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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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LONG-LASTING EFFECTS OF EARLY POSTNATAL DYSFUNCTION OF THE BRAIN MUSCARINIC CHOLINERGIC SYSTEM ON LEARNING AND MEMORY AND ADULT HIPPOCAMPAL NEUROGENESIS

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Abstract.

Aim: The present investigation aimed to explore in rats the early postnatal dysfunction of the brain muscarinic cholinergic system (EPDMChS) during the most vulnerable period of postnatal development, as the possible main factor for changes in adult hippocampal neurogenesis and disorders in hippocampus-dependent spatial learning and memory.

Methods: White inbred rats (n=15 in each group) were used. EPDMCHS was produced by a new method, which includes early postnatal blocking of M1-M5 muscarinic acetylcholine receptors in the rat pups, using subcutaneous injection of Scopolamine during postnatal days 7-28. Control rat pups received the same volume as Saline. Researches were started in adult age (2.5–3 months).

Results and conclusion: It was shown for the first time that EPDMCHS exerts long-lasting effects manifested in adult age in the impairment of hippocampal neurogenesis and significant deterioration of spatial long-term declarative memory in the MWM. The possible causal link between the EPDMCHS and two types of resulting disorders is underlined.

Key words. Muscarinic cholinergic system, postnatal dysfunction, adult hippocampal neurogenesis, spatial learning and memory.

Introduction.

Acetylcholine (ACh) has attracted the attention of researchers due to its dual actions in the CNS, both as a neurotransmitter and as a neuromodulator [1]. Studies about the involvement of the brain's muscarinic and nicotinic cholinergic systems in the basic mechanisms of various physiological functions and their disorders have a long history. As a result, it was shown that cholinergic dysfunction significantly modifies the normal functioning of the hippocampus [2-5], hippocampal ACh is important for the normal course of cognitive functions [6] including coding of location and movement speed, learning and memory in spatial memory tasks [1,4,7-14] the level of hippocampal ACh increases when the animals are exposed to a novel spatial environment [15,16]. The involvement of the muscarinic cholinergic system (MChS) in spatial navigation or spatial conditioning was extensively studied by different methodical approaches in rodents [13,17-20] and cats [21-24].

ACh-producing neurons are found widely throughout the brain - in the brain stem, basal forebrain, striatum, medial habenular nucleus [25], pedunculopontine and laterodorsal tegmental nuclei, basal ganglia, tectum, and basal forebrain [17,26-28]. The selective lesion of basal forebrain cholinergic neurons significantly impairs hippocampus-dependent memory function, suggesting a role for septo-hippocampal cholinergic projections in memory formation [3,6,13,11,12,17,29,30]. Consequently, the main body of the studies was so far devoted to the investigation of causal involvement, in the various tasks of learning and memory, of the septo-hippocampal, basal forebrain, and other regions, where the acetylcholine-producing neurons lie, or its cholinergic projections are ending.

It has been hypothesized that changes in adult hippocampal neurogenesis may be partially regulated by MChS, and its disturbances may be related to cognitive impairment since selective damage to the basal forebrain cholinergic neurons with the immunotoxin 192IgG-saporin causes cognitive deficits and inhibits neurogenesis in the dentate gyrus (DG) [31-34]. The cholinergic system is believed to regulate adult hippocampal neurogenesis by positively promoting the proliferation, differentiation, integration, and potential survival of newborn cells [32].

Of the neurogenic areas of the adult brain, hippocampal (mainly DG) neurogenesis has received the most attention because of its involvement in higher cognitive function; particularly spatial memory processes [5,33]. DG is one of the most plastic regions that generate principal neurons integrating into the pre-existing networks in the hippocampal layers throughout life [34]. ACh extensively impacts neuronal circuits by affecting neurogenesis, spine formation, synapse formation [6,21,32], and synaptic plasticity by modulating the spiking activity of neurons and neurotransmitter release [35,36].

Basic information about the participation of MChS in cognitive functions and adult hippocampal neurogenesis has been obtained in animals by the methods of electrolytic or immunotoxin lesions of ACh neurons in various brain regions, which are followed by the significant side effects because of the retrograde degeneration and sprouting of the nerve endings. We believe that to receive relatively pure effects of the MChS dysfunction it is necessary to make it using the method excluding damage to the cholinergic cells by any exogenous factors.

In the present investigation, we are dealing with the brain MChS, overall, because we aim to produce dysfunction of the whole brain MChS during the most significant period of postnatal development. It is necessary to mention that other authors have not yet studied this question. The method developed by Nachkebia et al. produces EPDMChS by the early postnatal blocking of M1-M5 acetylcholine receptors in all brain areas [37]. Using this method we have shown earlier that EPDMChS in rat pups causes adult-age disturbances of open-field behavior and significant sleep disorders [37], enhancement of the level of anxiety in adult age, and complication in the acquisition of information in fear-motivated non-declarative learning and memory (active and passive avoidance), with the normal course of its consolidation and long-term retention [38].

In the present research, we used the described method of production of EPDMChS for the first time to investigate the long-lasting effects of this procedure on the formation of spatial

declarative and non-declarative memory in Morris Water Maze (MWM) and adult hippocampal/DG neurogenesis. One of the aims was to assess the causal relationship between the possible disorders of spatial declarative learning and memory and adult hippocampal neurogenesis.

Materials and Methods.

Experiments were carried out on white albino rats (n=40), approximately 2.5-3 months of age, weighing 220-250 g at the start of the experiments. Rats were supplied from the Vivarium of Ivane Beritashvili Center of Experimental Biomedicine. One male and one female rat were housed in standard cages (total 5 families) on a natural light/dark cycle, with food and water ad libitum.

The protocol of experiments was approved by the "Animal Studies Committee of Ivane Beritashvili Center of Experimental Biomedicine".

