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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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BEHAVIORAL AND NEUROCHEMICAL CHANGES DURING INTRANASAL ADMINISTRATION OF ALPHA-GLUTAMYL-TRYPTOPHAN AND CHELATE COMPLEX OF ZINC ARGINYL-GLYCINATE ON MONOAMINE SYSTEMS DYSFUNCTIONS KNOCK-OUT MODELS

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Abstract.

Monoamine neurotransmitter system dysfunctions lead to behavioral disorders, cognitive metabolic, and other pathological conditions. In this case, different amino acids are precursors of monoamines, while the parenteral path of monoamine administration has pharmacological restrictions. Therefore, intranasal administration one of the most promising methods of delivering an active substance is. The purpose of the work is to study the effect of intranasal administration of a chelate complex of zinc arginyl-glycinate and alpha-glutamyl-tryptophan dipeptide on behavioral and neurochemical changes in acute and chronic experiments.

Materials and methods: The studies used outbred Wistar and DAT-KO rats, and inbred C57Bl6 and TAAR1-KO mice. Using intranasal administration of a chelate complex of zinc arginyl-glycinate and alpha-glutamyl-tryptophan dipeptide we tested methods for evaluating different behavioral indicators and the level of cerebral monoamines and their metabolites.

Results: An anxiolytic effect of zinc arginyl-glycinate and its combination with alpha-glutamyl-tryptophan was revealed. Both drugs have a physiological effect on the autonomic nervous system, but the determination of their operating mechanisms requires further research.

Conclusion: Thus, these data indicate that intranasal delivery of the dipeptides is effective during acute and chronic intranasal administration in rodents, the latter showed a change in the anxiety indicator. Acute AG intranasal administration demonstrated signs of lower anxiety and depressive-like behavior in C57Bl6 mice.

The acute intranasal administration of a chelate complex zinc arginyl-glycinate and combination with alpha-glutamyl-tryptophan in doses of 50–100 mg/kg of body weight may be used for pre-clinical studies as a new anxiolytic/antidepressant.

Key words. Alpha-glutamyl-tryptophan, Zinc arginyl-glycinate, Intranasal administration, Knock-out rats and mice, Dopamine reuptake, TAAR1, Depression, ADHD, Anxiety.

Introduction.

Monoamine neurotransmitter system dysfunctions lead to behavioral disorders, cognitive, metabolic, and other pathological conditions [1-4]. Trace amines and dopamine are among the most important regulators of complex forms of behavioral disorders [2,5].

Trace amines (TA) are a group of endogenous amine metabolites that are formed as a result of the decarboxylation of all known amino acids and are also neurotransmitter metabolites (dopamine, serotonin, noradrenaline, etc.) [1,2,6,7].

Trace amine-associated receptor 1 (TAAR1) is a G-protein coupled receptor (GPCR) that is expressed in the monoaminergic regions of the cortex, limbus, and mesencephalon [7-15]. It is activated by endogenous trace amines and plays an important role in modulating dopaminergic, serotoninergic, and glutamatergic chains. The important role of the trace amine system in regulating the operation of the dopamine systems was shown using TAAR1-KO mice. In particular, TAAR1 activation could regulate dopamine release in the adjacent core of the prosencephalon [1,2]. The drug Ulotaront (SEP-363856) is currently being developed; it is a receptor 1 agonist, with receptor 5-HT1A agonist activity that is in the 3rd phase of FDA clinical trials for groundbreaking therapy to treat schizophrenia [16-19].

Trace amines and dopamine break down under the influence of monoaminoxidase A and B enzymes (MAO-A and MAO-B) that display activity in various organs and tissues, including enterocytes [1,12,20-26]. Consequently, the parenteral path for monoamine administration has its pharmacological restrictions. Therefore, one of the most promising methods of delivering an active substance is the intranasal path.

Dopamine (DA) is an important regulator for behavioral and metabolic disorders. The dopamine transporter DAT is coded by the *SLC6A3* gene and plays a key role in the DA reuptake mechanism. Consequently, its normal activity level is important for the correct functioning of the dopaminergic systems [27,28]. Disorders in dopaminergic transmission regulation and prosencephalon functions are observed during neurodegenerative, mental, and behavioral disorders, such as schizophrenia, attention deficit and hyperactivity disorder (ADHD), obsessive-compulsive disorders (OCD), and Parkinson's disease (PD) [29].

DAT-KO rats are a good translational model for studying hyperactivity and other human illnesses related to changes in DA functioning. Due to this genetic defect, dopamine slowly accumulates in the synaptic fissure. Rats in this model display a higher concentration of extracellular DA in the striatum, while the total DA content in the tissues is noticeably diminished. Animals of this develop normally but display elevated impulsiveness, hyperactivity, stereotypical behavior, and cognitive disorders. Additionally, these rats show metabolic changes since with an equivalent diet they have lower body weight compared to heterozygotes (HET) and rats without the knock-out gene (WT) [4].

Different peptides are currently used widely to treat a broad spectrum of neurological and alimentary pathological conditions [30-33]. Most of these medicinal forms are given perorally, which significantly restricts the therapeutic effect due to the effect of intestinal microflora and the hepatic detoxication system.

α-Glutamyl-tryptophan (GT) is a synthetic peptide immunomodulatory. The drug modulates metabolic processes in the cells; stimulates the functional activity of immune system

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cells; stimulates tissue regeneration; accelerates wound healing; activates the functions of endotheliocytes, macrophages, and leukocytes in the infection focus; and inhibits the generation of histamine and serotonin during inflammation [34].

