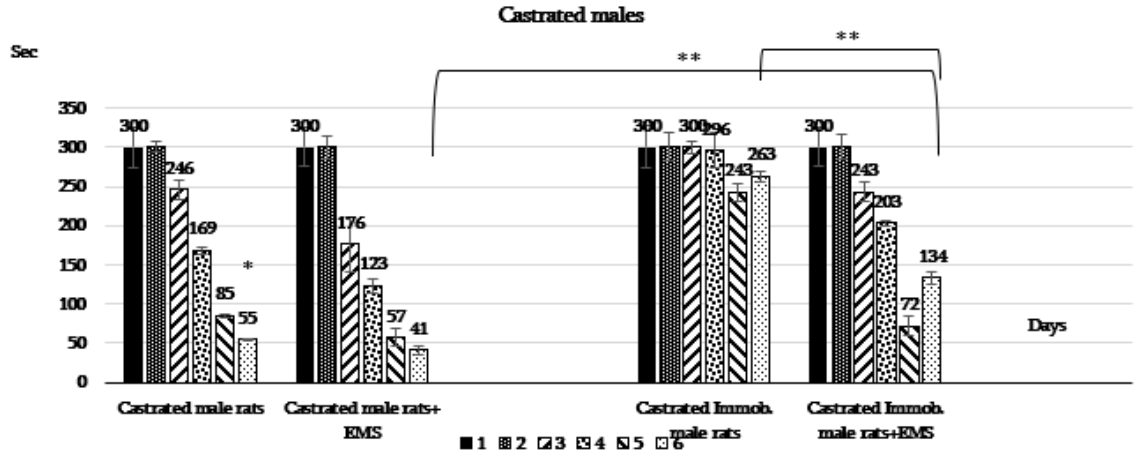

Figure 4. *Learning dynamics of intact stressed and unstressed castrated male rats with and without EMS in the multi-brunch maze test.*

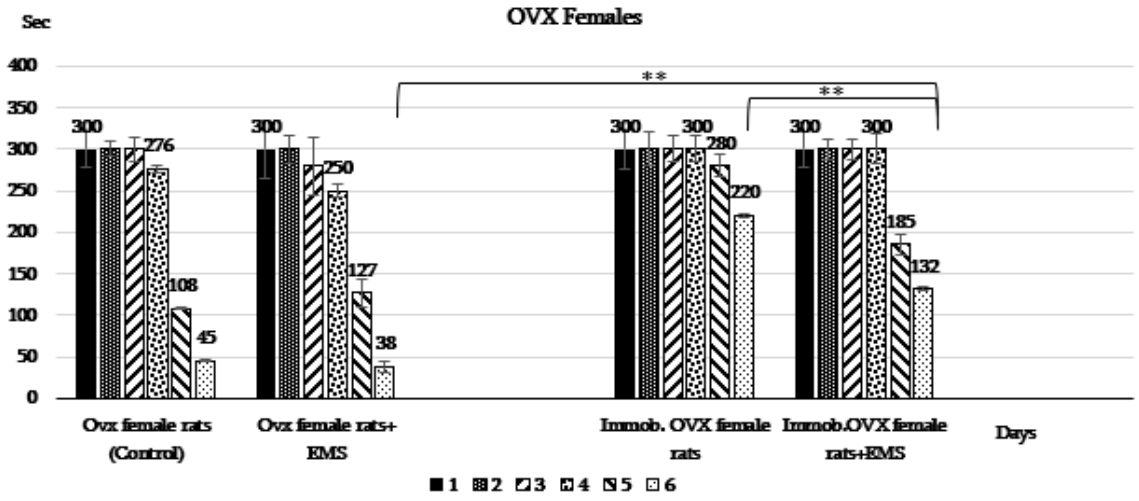

Figure 5. *Learning dynamics in intact stressed and unstressed ovariectomized female rats with and without EMS in the multi-brunch maze test.*