Procedure for antagonizing M1-M5 muscarinic acetylcholine receptors. The method for EPDMChS [37] implies subcutaneous injection of the non-selective antagonist of M1-M5 mAChRs, Scopolamine (SCOP), at the dose of 30 mg/kg, two times daily, started from the postnatal day 7 (P7) to P28 (experimental EPDMChS group, n=15). Newborn pups were with the dam during the period of breastfeeding.

Control rat pups received (from P7 to P28) subcutaneous injection of saline [control with early postnatal saline treatment (CPST) n=15] in the same volume as SCOP solution in EPDMChS pups. Experiments started in both groups reaching their adult age, 2.5-3 months.

Behavioral apparatus and procedure. Spatial declarative, and non-declarative learning and memory were studied using MWM [39,40], consisting of a circular tank (1.5 m in diameter and 0.5 m in height) filled with opaque (white-colored) water. The escape platform (10 cm in diameter) was located 2 cm beneath the surface, on invisible platform training days, and raised 2 cm above the water surface, on visible platform training days. A special video tracking system was used to measure the escape latency, and the speed to find the visible or invisible platforms. The procedure for a 10-day investigation was standard as determined by the authors of the MWM method [39,40].

Studying the changes in adult hippocampal neurogenesis. Changes in adult hippocampal neurogenesis were assessed by quantitative analysis, counting the number of newborn granular cells in the DG and hippocampal neurons in the upper (U), middle (M), and lower (L) areas of the CA1 and CA3 fields. In rats of CPST and EPDMChS groups (n=5 in each), the brain was removed under general anesthesia (in adult age for hippocampal neurogenesis, 1 month age for DG neurogenesis) and maintained in 10% formalin for approximately 1 month. Then the brain was cut in the coronal plane on a freezing microtome. Appropriate brain sections were stained by the Cresyl Violet (Nissl staining) method. The number of neurons in the studied sub-fields of the hippocampus and granular cells of the DG were calculated using a fluorescence optic microscope Leica MM AF. 30 sections from the hippocampus and DG from CPST and EPDMChS groups were selected Statistical processing.

The obtained data were treated statistically by the Student's t-test with standard deviations (SD). **= $p<0.05$ was taken as the index of significance.

Results and Discussion.

Results obtained by us show that there is a significant difference in the speed needed to find the visible platform location in the first-day session of MWM between the rats of EPDMChS and CPST groups; the time required to find the escape platform for EPDMChS rats becomes shorter with the launching from each subsequent starting position of the MWM, but significantly exceeds the value of time needed for rats of CPST group to find safe platform [North (N) 35 sec in CPST/55 sec in EPDMChS, $p<0.05$; South (S) 30 sec in CPST/45 sec, in EPDMChS, $p<0.05$; East (E) 28 sec in CPST/32 sec in EPDMChS, $p<0.05$; West (W) 25 sec in CPST/30 sec in EPDMChS, $p<0.05$, Figure 1A].

Data obtained on the second-day visible platform session, quantitatively are the same as in the first-day session – time to find the platform reduces in both groups but there is more retardation of the learning process in rats of EPDMChS group during launching from all starting positions in the MWM (Figure 1B,N,S,E,W). Thus, the obtained results show the disturbance in the formation of non-declarative spatial long-term memory in the EPDMChS group.

In the third day session, in the invisible platform version of MWM, it was found that in the animals of the EPDMChS group, the time required to find the invisible platform sharply increased in the cases of launching from all four starting positions (Fig.2, mean: N – 34 sec in CPST/50 sec in EPDMChS; S – 32 in CPST/48 sec in EPDMChS; E – 25 sec in CPST/44 sec in EPDMChS; W – 21 sec in CPST/46 sec in EPDMChS). Learning and memory processes were again studied in the visible platform version in the fourth and fifth-day sessions. It was found that the time to find the visible platform varies between daily sessions with each subsequent launching from the quarters; animals of the EPDMChS group require significantly more time to find the visible platform than the animals of the CPST group (Figure 3A and 3B).

In the sixth-day invisible platform sessions, the speed of platform finding was significantly longer in rats of the EPDMChS group after launching from all the fourth positions (N – mean: 30 sec in CPST/60 sec in EPDMChS; S – 28 sec in CPST/55 sec in EPDMChS; E – 20 sec in CPST/52 sec in EPDMChS; and W – 18 sec in CPST/50 sec in EPDMChS; Figure 5A).

In the seventh-day visible platform session, after starting from the first two starting positions, rats of the EPDMChS group spent more time finding the escape platform, compared to the data of the CPST group. After launching from the third (E) and fourth (W) starting positions, the difference disappears and rats from both groups found the visible platform at the same speed (Figure 4A). The data of the eighth-day visible platform session does not differ significantly from the data of seventh-day session (Figure 4B) indicating to the completion of the learning process in the visible platform task.

In the ninth-day session, the long-term declarative spatial memory about the location of the invisible platform was tested for the third time. The extent to which rats can use visual cues, during navigating in a maze, to learn the appropriate location of a platform with distant visual stimuli was tested. It was found, that rats of the EPDMChS group, found the invisible platform with a significant delay when launching from all starting