It is important to note that in the study on animals conducted by two other groups of authors, GT promoted faster wound healing via modulating the activity of different proteinases [35] and demonstrated an anti-angiogenic effect on models *in vitro*, *ex vivo*, and *in vivo* [36].

There is less information in the literature about the chelate complex of zinc arginyl-glycinate dihydrochloride (AH). It is part of the drug used to treat patients with chronic prostatitis and comorbid disorders of sexual and reproductive functions. The chelate complex has anti-inflammatory and antioxidant effects and reduces the level of oxidative stress [37]. Another interesting example is β-lactolin - a glycine-containing peptide that improved depression-like behavior via dopamine-D1-like receptor [38].

Despite the accumulated experience in using the GT dipeptide and chelate AG complex in clinical practice, the operating mechanism of these substances with intranasal administration continues to be actively studied. Also considering their amino acid nature (monoamine precursors), the task was set to studying the possible link between both drugs and key dopamine system modulators — TAAR1-mediated regulation of dopamine release and dopamine reuptake by DAT.

The purpose of the study was to investigate the impact of a chelate complex of zinc arginyl-glycinate and alpha-glutamyl-tryptophan on behavioral and neurochemical changes during acute and chronic intranasal administration on rodent knock-out models of cerebral diseases.

Materials and Methods.

Animals:

The studies were made on male outbred Wistar rats (n=56), DAT-KO rats (n=30), TAAR1-KO mice (n=19), and inbred C57Bl6 mice (n=16). During all the experiments, the rats and mice were kept in groups of 6–8 individuals in cages on sawdust bedding at temperature 20–22°C and lighting conditions 12/12 h. Work with the animals was in compliance with the Guidelines and Care and Use of Laboratory Animals (ILAR, DELS). All the experiments were approved by the Saint Petersburg State University Ethical Committee for Animal Research (No. 131-03-10 of 22 November 2021).

Active substances, doses, and administration method:

A suspension of freeze-dried alpha-glutamyl-tryptophan (GT) and chelate complex of zinc arginyl-glycinate (AG) preparations (MBNKB "Cytomed JSC", Russia) was dissolved in a physiological solution before each experiment.

Doses and methods of administration of the studied active substances are presented in Table 1.

All the substances under consideration were administered daily to the test animals in the aforementioned doses intranasally in acute (single) and chronic (2 weeks daily) experiments in a volume of 50–200 μl depending on the animal species and the solubility of the substances under consideration.

Methods for evaluating behavioral indicators.

Circular Open Field Test.

The Open Field (OF) test evaluates locomotor and search activity indicators, as well as other behavioral changes. The arena lighting was 600 lux. DAT-KO rats were placed in the center of the arena for 30 minutes to reach the greatest level of dopamine in the cerebral striatum (warm-up). Within 30 minutes, the substances under consideration were administered intranasally in the test doses and the experiment continued for the next 45 minutes. During both time periods (within 30 and 45 minutes), the total distance run by the animals and divided by field sectors, speed, trajectory, number of rests, grooming microstructure, and other behavioral indicators were measured.

Square Open Field Test.

To test the acute effect of the drugs, the Square Open Field test was used to record motor and inquisitorial activity.

In order to evaluate the chronic effect of the drug, a round arena was used with 12 holes, each 2 cm in diameter. The arena lighting was 600 lux. The behavior was recorded for 5 minutes. The motor activity parameters, such as overall traversed path, motion speed, and time spent in the center were recorded automatically using the Noldus Ethovision (Noldus, Spain) video tracking system. Rests and grooming time were evaluated visually.

Elevated Plus Maze Test.

The anxiety level in the animals was evaluated using Open Science (Russia) equipment. The Elevated Plus Maze (EPM)

Table 1. *Doses, duration of the experiment, and methods of administration of the studied active substances.*

Intranasal administration	Active substances	Rats/mice line	Doses, mg/kg body weight	Duration of the experiment, days
Acute	GT	DAT-KO rats	10	1
		Wistar rats	10, 50 and 100	1
		C57Bl6 mice	10	1
		TAAR1-KO mice	10	1
	AG	DAT-KO rats	10	1
		Wistar rats	10, 50 and 100	1
		C57Bl6 mice	10	1
		TAAR1-KO mice	10	1
	AG+GT	TAAR1-KO mice	100 per each	1
Chronic	GT	Wistar rats	50	14
	AG	Wistar rats	50	14

test made it possible to assess of the degree of the emotional reaction of fear and anxiety, motor activity, rate of orientation reactions, and other behavioral changes.

Wistar rats were placed in the center of the arena for 5 minutes. The anxiety level of the animals was evaluated in the Elevated Plus Maze test, which makes it possible to assess the degree of the fear and anxiety reaction and motor activity. Within 5 minutes of the laboratory animals being in the labyrinth, a determination was made of the number of transitions from one zone to another, distance traversed in closed (CA) and open (OA) labyrinth arms (and their ratio CA/OA, a decrease in which indicates a drop in anxiety level), number of overhangs in open chutes, number of entries and time spent in open and closed chutes, grooming microstructure indicators, etc.

After each animal, the arena was wiped with a sponge moistened with alcohol.

Light/Dark Transition Test.

