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QİDA ƏLAVƏLƏRİ KOMPLEKSİNİN KOQNİTİV FUNKSİYAYA VƏ ŞƏRTİ REFLEKSLƏRİN YARANMA SÜRƏTİNƏ TƏSİRİ

S.M.Bilaş[1], Ya.O.Oliyniçenko[1], O.M.Pronina[1], S.V.Donçenko[1], M.M.Koptev[1], A.V.Piroq-Zakaznikova[1], O.V.Davıdova[2], M.O.Oliyniçenko[1], O.V.Bezeha[1], O.V.Mamay[1], N.S.Kopıtko[1]

[1]Poltava Dövlət Tibb Universiteti, Poltava ş., Ukrayna;
[2]M.Ostroqradski ad. Kremençuq Milli Tibb Universiteti, Kremençuq ş., Ukrayna

Xülasə. *Məqalədə siçovullarda kimyəvi qida əlavələri kompleksinin (natrium qlutamat, natrium nitrat, ponso 4 R) müxtəlif müddətlərdə qəbul edildiyi şəraitdə hərəkət tərzinin dəyişikliklərini öyrənmək məqsədilə aparılmış tədqiqat işi haqqında məlumat verilmişdir. Tədqiqat zamanı laboratoriya şəraitində sıçovullara eksperimentlərin qruplarına müvafiq olaraq 1, 4, 8, 12, 16 və 20 həftə ərzində gündə 1 dəfə olmaqla kimyəvi qida əlavəsi kompleksi yeridilmişdir. Mürəkkəb quruluşlu labirint daxilində qida qəbuluna qarşı hərəkət tərzi refleksinin yaranmasına əsasən koqnitiv funksiyanın dəyişmələri qiymətləndirilmişdir.*

Mürəkkəb quruluşlu labirint daxilində heyvanların hərəkət tərzinin qiymətləndirilməsi göstərmişdir ki, qida əlavələrinin yeridilməsinin ilk müddətlərində (8 həftəyə qədər) alınmış nəticələr müsbət baxımdan etibarlıdır ($p<0,05$). Eksperimental tədqiqatın 12-ci həftəsindən başlayaraq, öyrənilən parametrlər mənfi istiqamətə meyl etmişdir. Statistik etibarlı ($p<0,05$) dəyişikliklər isə eksperimental tədqiqatın başlanmasından sonrakı 20-ci həftədə müşahidə edilmişdir.

Aydın olmuşdur ki, qida əlavələri kompleksinin uzunmüddətli tədbiqi siçovullarda koqnotiv disfunksiyaya səbəb olur. Bu isə yaddaşın və təlim vərdişlərinin yaranmasının pozulması ilə təzahür edir.

Açar sözlər: *koqnitiv disfunksiya, qida əlavələri, natrium qlutamat, natrium nitrit, ponso 4 R, hərəkət tərzi reaksiyaları, şərti reflekslər*

Ключевые слова: *когнитивная дисфункция, крысы, пищевые добавки, глутамат натрия, нитрит натрия, Понсо 4R, экспериментальное исследование, поведенческая реакция, условный рефлекс*

Key words: *cognitive dysfunction, rats, food additives, monosodium glutamate, sodium nitrite, Ponceau 4R, experimental study, behavioral response, conditioned reflex*

THE EFFECT OF A COMPLEX OF FOOD ADDITIVES ON COGNITIVE FUNCTIONS AND THE SPEED OF CONDITIONED REFLEX FORMATION

S.M.Bilash[1], Ya.O.Oliinichenko[1], O.M.Pronina[1], S.V.Donchenko[1], M.M.Koptev[1], A.V.Pirog-Zakaznikova[1], O.V.Davydova[2], M.O.Oliinichenko[1], O.V.Bezeha[1], O.V.Mamai[1], N.S.Kopytko[1]

[1]Poltava State Medical University, Poltava, Ukraine
[2]Kremenchuk Mykhailo Ostrohradskyi National University, Kremenchuk, Ukraine

The article provides information about a research study conducted to establish changes in behavioral reactions of rats at different times of use of a complex of chemical food additives (monosodium glutamate, sodium nitrite, Ponceau 4R).

The study was based on administering experimental laboratory rats subgroups for 1, 4, 8, 12, 16, and 20 weeks of a complex of food additives once a day orally. Cognitive function was determined by assessing the formation rate of a conditioned food-retrieval reflex in a complex maze.

The assessment of the behavior of experimental animals in a complex maze showed that at the initial time of introducing a complex of food additives (up to week 8), the obtained results had positive dynamics, with values of $p<0.05$. All evaluated parameters had a negative trend from the 12th week of the experimental study. Significantly ($p<0.05$), the worst indicators were registered after the 20th week of the experimental

study.