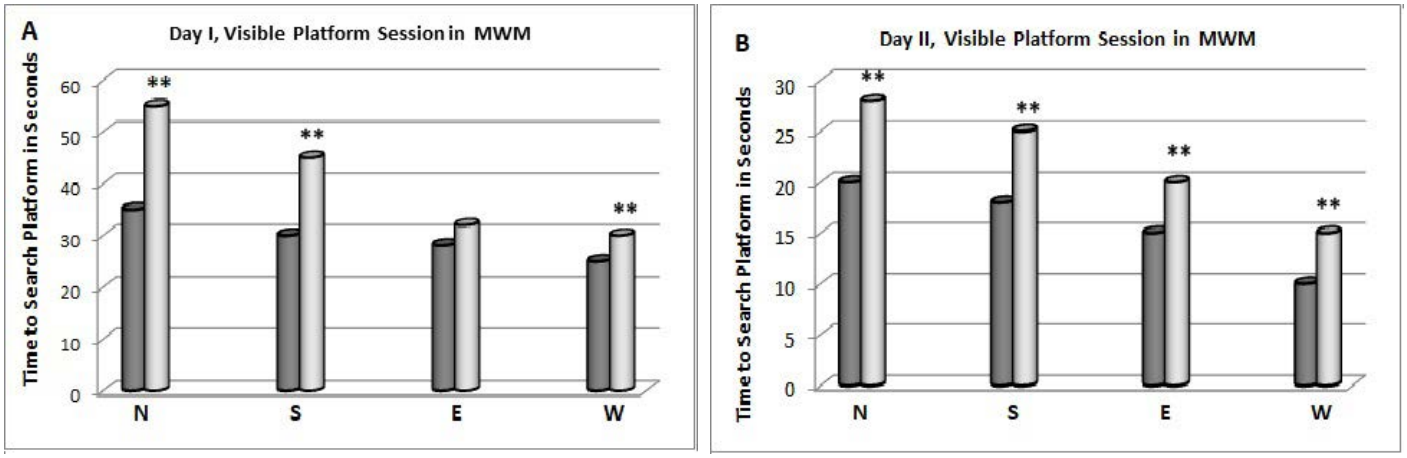

Figure 1. *Acquisition of spatial non-declarative task in MWM. Dark grey columns represent the data from CPST group, light gray columns – from PDMChS group. Designations on the abscissa: N-north, S –south, E – east, W – west. **=p<0.05.*

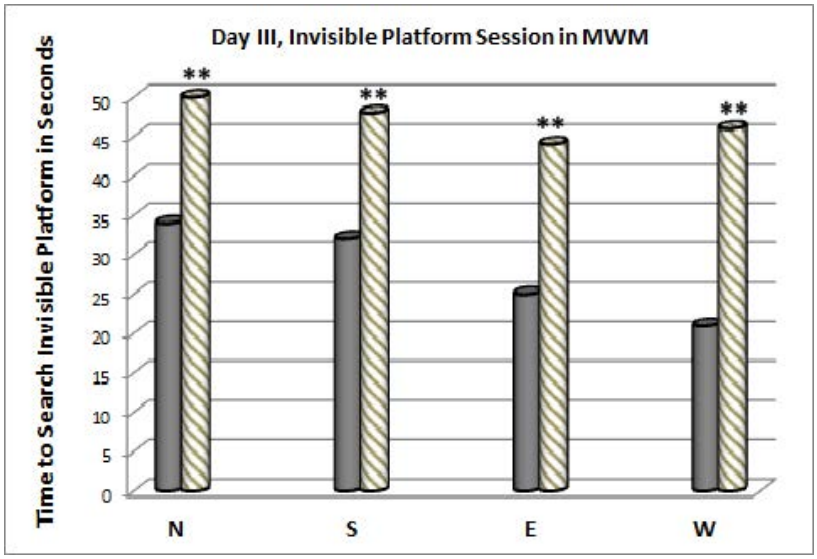

Figure 2. *Acquisition of spatial declarative task in MWM. Dark gray columns – data from CPST group; Hatched columns – data from EPDMChS group; on the abscissa: N-north, S –south, E – east, W –west. **=p<0.05.*

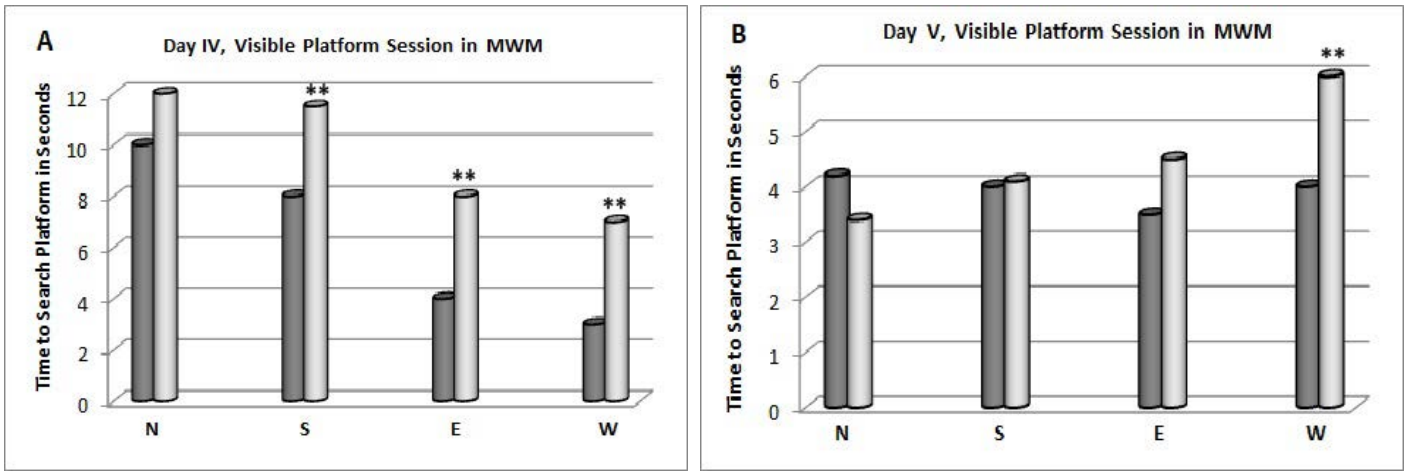

Figure 3. *Acquisition of spatial non-declarative task in MWM. Black columns – data from CPST group, Gray columns – data from EPDMChS group; N - north, S – south, E – east, W – west. **=p<0.05.*