The Light-Dark Transition test is conducted to evaluate the anxiety level of animals. The arena consists of two parts, each 20 x 20 cm in size, wall height of 20 cm connected by an opening 5 x 5 cm in size. The bright light intensity was 600 lux.

The black zone has an opaque roof, so it is dark, while the white zone is brightly lit. The animal was placed in the light zone and left to freely examine the situation for 3 minutes. At this time, an evaluation was made of the time spent by the animals in the light part of the unit, using the Noldus Ethovision (Noldus, Spain) video tracking system.

Forced Swim Test.

A Porsolt forced swim test [39] was conducted to assess the depressive-like behavior parameters. The animals were placed for 6 minutes in a transparent vessel (diameter 10 cm, height 25 cm) filled by 2/3. The duration of swimming and the immobility (stopping) were recorded.

Evaluating the grooming microstructure indicators.

The grooming microstructure indicators (latency (in s) of the start of grooming (LG) and total time (in s) spent on grooming (TGT)) were analyzed visually based on analysis of a video recording of the Open Field test in slow motion [40].

Grooming analysis also included the depression ratio (DR) — the ratio of total time spent on grooming to the start of grooming latency (TGT/LG) [6].

Stress-induced Hyperthermia Test.

The Stress-induced Hyperthermia (SIH) test [1] was designed to make a screening evaluation of the drugs and their candidates and was based on measurement (and change) in rectal temperature caused by the reaction of the autonomic nervous system to the stress factor (neurogenic hyperthermia). In this case, an increase or decrease in the rectal temperature indicators (compared to the control) within 20 minutes and/or 1 hour after administering the test drugs indicates a stress-induced response by the autonomic nervous system to the administered substance and its overall neurotropic effect.

The SIH test was given to Wistar rats with a slight addition to the technique. Body temperature was measured three times (thermometer error ±0.1 °C). The temperature of each rat was measured: t = 0 min (T1), t = +20 min (T2), and t = +60 min (T3). The rectal method of temperature measurement (T1) was the main stress factor. The difference in temperature (T2 – T1) was considered the main SIH development value. The difference in temperature (T3 – T2) was considered the auxiliary SIH development value. The total temperature difference (T3 – T1) was also calculated to evaluate the temperature change during the entire experiment. The experimental rat group received the aforementioned trace amines, GT, and AG in a dose of 10 mg/kg intranasally immediately after measuring T1. The control group received a physiological solution (0.9 % NaCl).

Evaluating the level of cerebral monoamines and their metabolites.

In order to evaluate the neurochemical parameters in the brains of the knock-out animals, we determined the monoamine level in the cerebral tissues of Wistar rats. For this purpose, we measured the concentration of dopamine, serotonin, noradrenaline, and their metabolites in different parts of the brain: the striatum and frontal cortex. Individual cerebral structures were isolated and then frozen in liquid nitrogen. The frozen samples were weighed and homogenized in the corresponding volume of $HClO_4$, centrifuged, and filtered using centrifuge filters. Monoamine concentrations were measured in the resulting samples using highly effective liquid chromatography with electrochemical detection (HELC-ED) on an Eicom HTEC-500 chromatograph with carbon electrode WE-3G and potential +650 mV. Separation used a CA-50DS column (150 x 2.1 mm, Eicom, Japan). The concentration of the following monoamines was measured: noradrenaline (NE), dopamine (DA), serotonin (5HT), homovanillic acid (HVA), 5-indoleacetic acid (5-HIAA), 3,4-dihydroxyphenylacetic acid (DOPAC).

Statistical processing of results.

A statistical analysis was made using non-parametric Mann-Whitney criteria (U-test) and Kruskal-Wallis (H-test) using the SPSS 16.0 and Prism GraphPad 6.0 programs. The results were presented as average values ± standard mean error.

Results.

An experiment was conducted on Wistar rats to study the impact of intranasal administration of GT and AG on different behavioral indicators (locomotor activity, anxiety level, grooming microstructure indicators, etc.) in the Elevated Plus Maze test.

No reliable differences were found in the anxiety indicators. Nevertheless, the average number of rests detected in the Wistar rat group that received GT intranasally was reliably higher than in the control group.

The grooming microstructure indicators did not reveal reliable differences between the Wistar rat group that received intranasally and the control group. The average grooming latency values did not reveal any differences between the control and the studied group.

In the Open Field test, the level of locomotor activity in DAT-KO rats, in the group that received GT intranasally, was a reliable increase compared to the control group only at the 50th minute of the experiment (Figure 1A).

In the group that received AG intranasally, the locomotor activity indicators changed reliably compared to the control group in the last 5 minutes of the experiment (Figure 1).