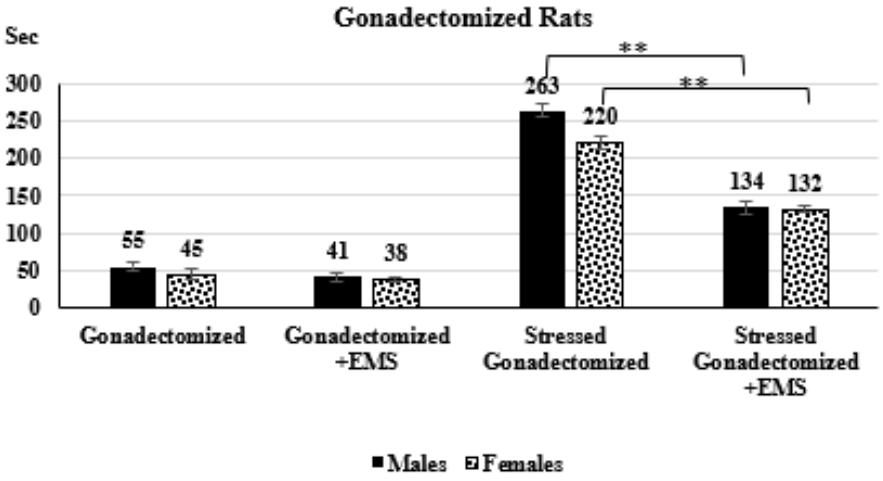

Figure 6. *The effect of CIS on maze passage time in gonadectomized rats of both sexes exposed to EMS.*

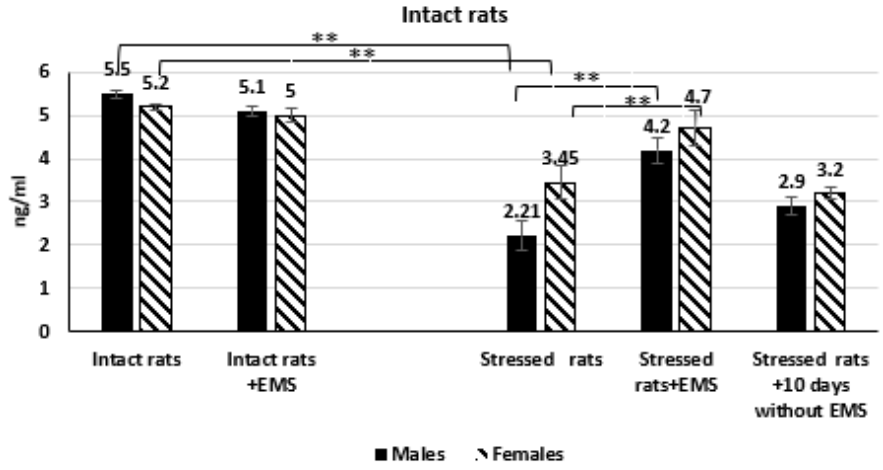

Figure 7. *EMS effects on Hippocampal GluR levels (ng/ml) in intact stressed and unstressed male and female rats.*

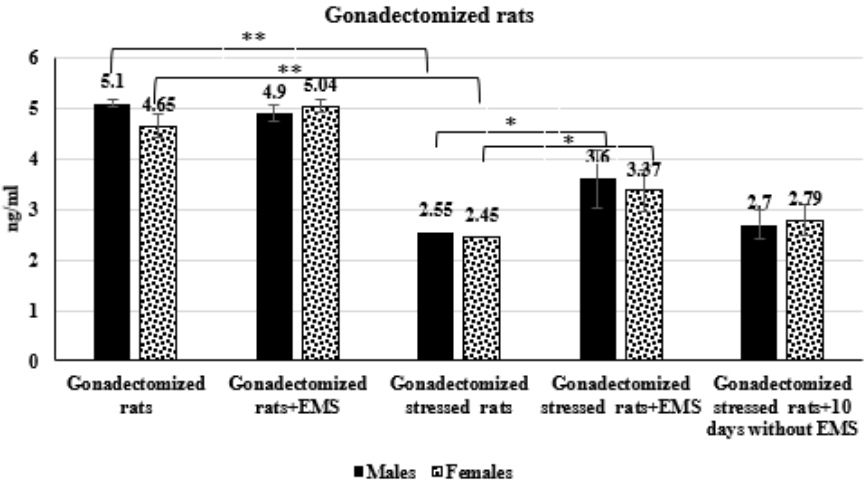

Figure 8. *EMS effects on Hippocampal GluR levels (ng/ml) in gonadectomized stressed and unstressed male and female rats.*

time to pass the elevated maze by both sexes, albeit with a more significant effect in intact female than in male rats.