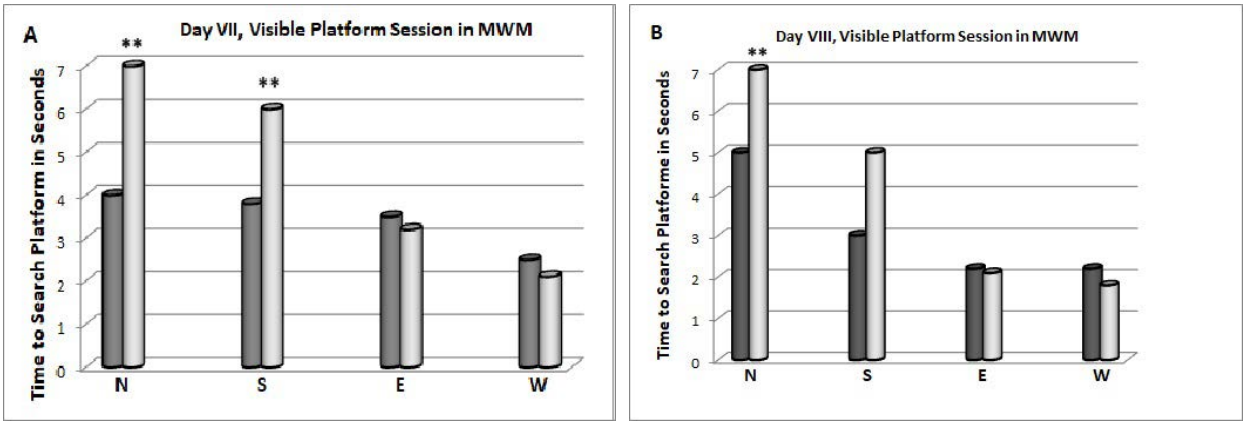

Figure 4. *Acquisition of spatial non-declarative task in MWM. Black columns – data from CPST group, Gray columns – data from PDMChS group N- north, S –south, E – east, W –west. **=p<0.05.*

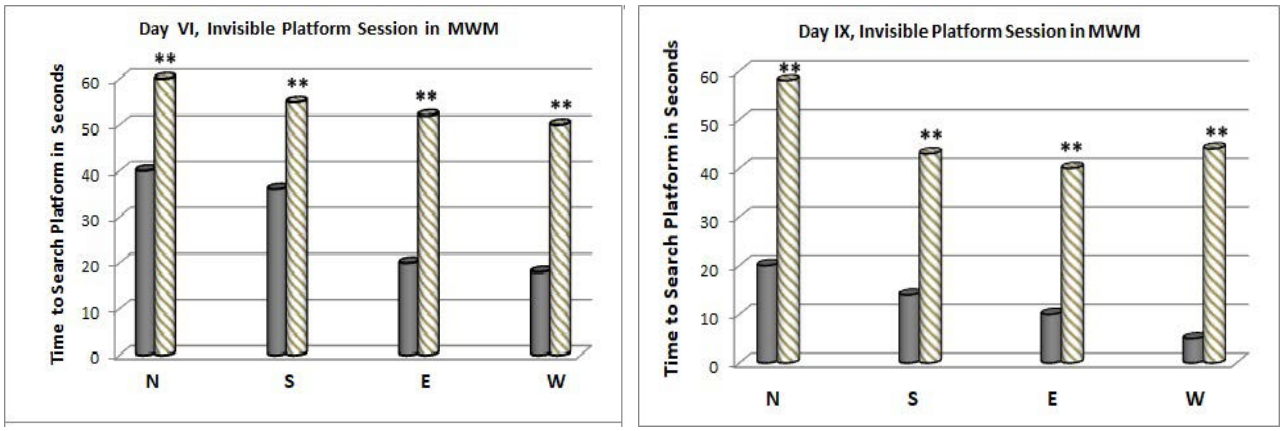

Figure 5. *Acquisition of spatial declarative task in MWM. Gray Columns – data from CPST group; hatched columns – data from EPDMChS group; N -north, S – south, E – east, W – west. **=p<0.05.*

positions (Figure 5B; Mean: N-20 sec in CPST group/58 sec in EPDMChS group; S- 14 sec in CPST/43 sec in EPDMChS; E- 10 sec in CPST/40 sec in EPDMChS; W- 5 sec in CPST/44 sec in EPDMChS; p<0.05).

After completing the invisible platform test for measuring the space tendency, animals were given in the tenth-day session a prob trial in which the platform was removed from MWM. In this case, in rats of the EPDMChS group, a significant impairment of spatial long-term declarative memory was found. After launching from the first starting position, experimental rats spent only 17% of the entire sample time in the quarter of the previous localization of the platform, and 13% after launching from the second starting position (Figure 6), which indicates a deficit of spatial long-term declarative memory in the MWM.

In the second part of our experiments, we investigated the possible long-lasting effects of EPDMChS on the adult neurogenesis of DG and hippocampal CA1 and CA3 fields. With these experiments, it was shown that EPDMChS suppresses adult neurogenesis in the DG which is manifested in the reduction of the number of granular cells (25±2 in CPST; 15±2 in PDMChS; p<0.05, Figure 7A). It is more evident in the percentage changes of the granular cells in the DG – mean 40% reduction in the adult rats of the EPDMChS group versus the data obtained in the CPST group (p<0.05, Figure 7B).

Quantitative analysis of the number of neurons in the hippocampal fields showed a significant reduction in the EPDMChS group in all studied areas of hippocampal CA1 and CA3 fields (p<0.05; Figure 8A). Percentage of the total number of neurons calculated from all studied sub-fields of the CA1 and CA3 layers reduces significantly in animals of the EPDMChS group (by 17% than in CPST group (Figure 8B).

It isn't a new fact that intact rats exposed to a novel environment, especially if it is dangerous, start active exploration and search for ways to avoid it. It becomes harder if the new environment is a water maze where the animals are forced to swim to find a safe route for survival. The intrinsic mechanism underlying such an important behaviour is not completely clear yet and retains topicality for further intensive research. For this aim, we investigated for the first time the role of the normal functioning of the brain MChS during the early postnatal development (P7-P28 days), which is a more vulnerable period for brain maturation and neurogenesis, for the normal course of declarative and non-declarative fear-motivated memory in MWM in the adult age.