It was found that consumption of a complex of food additives leads to cognitive dysfunction in rats, which was characterised by impaired memory function as well as learning ability.

Introduction. The question of the quality of food products is one of the most acute problems of our time. It is significantly related to food additives that improve the appearance and taste of food products, extend their shelf life, and prevent rapid spoilage. Although only those food additives that do not pose a threat to human health can be used in the food industry, in modern literature, there is a significant number of works devoted to the description of changes in various organs and systems during daily use of these substances, even in acceptable doses [1].

The conducted studies show that using food additives leads to various morphological changes in organs and systems, thereby causing disruption of their functioning [2-5]. Against this background, quite often, a violation of the regulatory influence of the nervous system develops at various levels, in particular, changing the course of behavioral and cognitive reactions.

Given the large number of chemicals classified as food additives, we searched to determine the frequency of their use. The results showed that monosodium glutamate (E621), sodium nitrite (E250), and Ponceau 4R (E124) are among the most common. Therefore, when conducting our research, we stopped our choice of them. In addition, it is known that a person often eats food containing a whole complex of food additives. Thus, studying their complex impact on the body as entire and various functions separately to reduce their harmful effects is a particularly urgent problem today, requiring significant attention and further research.

The study aimed to establish changes in the rate of conditional reflex formation and manifestations of cognitive dysfunction in rats on different terms of consumption of chemical food additives (monosodium glutamate, sodium nitrite, Ponceau 4R) exactly in the complex.

Material and methods. The study was conducted on 70 white, sexually mature rats weighing (180-202) g, which were kept in standard conditions of the Poltava State Medical University vivarium. All experimental animals were divided into two groups: control and experimental, last one in turn was divided into six subgroups depending on the period of use of the complex of food additives. There were ten animals in each group. The control group included rats that received saline orally, while the experimental animals received a complex of food additives. The experimental group had animals that received a complex of food additives (monosodium glutamate, sodium nitrite, Ponceau 4R) prepared immediately before administration. Before the main feeding, the animals received 0.5 ml of the solution, which consisted of 20 mg/kg monosodium glutamate, 0.6 mg/kg sodium nitrite, and 5 mg/kg Ponceau 4R. The doses of food additives were two times lower than the maximum permissible norms approved by the legislation of Ukraine. The animals received the solution for 1, 4, 8, 12, 16, and 20 weeks. After introducing a complex of food additives, the rats had free access to water and standard food for keeping animals in a vivarium during the day. At the end of the appropriate period, the rats were removed from the experiment under ether anesthesia. All manipulations were carried out following the "Rules for the Use of Laboratory Experimental Animals" (2006, Annex 4) and the Helsinki Declaration on the Humane Treatment of Animals, the Law of Ukraine "On the Protection of Animals from Cruelty" (№ 3447-IV of February 21, 2006) in compliance with the requirements of the bioethics commission of the Poltava State Medical University (protocol № 208 dated 22.09.2022), consistent with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986).

Cognitive function was determined by assessing the formation rate of a conditioned food-retrieval reflex in a complex maze.

Developing a conditioned food-seeking reflex in a complex maze allows us to evaluate memory functions, the ability to learn, and the speed of forming a conditioned reflex. The complex maze was a quadrant structure divided by five partitions into six corridors. Each partition contained a rectangular hole offset relative to the holes in the adjacent ones. A food bait was placed in the most distant corridor. Before the experiment, the animals were in conditions of 24-hour deprivation. The rat was placed in the maze for 30 minutes for the first time to adapt and form an orientation-search reaction. Later, the rats were placed in the maze five times a row, lasting 3 minutes. Visually recorded the

time of exit from the starting compartment (latent time of exit), reaction time (latent period of grasping food bait), number of executed reactions (number of cases when the animal found food bait within three minutes), number of errors (number of deviations from the optimal movement trajectory when the experimental animal after passing the next hole in the partition, it turned in the "wrong" direction) and the number of returns to the previous compartment. The total duration of the experiment was five days.

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 and their discussion. According to the study results, the assessment of signs of the formation of a conditioned reflex occurred on the 5th day of the experiment. The results established within 1-4 days did not show significant intergroup differences.

In the dynamics of the experiment, an increase in the exit time from the starting compartment (latent exit time **(Figure 1)**) was found. After introducing food additives for one week, no significant difference in the indicators was found. For the control group, it was 16.6±0.89 s, and after one week – 16.5±0.7 s. After the fourth week, the latent exit time was 15.9±0.9 s, which was 4.22% lower compared to the control group and first experimental subgroup. The time of exit from the starting compartment after eight weeks of administration of a complex of food additives to animals was 16.7±0.7 s, which is 5.03% higher in comparison with the previous observation period and reliably at $p<0.05$ did not differ from the control groups. When comparing the indicators established in animals that were given food additives for 8 and 12 weeks, we found negative dynamics. It was manifested by an increase in the latent time of exit 22.7±1.02 s. by 26.43% compared to the experimental subgroup after eight weeks of administration of the complex of food additives and by 26.87% compared to the control. The indicator of the exit time from the starting compartment – 29.6±0.85 s. after the 16th week of the experimental study increased by 30.4% compared to the previous experimental time and by 43.92% compared to the control. The worst indicator was found after 20 weeks of administration of food additives complex to laboratory animals 38.7±1.27 s., which corresponded to an increase in the latent time of exit by 23.51% compared to the 16th week and by 57.1% compared to the control.