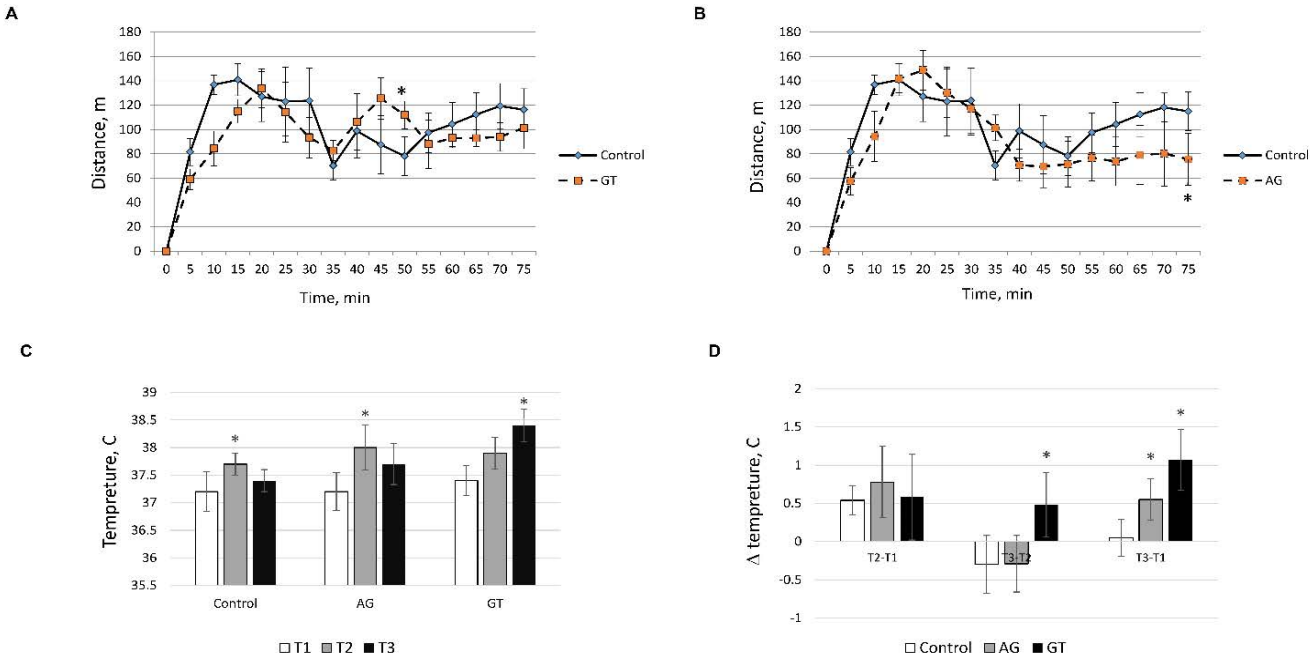

Figure 1. *Dynamics of changes in the locomotor activity of DAT-KO rats that received alpha-glutamyl-tryptophan (A) and zinc arginyl-glycinate (B) intranasally in the Open Field test and change in autonomic nervous system indicators (C and D) in Wistar rats in the Stress-induced Hyperthermia test. Data are expressed as mean ± SEM. * — $p < 0.05$ compared to the control group, non-parametric Mann-Whitney criterion (U-test).*

