

DOI: <https://doi.org/10.34921/amj.2024.3.004>**KİMYƏVİ QIDA ƏLAVƏLƏRİ KOMPLEKSİNİN TƏSİRİNDƏN STRESƏ
DAVAMLILIĞIN FORMALAŞMASI VƏ KOQNİTİV FUNKSİYALARIN
DƏYİŞİKLİKLƏRİ****S.M.Biləş¹, Ya.O.Oliyniçenko¹, O.M.Pronina¹, S.V.Donçenko¹, M.M.Koptev¹,
A.V.Piroq-Zakaznikova¹, V.V.Onipko², M.O.Oliyniçenko¹, O.V.Bezeha¹, O.V.Mamay¹**¹*Poltava Dövlət Tibb Universiteti, Poltava ş., Ukrayna*²*Poltava Dövlət Aqrar Universiteti, Poltava ş., Ukrayna*

Xülasə. Məqalədə müxtəlif müddətlərdə kimyəvi qida əlavələri (natrium-qlutamat, natrium-nitrit, Ponso-4R) qəbul etmiş siçovullarda davranış reaksiyalarının və stresə davamlılığın öyrənilməsi məqsədilə aparılmış tədqiqat işi haqqında məlumat verilmişdir.

Tədqiqatın gedişində eksperimental heyvanların müxtəlif yarımqruplarına peroral yolla, 1, 4, 8, 12, 16 və 20 həftə ərzində kimyəvi qida əlavələri verilmişdir. Koqnitiv funksiyalar “ekstrapolyasion azadolma” testindən istifadə edilməklə qiymətləndirilmişdir.

Alınmış nəticələrin təhlilindən aydın olmuşdur ki, eksperimentin başlanğıc mərhələlərində (8 həftəyə qədər) öyrənilən göstəricilər müsbət dinamikaya malik olmuşdur; bu, reaksiyaların müddətinin qısalması ilə təzahür etmişdir. Kimyəvi qida əlavələrinin 12 həftə ərzində qəbulundan sonra eksperimental heyvanlarda neqativ istiqamətli dəyişikliklər müşahidə edilmişdir. Bu, özünü tədqiq edilən göstəricilərin ləngiməsi ilə göstərmişdir. Eksperimentin başlanmasından 20 həftə sonra göstəricilərin xüsusilə pis olduğu nəzərə çarpmışdır.

Müəyyən edilmişdir ki, kimyəvi qida əlavələrinin uzunmüddətli qəbulu stres vəziyyəti zamanı reaksiyaların kütləşməsinə, həmçinin kəskin stress vəziyyətindən azad olmağa cəhd edilməsinin zəifləməsinə səbəb olur.

Açar sözlər: stresə davamlılıq, koqnitiv disfunksiya, qida əlavələri, natrium qlutamat, natrium nitrit, Ponso 4R

Ключевые слова: стрессоустойчивость, когнитивная дисфункция, пищевые добавки, глутамат натрия, нитрит натрия, Понсо 4R

Key words: stress resistance, cognitive dysfunction, food additives, monosodium glutamate, sodium nitrite, Ponceau 4R

**FORMATION OF STRESS RESISTANCE AND CHANGES IN COGNITIVE
FUNCTIONS UNDER THE INFLUENCE OF A COMPLEX OF CHEMICAL FOOD
ADDITIVES****S.M.Bilash¹, Ya.O.Oliinichenko¹, O.M.Pronina¹, S.V.Donchenko¹, M.M.Koptev¹,
A.V.Pirog-Zakaznikova¹, V.V. Onipko², M.O.Oliinichenko¹, O.V.Bezeha¹, O.V.Mamai¹**¹*Poltava State Medical University, Poltava, Ukraine*²*Poltava State Agrarian University, Poltava, Ukraine*

Summary. The article provides information about a study conducted to investigate behavioral reactions and stress tolerance in rats that were administered chemical food additives (monosodium glutamate, sodium nitrite, Ponso-4R) for different durations.

In the study, experimental subgroups of laboratory rats were administered complex chemical food additives daily once orally for 1, 4, 8, 12, 16 and 20 weeks. Cognitive functions were assessed using the “Extrapolation Escape” test.

The analysis of the obtained results showed that the estimated indicators at the initial stages of the experiment (up to 8 weeks) had positive dynamics, which was characterised by a reduction in the time of occurrence of reactions. After 12 weeks of introducing the complex of food additives, negative changes occurred, which were manifested by a gradual postponement of the studied indicators. The worst

indicators were recorded after week 20 (significant at $p < 0.05$).