As a result, we have shown for the first time that EPDMChS, applied in rat pups, dos does not disturb the process of non-declarative long-term spatial memory formation in adult age and despite its initial retardation proceeds finally with the same speed as in animals of CPST group. However, long-

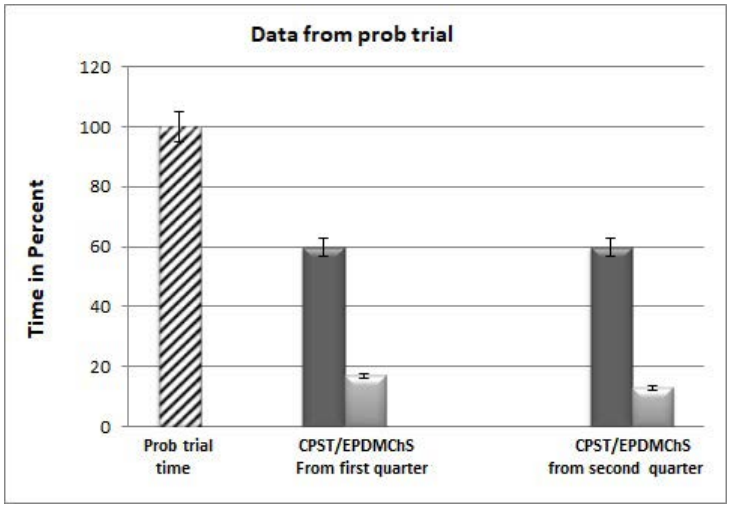

Figure 6. *Changes in the time spent on the quarter of the previous localization of the platform in the prob trial, Hatched column – prob trial time in percent; black columns – data from CPST group; gray columns – data from EPDMChS group.** =p<0.05.*

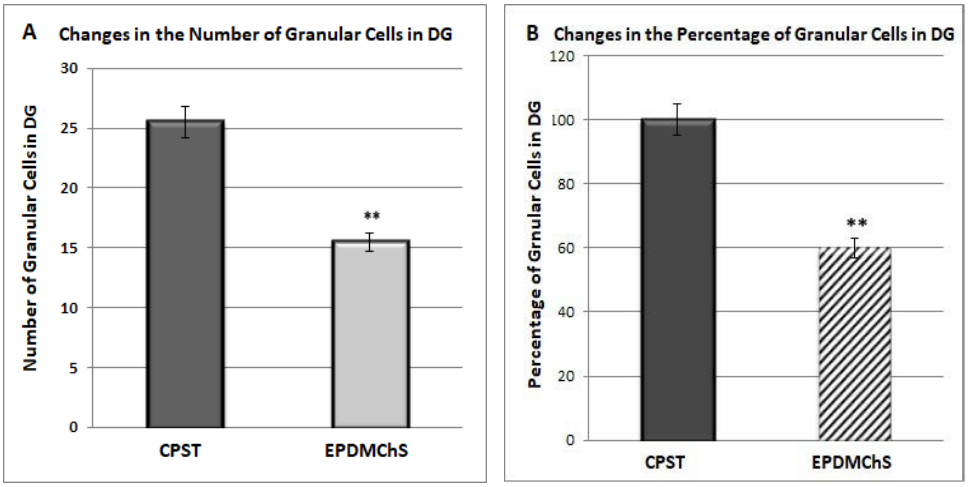

Figure 7. *Changes in the number and percentage of granular cells in the DG. p<0.05 is taken as an index of significance. More detail in the Text.*

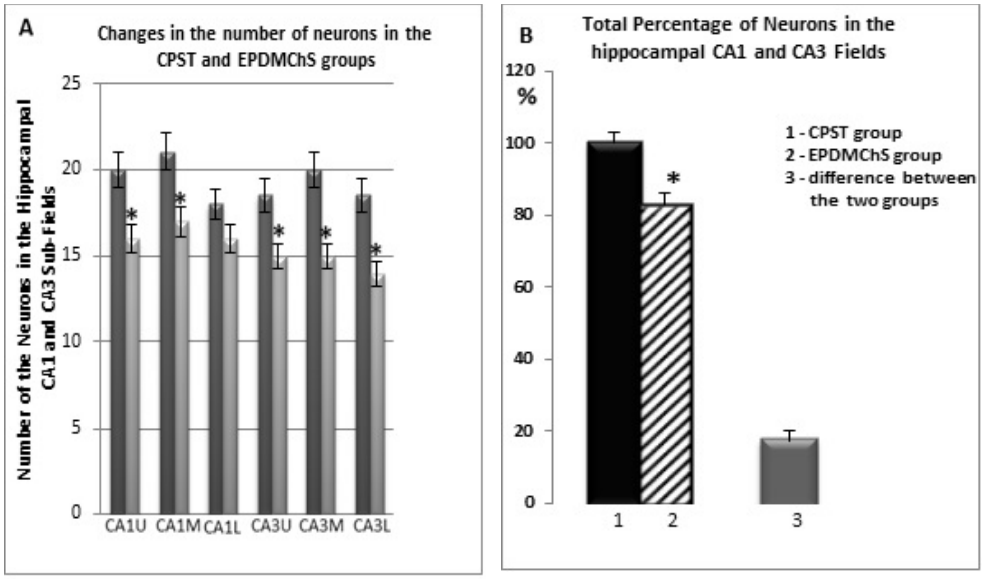

Figure 8. *Changes in adult hippocampal neurogenesis calculated by the number (A) and total percentage (B) of neurons in the CA1 and CA3 sub-fields of the hippocampus in adult rats. On the A: Black columns – data from the CPST group, Gray columns – data from the EPDMChS group. On the B: Total Percentage of Neurons in the hippocampal CA1 and CA3 Fields. p<0.05 is taken as the indicator of significance.*

term learning about the appropriate location of a safe platform concerning distant visual stimuli is impaired in the EPDMChS group, indicating the disturbance of long-term declarative memory in MWM in the adult age. These new data certify the high significance of normal functioning of the brain MChS, in the early period of postnatal development, for the formation in the adult age of hippocampus-dependent learning and memory in the spatial navigation test of MWM.