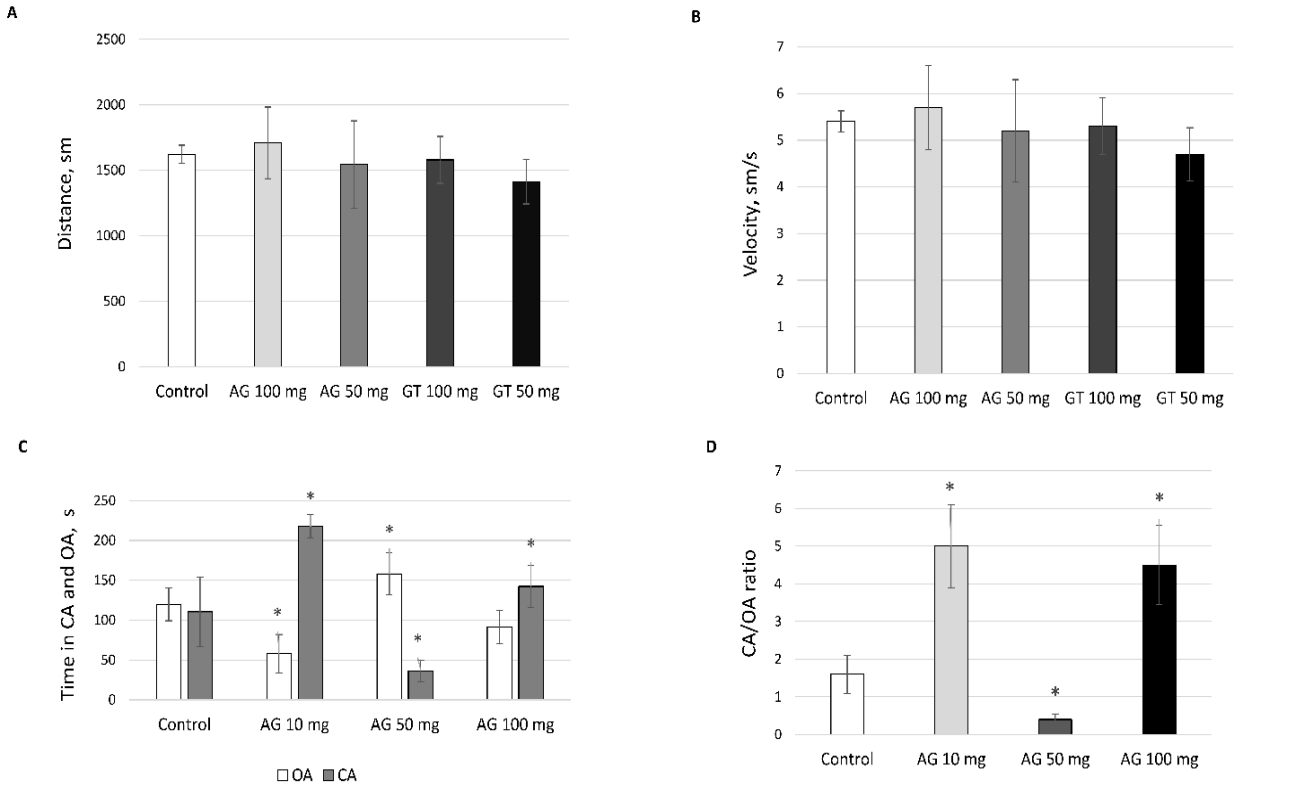

Figure 2. *Distance (A) and velocity (B) traversed by Wistar rats that received different doses of alpha-glutamyl-tryptophan (GT) and zinc arginyl-glycinate (AG) intranasally in the Open Field test, time spent in closed (CA) and open (OA) arms (C) and ratio of time CA/OA (D) spent by the Wistar rats in the labyrinth and received a combination of AG and GT in the Elevated Plus Maze test. Data are expressed as mean ± SEM. * — $p < 0.05$ in relation to the Control group, non-parametric Kruskal-Wallis criterion (H-test).*

Nevertheless, in the last 20 minutes of the experiment, a stable trend was observed towards a drop (by 20–30 %) in the locomotor activity of the DAT-KO rats in the group that received AG intranasally compared to the control group.

An experiment was conducted on Wistar rats to study the impact of intranasal administration of AG and GT on the neurogenic hyperthermia indicators in the Stress-induced Hyperthermia (SIH) test. The results of the effect of alpha-glutamyl-tryptophan on the neurogenic hyperthermia indicators in Wistar rats with intranasal administration in the SIH test are given in Figure 1.

Neurogenic hyperthermia, similar to the control group indicators, was observed with intranasal administration of GT and AG (T2), however, in the case of GT, this effect occurred more intensively not only within 20 minutes (T2) but also within an hour after the start of the experiment (T3) (Figure 1C). It follows from Figure 2D that no reliable difference in the indicators of the main values (T2 – T1) for development of neurogenic hyperthermia was found between all the studied groups. Nevertheless, analysis of the auxiliary SIH indicators demonstrated a reliable rise in the values (T3 – T2) for GT and the total temperature change (T3 – T1) for both drugs under consideration GT and AG.

The grooming microstructure indicators (TGT, DR) and anxiety did not reveal any reliable differences between the groups that received GT and AG intranasally and the control animals.

The study was conducted on C57Bl6 mice. The animals were given GT and AG preparations in a dose of 10 mg/kg intranasally. Within 20 minutes after the first administration to the animals, the Square Open Field, Light-Dark Transition, Elevated Plus Maze, and Forced Swim tests were conducted to evaluate the acute effect of the drug. After this, the animals were given drugs intranasally for 14 days, after which a re-test was given within 20 minutes after the last administration in the Square Open Field, Light-Dark Transition, and Forced Swim tests. After this brain structures were collected from the animals for subsequent analysis of monoamine level.

In the Forced Swim test, after a single administration of drugs, a drop was observed in the mean stopping time in the group that received GT in a dose of 100 mg/kg of body weight. In the Forced Swim test after chronic administration of the drugs in a dose of 10 mg/body weight, no changes were observed in the stopping time, which could indicate the absence of an accumulative effect.

In the Square Open Field test, the animals did not have any differences in the traversed path.

The number of rests did not differ significantly. The findings demonstrated that acute administration of the drugs did not have a significant effect on the motor and study activity. The time spent in the central zone also did not differ. In this case, the AG group had a shorter grooming time, which may indicate a lower anxiety level.

Thus, during acute and chronic intranasal administration of drugs GT and AG, the latter showed a change in the anxiety indicator. With acute AG administration, the animals demonstrated signs of reduced anxiety and depressive-like behavior. Chronic AG administration in the Square Open Field test showed a slight tendency towards higher anxiety levels. Here, as in the evaluation of the acute effect of the drugs, a similar effect was not repeated in other tests.

In the group that received GT in a dose of 100 mg/kg body weight, a drop was observed in the mean indicators for the stopping time of changes for the stopping time (Figure 2A).

This confirms results obtained in behavioral tests in an acute experiment and requires further study of the anti-anxiety effect of AG.

An analysis was made of the dose-dependent impact of intranasal administration of GT and AG on the anxiety indicators in Wistar rats. In the Open Field test, no differences were detected in the locomotor activity levels in doses 50 and 100 mg of each peptide per kg body weight compared to the control group (Figure 2A and 2B).

Intranasal AG administration at a dose of 50 mg/kg body weight reliably reduced the time spent by the Wistar rats in the labyrinth closed arms, which could indicate a lower anxiety level (Figure 2C).

This result was also confirmed in the analysis and correlation of the time spent by the Wistar rats of this group in closed chutes to the maze's closed arms (CA/OA, Figure 2D). An analysis was made of the impact of intranasal administration of GT and AG on the anxiety indicators in Wistar rats. In the Elevated Plus Maze test the experimental group animals received the test drugs in a dose of 100 mg of each per kg of body weight (Figure 3). Administration of a GT and AG combination reliably elevated locomotor activity of mice with normal genotype (WT AG + GT) compared to the control group WT (Figure 3A).

Similar results were obtained for the average and maximum speed of mice with normal genotype, but not knock-out animals (Figure 3B and 3C, respectively).