It was found that the use of a complex of chemical food additives leads to blunted reactions under conditions of acute stress, as well as impaired ability to find a way out of the acute stress situation.

Introduction. The modern food industry is characterised by a wide use of food additives, including those of chemical origin, which not only improve the organoleptic properties of food products but also extend their shelf life and prevent rapid spoilage. The food industry uses only those food additives that do not pose a threat to the health of consumers. However, several scientific studies have shown that even these substances can cause changes in various organs and systems when used regularly, even in the permissible dosage. There is evidence that certain food additives cause allergic reactions, disturbances in the functioning of the digestive, respiratory, endocrine and nervous systems, hyperactivity in children, and an increase in the likelihood of cancer [1-5].

Chemical food additives have been shown to cause morphological changes in various organs and systems, impairing their functional properties [6]. For example, changes in cognitive or behavioural reactions may occur due to the negative effects of chemical substances on different levels of the nervous system.

There is a long list of substances that are classified as chemical food additives. A review of the literature showed that the most common are monosodium glutamate (E621), sodium nitrite (E250) and Ponceau 4R (E124). This is what prompted us to use them in this experimental study. Considering that quite often we consume foodstuffs that contain more than one chemical substance at a time, the study of their complex influence both on separate organs and on the organism as a whole is a particularly urgent problem that requires further research.

The study aimed to establish changes in behavioural responses and stress tolerance in rats at different durations of complex administration of chemical food additives (monosodium glutamate, sodium nitrite, Ponceau 4R).

Material and methods. The study was carried out on 70 white sexually mature rats weighing (180-202) g, which were kept in standard conditions of the vivarium of Poltava State Medical University. The experimental animals were divided into 2 groups: control and experimental. The latter in turn was divided into 6 subgroups depending on the period of introduction of the complex of chemical

food additives. Each group consisted of 10 animals. The control group was administered orally saline. Simultaneously, the experimental subgroups received a complex of chemical food additives (monosodium glutamate, sodium nitrite, Ponceau 4R) prepared immediately before administration. Before the main feeding, the animals were administered 0.5 ml of the solution, which consisted of 20 mg/kg of monosodium glutamate, 0.6 mg/kg of sodium nitrite and 5 mg/kg of Ponceau 4R. The dosage of food additives used was two times lower than the maximum permissible norms approved by the legislation of Ukraine. Animals were administered the solution during 1, 4, 8, 12, 16 and 20 weeks. After the administration of the complex of chemical food additives, the experimental animals had free access to water and food during the day, which were standard for keeping animals in the vivarium. After the expiration of the appropriate period, after the study, the rats were removed from the experiment by overdose with ether anaesthesia.

All manipulations of the experimental study were performed in accordance with the "Rules for the Use of Laboratory Experimental Animals" (2006, Annex 4) and the Declaration of Helsinki on the Humane Treatment of Animals, the Law of Ukraine "On the Protection of Animals from Cruel Treatment" (No. 3447-IV of 21.02.2006) in compliance with the requirements of the Bioethics Commission of Poltava State Medical University (Protocol No. 208 of 22.09.2022), agreed with the provision of the "European Convention for the Protection of Vertebrate Animals used in Experimental and Experimental Research" (Strasbourg, 1986).

Determination of behavioral reactions was carried out using "Extrapolation Escape" test.

The device for the "Extrapolation Escape" test was designed to study the cognitive functions of rats under conditions of acute stress and made it possible to assess individual differences in the cognitive style of solving the task (searching for a way out of an acute stress situation) and the influence of exogenous factors (a complex of food additives) on cognitive function disorders. The device consisted of 2 cylinders: an outer opaque one with an approximate diameter of 35 cm and an inner transparent one (diameter – 8 cm, height – 23 cm). The outer container was filled with water (22-23 °C), and the inner cylinder was placed to a depth of 2.5-3 cm. The rat was carefully placed into the water, lowering to the inner transparent cylinder with its tail down, preventing the rear paws from grabbing its edge. The latent period of the onset of aversive reactions

after boarding into the device, the number of jumps during the test period, and the latent period of immersion were recorded. The experimental animal was removed from the water (outer cylinder) immediately after immersion. The total time the rat stayed in the test conditions did not exceed 2 minutes.

The statistical analysis of the research results was carried out using a personal computer with the help of a package of application programs for statistical processing of data from medical, biological, and epidemiological studies "InStat." The program made it possible to obtain research results from the following predicted values: M – average value; σ – standard deviation; m is the standard error of the mean value. The Student's t -test was used to compare quantitative values. The difference was considered probable at values of $p < 0.05$.

Research results.