They agree with the early results of many other authors, obtained by the use of various other methodical approaches in mature animals, which are certified essential role of the MChS and hippocampus in the learning and memory [2-5,7-12,18], and also with the results of our early experiments with electrolytic lesion and/or section of the main hippocampal afferent systems (septal cholinergic and entorhinal glutamatergic) producing significant impairment of hippocampus-dependent spatial food motivated sound discrimination learning and memory [21-24].

More importantly, we find for the first time that the lasting effects of EPDMChS are also expressed in the significant worsening of adult hippocampal neurogenesis. This can help us to explain the intrinsic mechanisms of the causal interrelationship between the two significant long-lasting consequences of EPDMChS, a disorder of the hippocampus-dependent spatial memory and reduction in adult hippocampal neurogenesis.

Despite the widely accepted fact that the mAChRs in the hippocampus underlie memory formation, there is no consensus on which mAChRs subtypes are critical for memory processing. Interestingly, manipulations with higher receptor-specificity were generally less potent than those targeting multiple receptor subtypes, suggesting that mAChRs act in synergy to regulate memory processes [9]. It is known that five subtypes of muscarinic receptors, M1-M5. M1, M3, and M5 have a wide distribution in the brain [25,41]. M1 mAChRs, the most abundant sub-type in the hippocampus with expression mainly in the dendrites or somas [20,42], play a critical role in regulating the excitability of hippocampal neurons [20]. M2 and M4 mAChRs are mainly expressed at the synaptic terminals and modulate neurotransmitter release [28,30,36,43]. Though we have antagonized simultaneously, in the early postnatal period, all 5 subtypes of mAChRs that are widely distributed in the brain, the main focus of our attention must be paid to the M1, M2, and M4 mAChRs expressed highly in the hippocampus. Therefore, it could be hypothesized that despite the early postnatal simultaneous antagonizing of brain M1-M5 mAChRs, dysfunction of hippocampal M1, M2, and M3 receptors must play a major role in the spatial memory impairment and changes in adult hippocampal neurogenesis we obtained. At present, not all is known about the details of neuronal maturation, but it seems that after a period of 7 wk., the new neurons become indistinguishable from their older neighbour, so the entire period of adult neurogenesis takes approximately 7 wk. [34,44]. It is accepted that the numerous factors involved in the progression of adult hippocampal neurogenesis are connected through complex cross-talk signalling pathways [29,31,32,34,44].

In our experiments, we eliminated from the factors contributing to the adult neurogenesis, the muscarinic cholinergic one, during such a critical period of early postnatal development as the days P7-P28. More precisely, we are accented on the M1, M2, and M4 mAChRs that were antagonized at the level of the hippocampus, during this period. We believe it can be considered as a critical factor for the significant reduction in the number of granular cells in the DG and neurons in the CA1, and CA3 fields in the adult hippocampus. Disturbances obtained in our experiments in the hippocampus-dependent spatial declarative memory in the MWM might be produced by the reduction of adult hippocampal neurogenesis. Namely, it is known that the DG is one of the most plastic regions in the mammalian brain because it can generate principal neurons that integrate into the pre-existing networks throughout life [44]. Therefore, we can speculate at this stage that one possible explanation for our results may be the admission that the reduction of this integration/connectivity can be produced by the EPDMChS leading in adult age to the decrease in the number of granular cells in the DG and as a result the reduction in the number of neurons in the CA1 and CA3 fields of the adult hippocampus.

Conclusion.

Finally, with these experiments, we have shown that EPDMChS through antagonizing, during the three postnatal weeks (second, third, and fourth), of M1-M5 mAChRs have long-lasting effects revealed in the adult age in the reduction of adult hippocampal neurogenesis and significant impairment of hippocampus-dependent spatial, declarative learning and memory. We suppose a causal relationship between these two long-lasting effects of early postnatal dysfunction of the muscarinic cholinergic system.

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REFERENCES

1. Picciotto M.R, Higley M.J, Mineur Y.S. Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behavior. Neuron. 2012;76:116-129.
2. Berger-Sweeney J, Stearns N.A, Murg S.L, et al. Selective immune-lesions of cholinergic neurons in mice: effects on neuroanatomy, neurochemistry, and behavior. The Journal of Neuroscience: the official journal of the Society for Neuroscience. 2001;21:8164-8173.
3. Blokland A, Honig W, Raaijmakers W.G. Effects of intra-hippocampal scopolamine injections in a repeated spatial acquisition task in the rat. Psychopharmacology. 1992;109:373-376.
4. von Linstow Roloff, Harbaran D, Micheau J, et al. Dissociation of cholinergic function in spatial and procedural learning in rats Neuroscience. 2007;146:875-88.
5. Opello K.D, Stackman R.W, Ackerman S, et al. AF64A (ethylcholine mustard aziridinium) impairs acquisition and performance of a spatial, but not a cued water maze task: relation to cholinergic hypofunction. Physiology & behavior. 1993;54:1227-1233.
6. Parent M.B, Baxter M.G. Septo-hippocampal ACh: involved in but not necessary for learning and memory? Learn Mem. 2004;11:9-20.
7. Haam J, Yakel J.L. Cholinergic modulation of the hippocampal region and memory function. J. Neurochem. 2017;142:111-121.