Analysis of behavioral changes was also revealed in the Elevated Plus Maze test. The average number of overhangs for knock-out mice that received a GT and AG combination was reliably lower compared to the mice of normal genotypes that received both drugs intranasally. Here, the average indicators for the number of overhangs in mice with normal genotype obtained with a GT and AG combination intranasally rose reliably compared to the control group (Figure 3F).

The results of the effect of a GT and AG combination on TAAR1-KO mice with intranasal administration in the Elevated Plus Maze test are shown in Figure 3D and 3E. For mice with genotype TAAR1-KO, that received an intranasal GT and AG combination, a reliable increase was found in the average indicators of time spent in the labyrinth OA, which indicates lower anxiety indicators compared to the control group of knock-out animals (Figure 3D).

Similar results were obtained in relation to the time spent by the mice of both genotypes in closed arms to open labyrinth arms (CA/OA), that received a GT and AG combination intranasally compared to the control groups ($p < 0.05$) (Figure 3E).

The findings indicate the positive anxiolytic effect of the GT and AG combination in a dose of 100 mg of each peptide per kg of body weight with intranasal administration.

Evaluation of the grooming microstructure indicators (TGT,

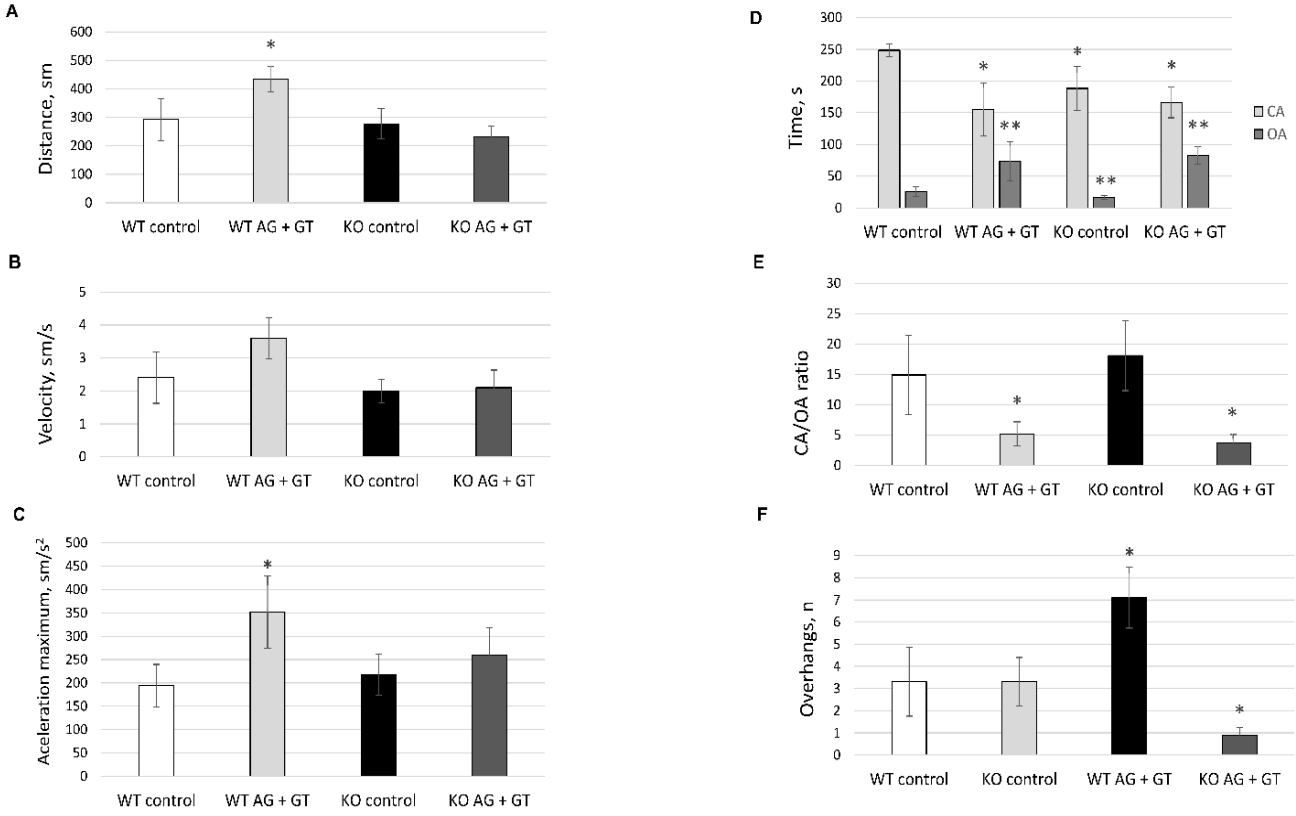

Figure 3. *Distance (A), velocity (B) and acceleration maximum (C) traversed by TAAR1-KO mice in the Open Field test and time spent by TAAR1-KO mice in closed (CA) and open (OA) labyrinth arms (D) and their correlation (E), and average number of overhangs (F) that received a GT and AG combination intranasally in the Elevated Plus Maze test. Data are expressed as mean ± SEM. * — $p < 0.05$ compared to the control group, Kruskal-Wallis criterion (H-test).*