In gonadectomized stressed rats, EMS upregulated GluR levels from 2.55±0.56 to 3.6±0.29 ng/ml and from 2.45±0.41 to 3.37±0.32 ng/ml in male and female rats, respectively, but the effect was significantly less than in intact CIS rats ($P \leq 0.01$). It was suggested that EMS-mediated regulation of GluR expression is more efficient in the presence of sex hormones.

Finally, the time-dependent restoration of GluRs was evaluated 10 days after CIS without EMS. Levels of GluRs were not restored in those rats. Thus, it might be assumed that EMS indeed contributes to the restoration of GluRs.

Discussion.

One key mechanism of diminishing HPA-axis activity by antidepressants is dysregulation of the HPA-axis [12]. GR-mediated gene transcription can also be inhibited by several antidepressants. In animal experiments, antidepressant medicine has been shown to increase GluR levels in the brain, decrease stress-induced glucocorticoid production, and improve HPA-axis feedback. In vitro research has shown that antidepressants can improve GR function [15,23]. Animal studies have also shown that dexamethasone can cause cell death, particularly in the striatum and the CA1 and CA3 parts of the hippocampus, and that long-term antidepressant medication can prevent this damage [14].

It should be noted that glucocorticoids exert their adaptive effects (such as changes in energy metabolism, gene transcription, and so on) through GluRs, and reducing GluRs signaling would affect not only negative feedback inhibition, but also the action of glucocorticoids on cellular processes. Furthermore, it has been established that stress exposure in children and adolescents increases the methylation process of the NR3C1 gene, resulting in downregulation of GR transcription and functionality and an increased risk of anxiety, sadness, social withdrawal, and fearfulness [4,24].

Since the stressful episode was extended (CIS rats were restrained for 20 days) due to impaired negative feedback, glucocorticoids remained elevated for a longer period, resulting in hippocampus damage that was reflected as increased trajectory crossing time in the spatial learning test. Moreover, male rats were more vulnerable to immobilization stress as their passing maze time increased and the number of errors was also higher than in female rats. At the same time, the levels of GluRs in males were substantially lower than in females. It is possible to conclude that GluR changes are critical for the manifestation of stress-induced responses.

GluR deficiency in the hippocampus reduces negative input to the HPA-axis. As a result, CORT and ACTH secretion does not decrease, but rather hypersecretion of corticosterone damages several brain areas, including the hippocampus, resulting in cognitive impairment. Thus, chronic stress initiates a cycle (glucocorticoids-hippocampus-HPA axis) that worsens hippocampal damage.

EMS restored hippocampal GluR levels in rats of both sexes after immobilization stress. As a result, it is reasonable to believe that EMS can restore the negative feedback of glucocorticoids to the HPA axis. Restored corticosteroid receptor signaling leads to a reduction in stress-induced deterioration of spatial learning.

As a result of gonadectomy, the trajectory passing time increased, while the GluR content in the hippocampus decreased ($P \leq 0.05$). We hypothesize that in gonadectomized rats, under conditions of peripheral sex hormone deficiency, the negative feedback of glucocorticoids on the HPA axis is reduced. The content of glucocorticoids increases in the blood, that has a damaging effect on the hippocampus and other structures of the brain. This increases the risk of emotional background formation, neuronal loss, and impaired neurogenesis. Furthermore, sex steroids have been shown to be neuroprotective by controlling hippocampal structural integrity, amyloid beta production, neuronal loss, and neurogenesis [25,26].

Thus, in our study, we discovered a correlation between spatial memory and alterations in GluRs in the hippocampus, which is crucial for understanding the processes of stress-induced reactions. Also, the beneficial effect of EMS was to minimize the negative impacts of CIS. Because EMS-mediated effects on GluR levels were more efficient in the presence of sex hormones, we postulate that those hormones are involved in the implementation of EMS effects.