Assessment of the cognitive functions of rats under stress conditions using a device for the "Extrapolation Escape" test revealed negative changes in the course of escape reactions that progressed in the dynamics of the experiment.

For the control group, the average indicator of the latent period of the onset of aversive reactions was (5.9 ± 0.38) s, and the latent period of immersion was (18.8 ± 0.49) s, after one week of the introduction of a complex of food additives - (5.1 ± 0.23) s and (18.7 ± 0.45) s, after four weeks – (5 ± 0.3) s and (18.1 ± 0.31) s, after eight weeks – (4.7 ± 0.3) s and (17.8 ± 0.36) s, after 12 weeks – (8.3 ± 0.42) s and (21.2 ± 0.65) s, after 16 weeks – (9.5 ± 0.5) s and (23.8 ± 0.87) s,

after 20 weeks – (11 ± 0.45) s and (26.3 ± 0.42) s, respectively.

Thus, during the registration of the latent period of the onset of aversive reactions after boarding into a device and the latent period of immersion, it was established that at the initial stages of the experiment (up to week 8), positive dynamics were observed, was characterized by a reduction in the time of occurrence of these reactions (**Figure 1**). In particular, it was established that after the 8th week of the experimental study, the indicator of the latent period of the onset of aversive reactions was 25.53% lower than the control group and 6.38% lower than the previous observation period. As for the latent period of immersion, the lowest indicator was also registered exactly after the 8th week of the study and was 5.62% lower than the control group and 1.69% lower than the indicator of the previous period of the experiment.

After the 12th week, negative changes occurred, manifested by the gradual delay of both the latent period of the onset of aversive reactions and the latent period of immersion (**Figure 1**). Thus, after 12 weeks of administration of a complex of food additives to laboratory animals, the indicator of the latent period of immersion was 19.1% higher than the previous experimental group and 12.77% higher than the control group. After the 16th week of the study, this indicator was higher than the

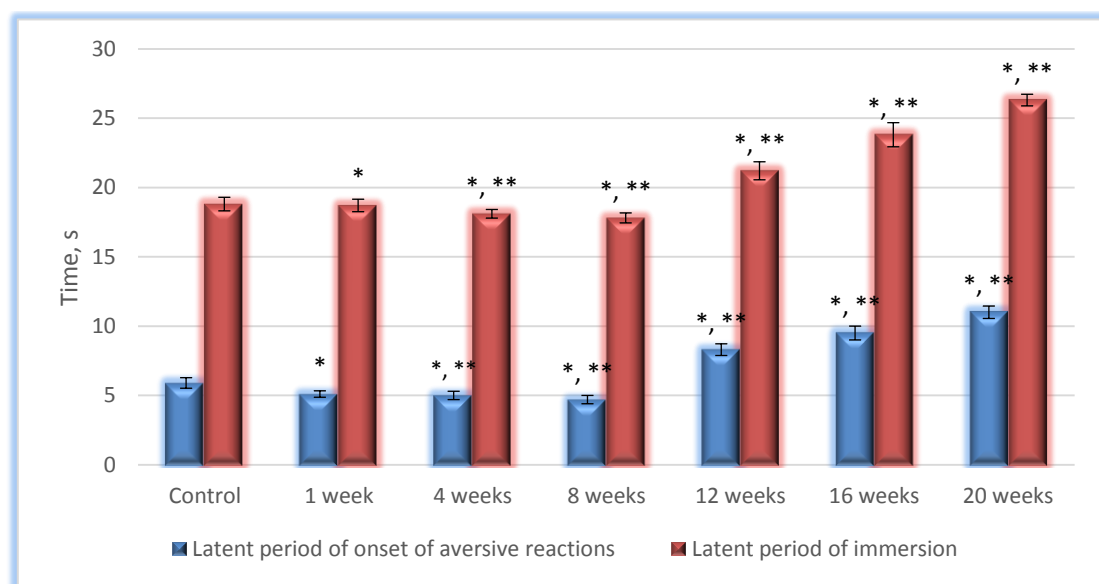


Figure 1. The time of registration of the latent period of the onset of aversive reactions and the latent period of immersion. Notes: * – $p < 0.05$ compared to the control group, ** – $p < 0.05$ compared to the previous term of the experimental study