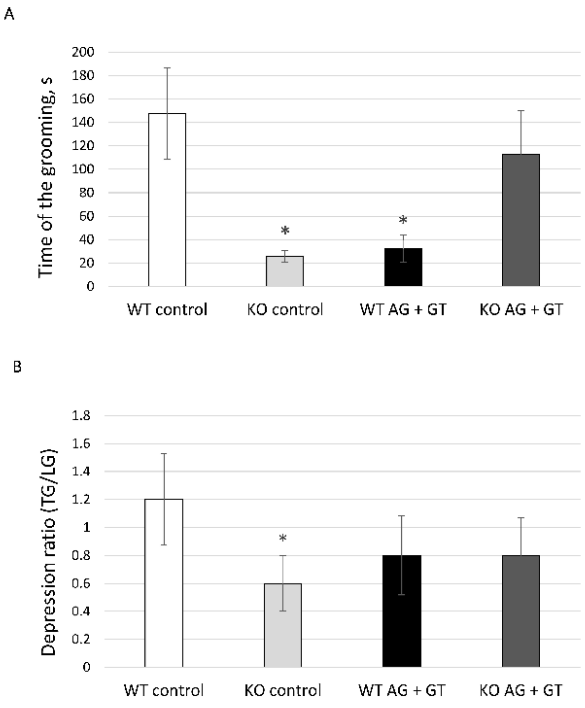

Figure 4. *Total grooming time (A) and correlation of total grooming time to latency of first grooming act (B) in TAAR1-KO mice that received a combination of alpha-glutamyl-tryptophan (GT) and zinc arginyl-glycinate (AG) intranasally in the Elevated Plus Maze test. Data are expressed as mean ± SEM. * — $p < 0.05$ compared to the control group (WT), Kruskal-Wallis criterion.*

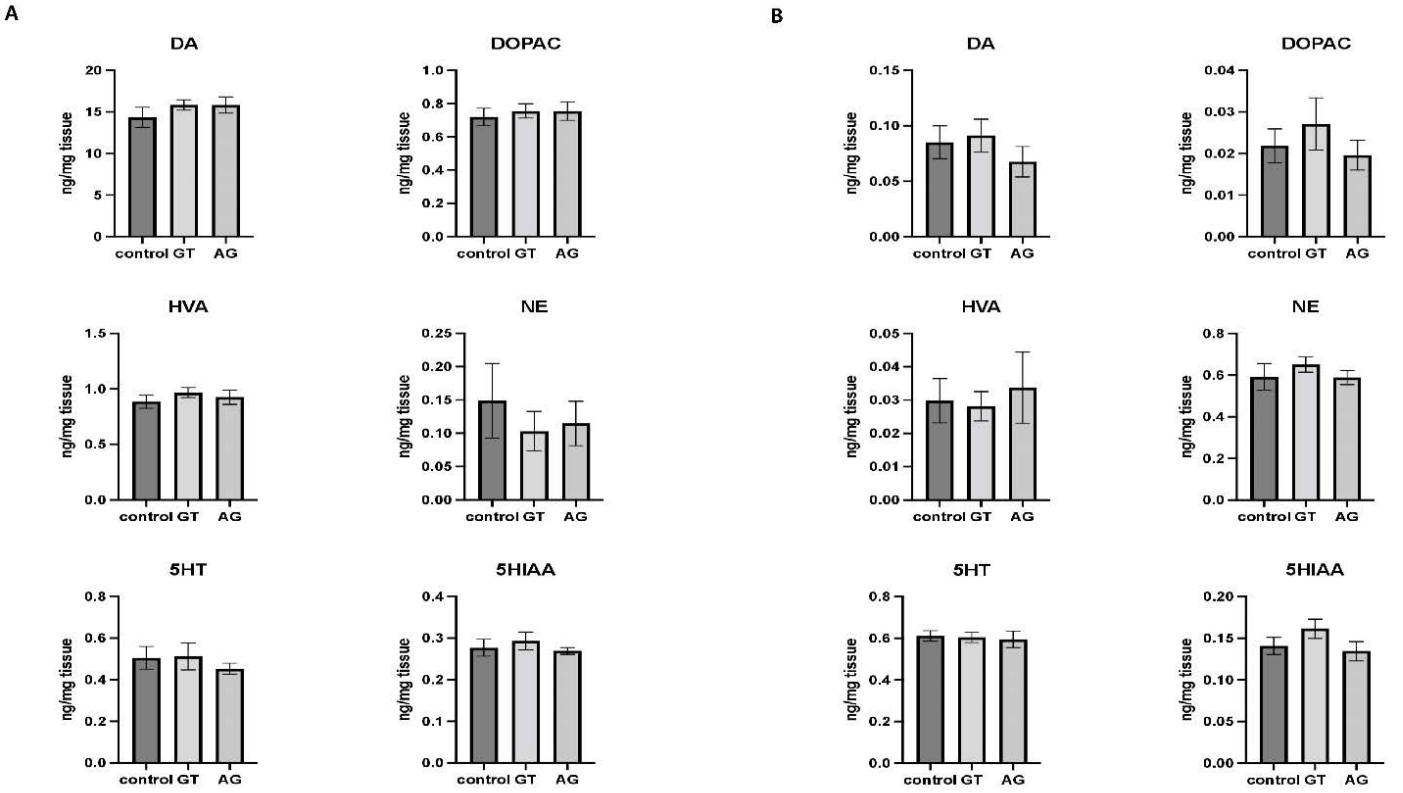

Figure 5. *Average monoamine levels in the striatum (A) and frontal cortex (B) of Wistar rats that received alpha-glutamyl-tryptophan (GT) and zinc arginyl-glycinate (AG) intranasally. The concentrations are presented in ng/mg of tissue. Data are expressed as mean ± SEM.*

TGT/LG) revealed differences between the wild-type (WT) groups that received a GT and AG combination and the control group with knock-out gene *TAAR1* (KO K) obtained in the Elevated Plus Maze test (Figure 4). The total grooming time in the group dropped reliably compared to the wild type control group (WT control) by approximately 6 times (Figure 4A), which indicates an elevated anxiety level and potentially depressive-like behavior of knock-out animals compared to the control group. Here, the intranasal administration of a GT and AG combination raised the average TGT indicators in knock-out animals and reduced them in mice with normal genotypes.