We consider that an important goal for future research would be the elucidation of the neuromediator systems that were activated or inhibited by EMS that restores the negative feedback of glucocorticoids on the hippocampus and HPA axis under stress conditions. As a result, hippocampus damage is reduced preventing stress-dependent deterioration of cognitive function.

Conclusion.

Chronic immobilization stress impairs the function of the hippocampus, lowers GluR levels, and destabilizes the functioning HPA axis in rats of both sexes. The observed difference between sexes is related to circulating gonadal hormones because gonadectomy changed the sex-dependent patterns of GluRs after CIS. EMS increases GluR levels in the hippocampus, restores negative feedback to the HPA axis, and prevents the decline of cognitive function, in particular spatial memory. By restoring GluR levels in the hippocampus and HPA axis activity, EMS may be able to correct long-term deficiencies in cognitive and endocrine reactivity. However, in the absence of sex hormones, the effects of EMS are less efficient.

Conflict of interest statement.

The authors have declared that no competing interests exist.

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Электромагнитная стимуляция предотвращает стрессом вызванное ухудшение пространственной памяти

Букия Н.Г., Буцхрикидзе М.П., Мачавариани Л.И., Сванидзе М.Дж, Нозадзе Т.Н.

Центр экспериментальной биомедицины им. Ив Бериташвили, Тбилиси, Грузия

Абстракт

В ответ на физический и психологический стресс активируются нейробиологические процессы для поддержания гомеостаза. Стресс изменяет активность гипоталамо-гипофизарно-надпочечниковой системы (ГГН). Механизм этого эффекта пока не ясен. Для формирования умеренного стресса была выбрана модель хронического иммобилизационного стресса- CIS (ограничение двигательной активности по 2 часа ежедневно в течение 20 дней).

Влияние CIS на когнитивную функцию (тест приподнятого многоветвевого лабиринта) и уровень глюкокортикоидных рецепторов (GluR) в гиппокампе изучали у взрослых крыс обоего пола. Электромагнитная стимуляция (EMS) является эффективным и неинвазивным методом лечения. После окончания CIS у интактных и гонадэктомированных крыс устанавливали параметры EMS (10 000–15 000 Гц, 1,5 м/Т, 20 мин, 10 сут) которые позитивно влияли на когнитивную функцию и изменения GluR. Цель исследования было определить: а. влияние CIS на пространственное обучение и содержание GluR в гиппокампе; б. способность EMS предотвращать или восстанавливать расстройства, развившиеся в ответ на стресс; и с. зависят ли возникшие реакции на стресс от половых гормонов.

Выявлено, что иммобилизационный стресс увеличивает время прохождения лабиринта и количество ошибок как у самцов, так и у самок крыс. Влияние стресса у самцов крыс было более значительным, чем у самок. Гонадэктомия

увеличивала время прохождения лабиринта независимо от пола. EMS в течение десяти дней положительно влиял на пространственное обучение. У самок, подвергшихся стрессу, время прохождения лабиринта полностью восстанавливалось до уровня интактных крыс, хотя у самцов это время оставалось относительно высоким. Время прохождения траектории увеличилось после десяти дней стресса без EMS. В результате иммобилизационного стресса как у интактных, так и у гонадэктомированных крыс содержание GluR снижалось в гиппокампе и восстанавливалось после EMS.

Заключение. CIS вызывает снижение GluRs в гиппокампе, что проявляется в ухудшении пространственной памяти. EMS восстанавливает экспрессию GluR и облегчает выполнение поставленной задачи. В отсутствие половых гормонов эффекты EMS на GluR минимальны.