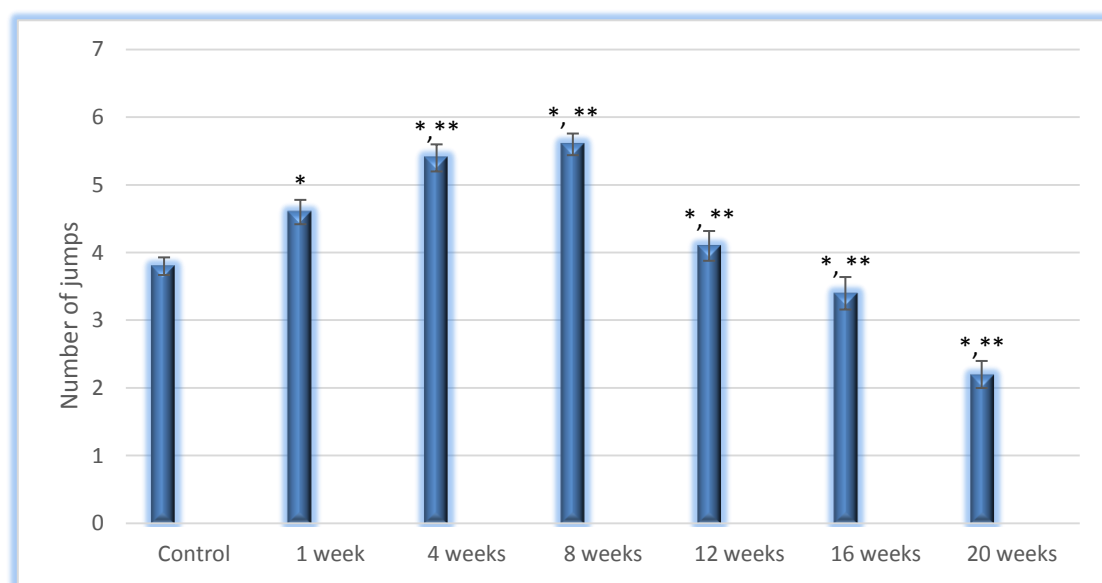


Figure 2. The number of jumps of rats during the test period. Notes: * – $p < 0.05$ compared to the control group, ** – $p < 0.05$ compared to the previous term of the experimental study

previous experimental group by 10.92% and the control group by 21%. Finally, the highest rate was established after the 20th week of the study, which was 28.51% higher than the control group and 9.5% higher than the previous experimental group. Regarding the latent period of the onset of aversive reactions, the worst indicator was also registered after the 20th week of the experimental study. It was 86.44% higher than the control group and 15.79% higher than the previous experiment period.

A similar dynamic was observed when evaluating the number of jumps of rats during the test period. In the initial stages, their number was higher compared to the later stages (**Figure 2**). The highest number of jumps of 5.6 ± 0.16 was registered after eight weeks of the experimental study, which was 47.37% higher than in the control group, for which this indicator was 3.8 ± 0.25 . Starting from the 12th week, the number of jumps of the rats decreased, reaching the lowest number of 2.2 ± 0.2 after the 20th week of the experiment, 42.1% lower than the control group and 2.8 times lower than the experimental group that consumed food additives for eight weeks. This trend may indicate an adverse effect of a complex of food additives on the orienting ability of rats exposed to an unknown aversive test situation.

When analyzing the data in Figures 4 and 5, it can be established that the use of a

complex of food additives has a negative effect on the cognitive functions of rats under conditions of acute stress, as well as on their ability to solve tasks, in particular, the search for a way out of a critical stressful situation.

A comparison of the results obtained during the assessment of behavioral reactions in the “Extrapolation Escape” test and the complex maze showed that at the initial stages of the experiment (up to the 8th week), there was an increase in cognitive function, which may indicate the activation of the course of nervous reactions under the influence of a complex of food additives. However, starting from the 12th week, all indicators had a negative trend, demonstrating manifestations of the inhibitory effect of monosodium glutamate, sodium nitrite, and Ponceau 4R on the nervous system.

Discussion of research results. A common cause of cognitive impairment is the influence of endogenous and exogenous factors [7, 8]. Among them, a significant role is played by the quality of food products, in particular, the content of food additives in them. Cognitive dysfunction is characterised by impaired memory, attention concentration, and the ability to make quick decisions under stress [9]. Some of these disorders were demonstrated in our study.

The starting mechanism for cognitive dysfunction is damage to neural tissue, cha-

racterised by the appearance of changes in both the grey and white matter of the brain. As noted, this can be caused by the harmful effects of chemicals, particularly food additives. Several works are linking cognitive disorders with nutritional peculiarities [10, 11]. For example, it has been found that metabolic syndrome, which is a consequence of obesity, is often a risk factor for moderate cognitive impairment and age-related decline in cognitive function [12].

The results of the literature search showed that the chemical food additives used in our experimental study may be the cause of cognitive dysfunction.