However, a trend was observed towards elevated anxiety levels in the KO control group, which manifested as a decrease in the average values of the ratio of total grooming time to the latency of the first grooming act (TGT/LG) in knock-out animals (depression coefficient [6]). Such differences were not found in the groups with WT and KO genotypes that received a GT and AG combination intranasally (Figure 4B).

Thus, a correlation is observed between the mean TGT indicators, and the time spent by the wild-type (WT) mice in closed arms to open arms, and a reverse correlation for the knock-out animals (EPM test).

In order to evaluate the neurochemical parameters in the brain of Wistar rats that received GT and AG intranasally in a chronic experiment in a dose of 10 mg/kg body weight/day for 10 days, the monoamine levels in the cerebral tissues were determined. For this purpose, we measured the concentration of dopamine, serotonin, noradrenaline, and their metabolites in different parts of the brain.

No reliable changes in the monoamine level were found in the striatum (Figure 5A) and frontal cortex (Figure 5B) in rats of the aforementioned.

Discussion.

Since peroral administration of amino acids and peptides has pharmacological limitations, we chose the intranasal route of administration. The primary objective of the study was to analyses effect of a chelate complex of zinc arginyl-glycinate and alpha-glutamyl-tryptophan on behavioral and neurochemical changes following acute and chronic intranasal administration, as we hoped to obtain evidence of a direct effect of the studied substances in the “nose-to-brain" projection. The conducted behavioral studies revealed the anxiolytic effect (lowered anxiety states) of alpha-glutamyl-tryptophan, chelate complex zinc arginyl-glycinate, and their combinations when administered intranasally.

Both drugs under consideration (AG and GT) have a physiological effect (neurogenic hyperthermia) on the autonomic nervous system. In this case, a single intranasal AG administration in a dose of 10 mg/kg body weight demonstrated signs of lower anxiety and depressive-like behavior in C57Bl6 mice in various behavioral tests. Thus, the intranasal form of zinc arginyl-glycinate and its combinations with GT in large doses may be used for pre-clinical studies as a new antidepressant and/or anxiolytic. The findings indicate a positive anxiolytic effect of a combination of GT and AG in a dose of 100 mg of each peptide per kg of body weight with intranasal administration. Thus, during acute and chronic intranasal administration of

drugs GT and AG, the latter showed a change in the anxiety indicator. Acute AG intranasal administration demonstrated signs of lower anxiety and depressive-like behavior in C57Bl6 mice.

In the Forced Swim test, a hypothetical antidepressant effect was found in GT intranasal administration in a dose of 100 mg/kg body weight in Wistar rats. However, the average number of rests detected in the Wistar rat group that received GT intranasally was reliably higher than in the control group, which could indicate the higher search activity in these animals.

Studying the AG effect on grooming macrostructure indicators did not reveal reliable differences between the control and experimental groups. In the last 20 minutes of the experiment, a stable trend was observed toward a drop (by 20–30 %) in the locomotor activity of the DAT-KO rats in the group that received AG intranasally. A decrease was revealed in the CA/OA ratio in the Elevated Plus Maze test indicating lower anxiety indicators in the group of rats that received AG in a dose of 10 mg/kg body weight compared to the control group.

Acute intranasal GT and AG administration to Wistar rats showed a reliable elevation in auxiliary SIH values (T3 – T2) for GT and of overall temperature change T3 – T1 for both test drugs in the Stress-induced Hyperthermia test. In the case of GT, the increasing effect of neurogenic hyperthermia continued not only within 20 minutes (T2) but also within an hour after the experiment started (T3). Thus, GT and AG drugs have a pronounced physiological effect (neurogenic hyperthermia) on the autonomic nervous system, however, determination of their operating mechanisms requires further research.

A direct correlation was observed between the average TGT indicators, and the time spent by the wild type (WT) mice in closed arms to open arms (EPM test) and a reverse correlation in TAAR1-KO knock-out mice and a trend towards higher anxiety levels in the mice group that received a GT and AG combination intranasally.

Conclusion.

Thus, the conducted behavioral studies revealed the anxiolytic effect (lowered anxiety states) of alpha-glutamyl-tryptophan, chelate complex zinc arginyl-glycinate, and their combinations during acute and chronic intranasal administration. Acute AG intranasal administration demonstrated signs of lower anxiety and depressive-like behavior in C57Bl6 mice.

Both drugs of a chelate complex zinc arginyl-glycinate and alpha-glutamyl-tryptophan with intranasal administration, as well as their composites, are promising for further preclinical studies as anxiolytics and antidepressants.

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Institutional Review Board Statement.

All the animal studies were carried out according to the guidelines of the Ministry of Health of the Russian Federation, FELASA, and RusLASA. All the experiments were approved by the Saint Petersburg State University Ethical Committee for Animal Research (No. 131-03-10 of 22 November 2021).

Conflicts of interest.

There are no conflicts of interest.

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