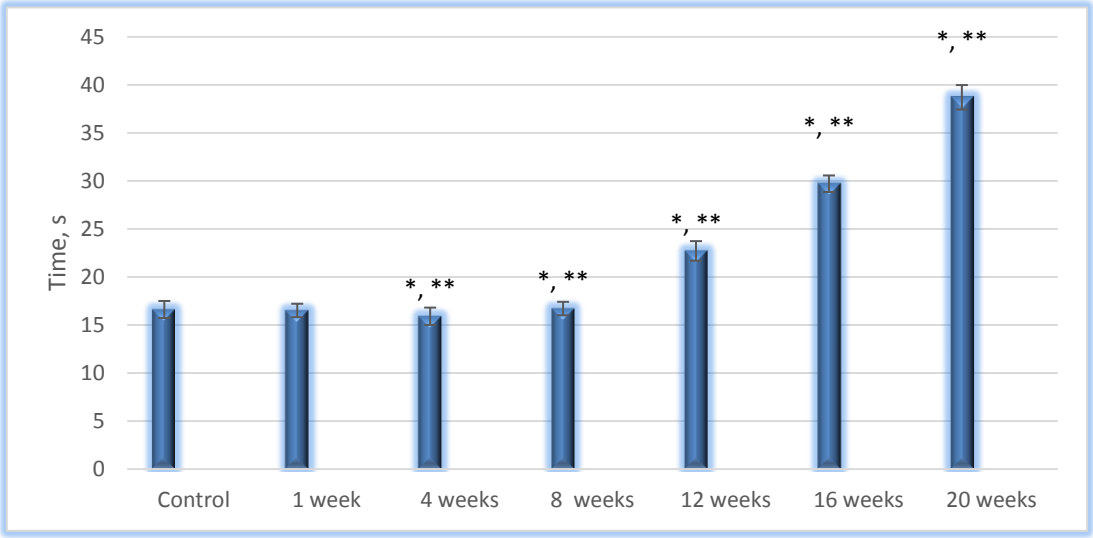

Figure 1. Time of exit from the starting compartment in the dynamics of the experiment.
***Notes:* * – p<0.05 compared to the control group, ** – p<0.05 compared to the previous term of the experimental study**

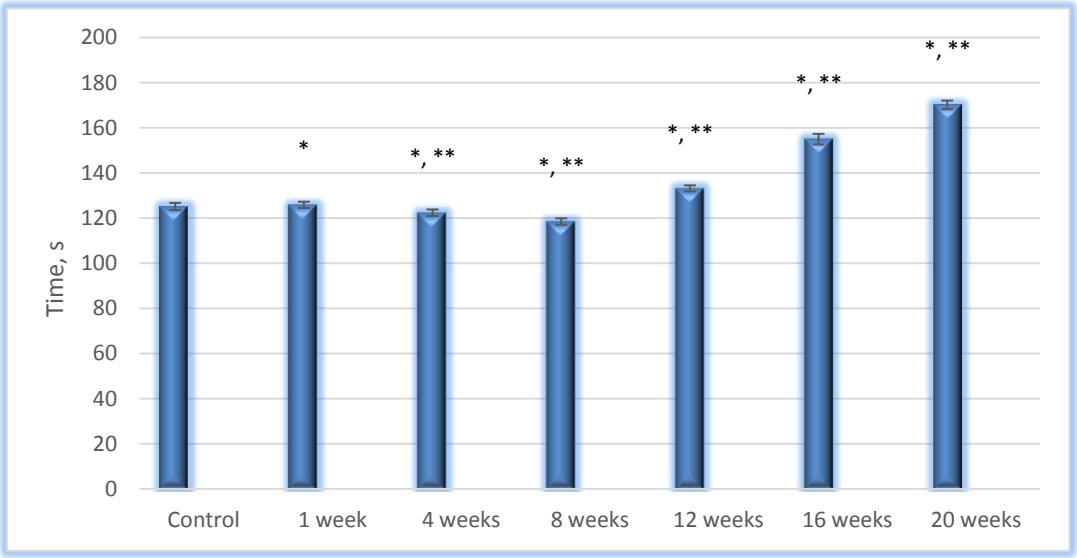

Figure 2. Latency period of grasping food bait in the dynamics of the experiment.
***Notes:* * – p<0.05 compared to the control group, ** – p<0.05 compared to the previous term of the experimental study**