Ключевые слова:
Электромагнитная стимуляция, гиппокамп, глюкокортикоидные рецепторы, стресс, пол

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ელექტრომაგნიტური სტიმულაცია ხელს უშლის სტრესით გამოწვეული სივრცითი მეხსიერების გაუარესებას

ბუკია ნ.გ., ბუცხრიკიძე მ.პ., მაჭავარიანი ლ.ი., სვანიძე მ.ჯ., ნოზაძე თ.ნ.
ივ.ბერიტაშვილის ექსპერიმენტლი ბიომედიცინის ცენტრი, თბილისი, საქართველო

აბსტრაქტი
ჰომეოსტაზის შესანარჩუნებლად ფიზიკური და ფსიქოლოგიური სტრესის საპასუხოდ აქტიურდება ნეირობიოლოგიური პროცესები. სტრესი ცვლის ჰიპოთალამურ-ჰიპოფიზურ-თირკმელზედა ჯირკვლის (HPA) ღერძის აქტივობას. ამ ზემოქმედების მექანიზმი ჯერ კიდევ არ არის ბოლომდე ახსნილი. ზომიერი სტრესის ფორმირებისთვის არჩეული იქნა ქრონიკული იმობილიზაციის სტრესის მოდელი - CIS (მოტორული აქტივობის შეზღუდვა 2-2 საათით დღეში, 20 დღის განმავლობაში).

CIS-ის ეფექტი კოგნიტურ ფუნქციაზე (ამაღლებული ლაბირინთის ტესტი) და გლუკოკორტიკოიდური რეცეპტორების (GluR) რაოდენობაზე ჰიპოკამპში შესწავლილი იყო ორივე სქესის ზრდასრულ ვირთაგვებში. ელექტრომაგნიტური სტიმულაცია (EMS) არის ეფექტური და არაინვაზიური მკურნალობის მეთოდი. CIS-ის დასრულების შემდეგ, ხდებოდა ინტაქტური და გონადექტომიზებული ვირთაგვების EMS (10,000-15,000 Hz, 1.5 მ/ტ, 20 წთ, 10 დღე), რამაც დადებითად იმოქმედა კოგნიტურ ფუნქციაზე და GluR ცვლილებებზე. კვლევის მიზანი იყო დაგვედგინა: ა. CIS-ის გავლენა სივრცულ დასწავლაზე და GluR-ის შემცველობაზე ჰიპოკამპში; ბ. EMS-ის უნარი, თავიდან აიცილოს ან შეცვალოს სტრესთან დაკავშირებული დარღვევები; და გ. დამოკიდებულია თუ არა სტრესზე აღმოცენებული რეაქციები სასქესო ჰორმონებზე.

გამოვლინდა, რომ CIS ზრდის ლაბირინთში გავლის დროს და შეცდომების რაოდენობას როგორც მამრ, ისე მდედრ ვირთაგვებში. მამრ ვირთაგვებში სტრესის ეფექტი უფრო მნიშვნელოვანია, ვიდრე მდედრებში. გონადექტომიამ გაზარდა ლაბირინთის გავლის დრო სქესის მიუხედავად. ათი დღის განმავლობაში EMS-მდადებითად იმოქმედა სივრცით დასწავლაზე. სტრესირებულ მდედრებში, ლაბირინთის გავლის დრო სრულად აღდგა ინტაქტური ვირთაგვების დონემდე. ეს დრო შედარებით გაზრდილი იყო მამრებში. EMS-ის გარეშე, ტრაექტორიის გავლის დრო გაიზარდა CIS-ის შემდეგ. იმობილიზაციური სტრესის შედეგად, როგორც ინტაქტური, ისე გონადექტომიური ვირთაგვების ჰიპოკამპში GluR-ის შემცველობა შემცირდა და აღდგა EMS-ის შემდეგ.

დასკვნა. CIS იწვევს ჰიპოკამპში GluR-ების შემცირებას, რაც გამოიხატება სივრცითი მეხსიერების გაუარესებით. EMS აღადგენს GluR -ების შემცველობას. სქესობრივი ჰორმონების არარსებობის შემთხვევაში, EMS-ის ეფექტი GluR-ზე მინიმალურია.

საკვანძო სიტყვები:
ელექტრომაგნიტური სტიმულაცია, ჰიპოკამპი, გლუკოკორტიკოიდული რეცეპტორები, სტრესი, სქესი

კვლევა ჩატარდა შოთა რუსთაველის ეროვნული სამეცნიერო ფონდის მხარდაჭერით N FR-19-185