8. Kesner R.P. Neurobiological views of memory. In J. L. Martinez & R. P. Kesner (Eds.), Learning and memory: A biological view. 1986:399-438.
9. Leaderbrand K, Chen H.J, Corcoran K.A, et al. Muscarinic acetylcholine receptors act in synergy to facilitate learning and memory. 2016;23:631-638.
10. Levey A.I. Muscarinic acetylcholine receptor expression in memory circuits: implications for treatment of Alzheimer disease. Proceedings of the National Academy of Sciences of the USA. 1996;93:13541-13546.
11. Martyn A.C, De Jaeger X, Magalhaes A.C, et al. Elimination of the vesicular acetylcholine transporter in the forebrain causes hyperactivity and deficits in spatial memory and long-term potentiation. Proc. Nat. Acad. of Sci., USA. 2012;109:17651-17656.
12. Nilsson O.G, Leanza G, Rosenblad C, et al. Spatial learning impairments in rats with selective immunolesion of the forebrain cholinergic system. Neuroreport. 1992;3:1005-1008.
13. Prado V.F, Janickova H, Al-Onaizi M.A, et al. Cholinergic circuits in cognitive flexibility. Neuroscience. 2017;345:130-141.
14. Solary N, Hangya B. Cholinergic modulation of spatial learning, memory and navigation. European Journal of Neuroscience. 2018;48:2199-2230.
15. Aloisi A.M, Casamenti F, Scali C, et al. Effects of novelty, pain, and stress on hippocampal extracellular acetylcholine levels in male rats. Brain Res. 1997;748:219-226.
16. Bianchi L, Ballini C, Colivicchi M.A, et al. Investigation on acetylcholine, aspartate, glutamate, and GABA extracellular levels from ventral hippocampus during repeated exploratory activity in the rat. Neurochem. Res. 2003;28:565-573.
17. Kimura H, McGeer P.L, Peng F, et al. Choline acetyltransferase-containing neurons in rodent brains demonstrated by immunohistochemistry. Science. 1980;208:1057-1059.
18. Kolisnyk B, Al-Onaizi M.A, Hirata P.H, et al. Forebrain deletion of the vesicular acetylcholine transporter results in deficits in executive function, metabolic, and RNA splicing abnormalities in the prefrontal cortex. J. Neuroscience. 2013;33:14908-14920.
19. Rashidy-Pour A, Motamedi F, Motahed-Larijani Z. Effects of reversible inactivation of the medial septal area on reference and working memory versions of the Morris water maze. Brain Research. 1996;709:131-140.
20. Yi F, Ball J, Stoll K.E, et al. Direct excitation of parvalbumin-positive interneurons by M1 muscarinic acetylcholine receptors: roles in cellular excitability, inhibitory transmission and cognition. The Journal of physiology. 2014;592:3463-3494.
21. Nachkebia N.G, Oniani T.N. The role of the septo-hippocampal system in the regulation of learning and memory. In: «Neurophysiology of motivation, memory and sleep-wakefulness cycle», (T.Oniani Ed.) Tbilisi, Metsniereba. 1985;N4:59-93.
22. Nachkebia N, Lotkipanidze. N, Oniani. T, et al. The influence of reversible functional inactivation of hippocampus on the elaboration of spatial sound discrimination in cats. In Neurobiology of sleep-wakefulness cycle. 2002;2:6-20.
23. Oniani T.N, Nachkebia N.G, Nachkebia A.J, et al. The influence of electrocoagulation of the septum and section of the entorhinal cortex on general behavior and memory in cats. Acta Physiologica Hungarica, Academiai Kiado, Budapest. 1989;74:9-25.
24. Oniani T, Nachkebia N. The role of the septo-hippocampal system and paradoxical phase of sleep, in the regulation of learning and memory. In: Neurobiology of Sleep-Wakefulness Cycle. 2001;1:64-94.
25. Fryer A.D, Christopoulos A, Nathanson N. M. Muscarinic receptors, in Handbook of experimental pharmacology (Allison D. Fryer, Arthur Christopoulos, Neil M. Nathanson, ed.), pp. 1 online resource (xiii, 499p). Springer, Berlin; New York. 2012.
26. Armstrong D.M, Saper C.B, Levey A.I, et al. Distribution of cholinergic neurons in rat brain: demonstrated by the immunocytochemical localization of choline acetyltransferase. The Journal of Comparative Neurology. 1983;216:53-68.
27. Mesulam M.M, Mufson E.J, Wainer B.H, et al. Central cholinergic pathways in the rat: an overview based on an alternative nomenclature (Ch1-Ch6). Neuroscience. 1983;10:1185-1201.
28. Woolf N.J, Butcher L.L. Cholinergic systems in the rat brain: III. Projections from the pontomesencephalic tegmentum to the thalamus, tectum, basal ganglia, and basal forebrain, Brain research bulletin. 1986;16:603-637.
29. Paez-Gonzalez P, Asrican B, Rodriguez E, et al. Identification of distinct ChAT(+) neurons and activity-dependent control of postnatal SVZ neurogenesis. Nature neuroscience. 2014;17:934-942.
30. Zheng F, Wess J, Alzheimer C. M2 muscarinic acetylcholine receptors regulate long-term potentiation at hippocampal CA3 pyramidal cell synapses in an input-specific fashion. Journal of neurophysiology. 2012;108:91-100.
31. Bruel-Jungerman E, Lucassen P.J, Francis F. Cholinergic influences on cortical development and adult neurogenesis. Behavioural Brain Research. 2011;221:379-388.
32. Corns L.F, Atkinson L, Daniel J, et al. Cholinergic Enhancement of Cell Proliferation in the Postnatal Neurogenic Niche of the Mammalian Spinal Cord. Stem Cells. 2015;33:2864-76.
33. Hvoslef-Eide M, Oomen C.A. Adult neurogenesis and pattern separation in rodents: a critical evaluation of data, tasks and interpretation. Frontiers in Biology. 2016;11:168-181.
34. Kempermann G, Song H, Fred H. Neurogenesis in the Adult Hippocampus. Cold Spring Harb. Perspect. Biol. 2015;7:a018812.
35. Dannenberg H, Pabst M, Braganza O, et al. Synergy of Direct and Indirect Cholinergic Septo-Hippocampal Pathways Coordinates Firing in Hippocampal Networks. Journal of Neuroscience. 2015;35:8394-8410.
36. McQuiston A.R, Madison D.V. Muscarinic receptor activity has multiple effects on the resting membrane potentials of CA1 hippocampal interneurons. J. Neuroscience. 1999;19:5693-5702.
37. Nachkebia N, Chkhartishvili E, Mchedlidze O, et al. Lasting effects of early postnatal dysfunction of the brain muscarinic cholinergic system and its' super-sensitivity in adult age – character of sleep disorders and behavioral disturbances J. of Physiology and Pharmacology. 2021;72:39-40.