Monosodium glutamate (MSG, E621) has been found to have neurotoxic effects [13, 14]. In addition, MSG-induced hyperactivation of glutamergic receptors leads to ionic imbalance, which causes oxido-nitrosative stress, neuroinflammation, and brain cell death, which may result in problems finding a way out of an acute stress situation [15]. E621 also affects the course of anxiogenic reactions.

Sodium nitrite may also be responsible for cognitive impairment by causing hypoxia, impaired redox reactions and the production of significant amounts of peroxides and free radicals, thereby further enhancing tissue damage and causing behavioural changes [16]. In addition, it has been found that the use of sodium nitrite can induce impairments

in memory and retrieval processes during certain stress reactions [17].

As for the effect of Ponceau 4R, as a representative of food colouring agents, it was found that its prenatal administration can cause increased mobility, decreased motivation, and anxiety in offspring [18].

The above described shows that separate administration of chemical food additives used by us can cause manifestations of cognitive dysfunction characteristic of experimental animals in our study. As for their complex effect on cognitive functions, the number of papers describing the results of such studies is rather limited.

Although it was found that long-term consumption of monosodium glutamate, sodium nitrite and Ponceau 4R in complex leads to increased anxiety, fear, blunted adaptive reactions [19], as well as the appearance of signs of impaired memory function and learning ability [20].

Conclusion. It was found that despite some signs of activation of the course of neural reactions during the initial stages of consumption of monosodium glutamate, sodium nitrite and Ponceau 4R, from week 12 to the end of the study, there were signs of progression of cognitive dysfunction. Thus, consuming a complex of food chemical additives leads to blunted reactions under acute stress and impaired ability to seek escape from an acute stress situation.

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ФОРМИРОВАНИЕ СТРЕССОУСТОЙЧИВОСТИ И ИЗМЕНЕНИЕ КОГНИТИВНЫХ ФУНКЦИЙ ПОД ВЛИЯНИЕМ КОМПЛЕКСА ХИМИЧЕСКИХ ПИЩЕВЫХ ДОБАВОК

**С.М.Билаш¹, Я.А.Олийниченко¹, Е.Н.Пронина¹, С.В.Донченко¹, М.М.Коптев¹,
А.В.Пирог-Заказникова¹, В.В.Онипко², М.А.Олийниченко¹, Е.В.Безега¹, О.В.Мамай¹**

¹*Полтавский государственный медицинский университет, г. Полтава, Украина*

²*Полтавский государственный аграрный университет, г. Полтава, Украина*

Резюме. В статье представлены результаты исследования, проведенного с целью установить изменения поведенческих реакций и стрессоустойчивости у крыс на разных сроках комплексного употребления химических пищевых добавок (глутамат натрия, нитрит натрия, Понсо 4R).

При проведении исследования экспериментальным подгруппам лабораторных крыс в течение 1, 4, 8, 12, 16 и 20 недель вводили комплекс химических пищевых добавок ежедневно однократно перорально. Оценка когнитивных функций была проведена с использованием теста "Экстраполяционное извлечение".

Анализ полученных результатов показал, что оцениваемые показатели на начальных этапах эксперимента (до 8 недель), имели положительную динамику, которая характеризовалась сокращением времени возникновения реакций. После 12 недели введения комплекса пищевых добавок произошли негативные изменения, что проявлялись постепенной отсрочкой исследуемых показа-

телей. Худшие показатели были зарегистрированы после 20 недели (достоверно при $p < 0,05$).

Было установлено, что употребление комплекса химических пищевых добавок приводит к притуплению реакций в условиях острого стресса, а также ухудшением способности к поиску пути избавления от острой стресс-ситуации.

ORCID of each author:

S.M. Bilash: <https://orcid.org/0000-0002-8351-6090>

Ya.O. Oliinichenko: <https://orcid.org/0000-0001-7724-7333>

O.M. Pronina: <https://orcid.org/0000-0002-8242-6798>

S.V. Donchenko: <https://orcid.org/0000-0001-7411-9897>

M.M. Koptev: <https://orcid.org/0000-0002-3726-8911>

A.V. Pyroh-Zakaznikova: <https://orcid.org/0000-0001-9070-1588>

V.V. Onipko: <https://orcid.org/0000-0002-2260-971X>

M.O. Oliinichenko: <https://orcid.org/0000-0003-2198-6318>

O.V. Bezeha: <https://orcid.org/0000-0002-9793-3880>

O.V. Mamai: <https://orcid.org/0009-0008-4561-3253>

Address for correspondence:

Oliinichenko Yaryna, Department of Anatomy with Clinical Anatomy and Operative Surgery,
Poltava State Medical University

E-mail: jarinaoliinichenko93@gmail.com