38. Shavgulidze M, Babilodze M, Rogava N, et al. Early postnatal dysfunctioning of the brain muscarinic cholinergic system and the disorders of fear-motivated learning and memory. Georgian Medical News. 2021;321:125-131.
39. Morris R.G.M. Spatial localization does not require the presence of local cues. Learning and Motivation. 1981;12:239-260.
40. Stewart C.A, Morris R.G.M. The water-maze. In: Sahgal A., editor. Behavioural Neuroscience, Volume I, A Practical Approach. IRL Press at Oxford University Press; Oxford: 1993:107-122.
41. Eglen R.M. Overview of muscarinic receptor subtypes. Handb. Exp. Pharmacol. 2012;208:3-28.
42. Wall S.J, Yasuda R.P, Hory F, et al. Production of antisera selective for m1 muscarinic receptors using fusion proteins: distribution of m1 receptors in rat brain. Molecular pharmacology. 1991;39:643-649.
43. Dasari S, Gulledge A.T. M1 and M4 receptors modulate hippocampal pyramidal neurons. Journal of Neurophysiology. 2011;105:779-792.
44. Bond A.M, Ming G.L, Song H. What Is the Relationship Between Hippocampal Neurogenesis Across Different Stages of the Lifespan? Front. Neurosci. 2022;16:891713.

Tavis tvinis muskarinuli qolinerguli sistemis adreuli postnataluri disfunqciis xanrgZlivi efeqtebi daswavlasa da mexsierebaze da hipokampis zrdasrul neirogenezzemelano SavguliZe, neli maRlakeliZe, nino rogava, xaTuna beJaniSvili, nargiz naWyebia*

ivane beritaSvilis eqsperimentuli biomedicinis centris Zil-RviZilis ciklis neirobiologiis laboratoria, Tbilisi, saqarTvelo

reziume

kvlevis mizani. naSromis mizans Tavis tvinis muskarinuli qolinerguli sistemis adreuli postnataluri disfunqciis (mqsapd), rogorc hipokamp-damokidebuli sivrciTi mexsierebis da hipokampis zrdasruli neirogenezis darRvevebis SesaZlo ZiriTadi faqtoris kvleva Seadgenda.

masala da meTodebi. Eeqsperimentebi Catarda TeTr, velur virTagvebze (n=15 jgufSi). mqsapd-s vaxdendiT laboratoriaSi SemuSavebuli axali meTodiT - m1-m5 muskarinuli qolinoreceptorebis adreul postnataluri (p7-p28 dReebi) dablokva, RlapebSi, skopolaminis yoveldRiuri ineqciiT; sakontrolo Rlapebi igive moculobis fiziologiur sxnars Rebulobdnen. sivrciTi mexsierebis da neirogenezis cvlilebebi zrdasrul asakSi Seiswavleboda.

Sedegebi da daskvnebi. pirvelad iqna naCvenebi, rom mqsapd xangrZliv efeqtebs avlens, rac zrdasrul asakSi, hipokampis neirogenezis da sivrciTi xanrgZlivi mexsierebis gauaresebaSi vlindeba. ganixileba mqsapd-is da am ori tipis darRvevis SesaZlo mizezobrivi urTierTkavSiri.

sakvanZo sityvebi – muskarinuli qolinerguli sistema, postnataluri disfunqcia, hipokampis neirogenezi, sivrciTi daswavla da mexsiereba

Длительные эффекты ранней постнатальной дисфункции мускариновой холинергической системы головного мозга на пространственное обучение и память и взрослый нейрогенез гиппокампа.

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РЕЗЮМЕ

Цель Исследования. Изучалась ранняя постнатальная дисфункция мускариновой холинергической системы (РПДМХС) как возможный фактор в вызове нарушений нейрогенеза гиппокампа и зубчатой извилины и гиппокамп-зависимого пространственного обучения и памяти во взрослом возрасте.

Материал и методы. Использовались белые дикие крысы (n=15 в каждой группе). РПДМХС вызывали новым, разработанным нами методом, включающим раннюю постнатальную (П7-П28 дни) блокаду холинорецепторов М1-М5 у крысят путем ежедневного подкожного введения скополамина. Контрольным крысятам вводили равный объем физиологического раствора. В зрелом возрасте изучали гиппокамп-зависимое обучение и память в контрольной и экспериментальной группах в водном бассейне Морриса, а нейрогенез гиппокампа и зубчатой извилины - в замороженных слоях, окрашенных по методу Ниссля.

Результаты и выводы. Впервые показано что РПДМХС, примененная в дни П7–П28, оказывает длительное воздействие, проявляющееся во взрослом возрасте в нарушениях взрослого нейрогенеза гиппокампа и пространственного обучения и памяти. Подчеркивается возможная причинно-следственная связь между РПДМХС и этими двумя типами нарушений.

Ключевые слова. Мускариновая холинергическая система, Постнатальная дисфункция, Нейрогенез взрослого гиппокампа, Пространственное обучение и память.