When rats were given a complex of food additives, an increase in the reaction time (latent period of grasping food bait) was observed in the dynamics of the experiment **(Figure 2)**. At the initial stages of the experiment (after one week), it was established that the difference between the indicators of the experimental 125.9±1.38 s. and the control group 125.2±1.58 s. was 0.56%. After the 4th week of the experimental study, positive dynamics were observed, characterized by a decrease in the latent period of grasping food bait by 2.29% compared to the control group and was 122.4±1.47 s. The lowest indicator – 118.5±1.43 s. was established after eight weeks of administration of a complex of food additives to laboratory animals, which was 4.8% lower compared to the control. In the future, the growth of negative dynamics was revealed. In particular, after the 12th week of the experimental study, the reaction time 133.2±1.34 s. increased by 6.01% compared to the control and 10.29% compared to the previous period of the experiment. After the 16th week, the indicator increased by 16.37% compared to the previous study period and by 23.8% compared to the control group of animals and amounted to 155±2.35 s. Finally, the worst indicator was established after the 20th week of administration of a complex of food additives to rats – 170.2±1.91 s, which was characterized by an increase in reaction time by 8.93% compared to the previous experimental group and by 26.44% compared to the control.

In the dynamics of the experiment, a deterioration of the orienting functions of the rats was also revealed, which was manifested in a decrease in the number of performed functions and an increase in both the number of errors and the number of returns to the previous compartment **(Figure 3)**. The highest number of performed functions was registered in the experimental group that used food additives for eight weeks and was 12.1±0.77, which was 24.74% higher than in the control group, for which this indicator was 9.7±0,83. Regarding the number of errors and returns to the previous compartment, the lowest rate was recorded after the 4th week of the experimental study. The number of errors registered at this experiment stage was 5.3±0.3, which was 16.98% lower than the indicator set in the control group – 6.2±0.33. After four weeks of administration of the complex of food additives to experimental animals, the number of returns to the previous compartment was 3.7±0.3, which is 18.92% lower than in the control group, for which the indicator was 4.4±0.45.

The worst indicators of orientation functions were established after the 20th week of the experiment. The number of performed functions decreased by 2.02 times compared to the control. The number of errors increased by 63.31% and was 16.9±0.6 compared to the control

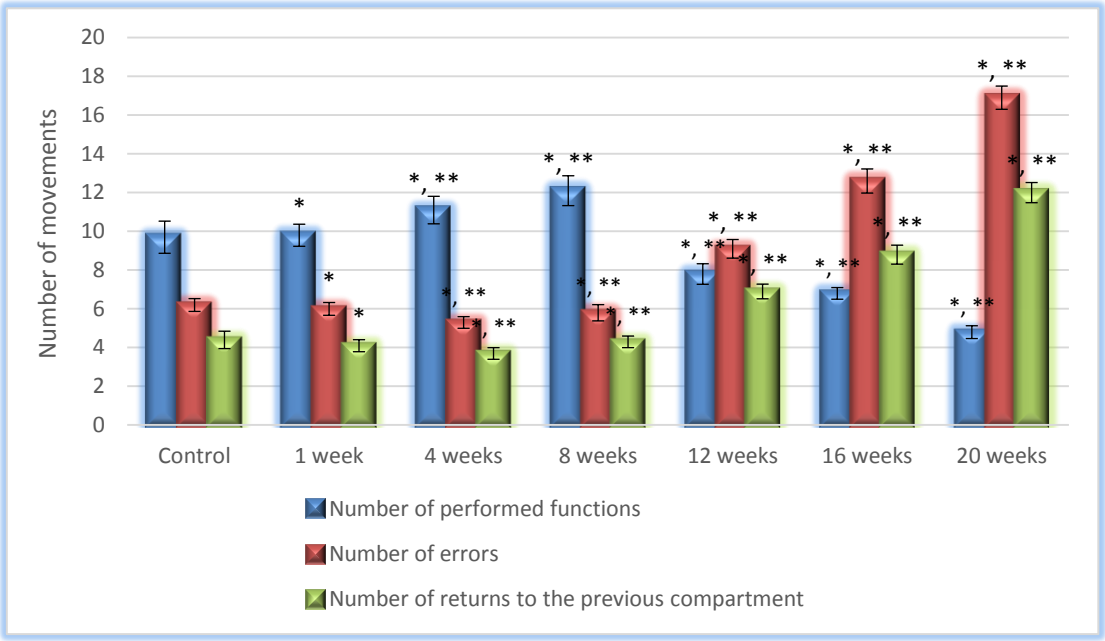

Figure 3. Assessment of orientational functions of rats in a complex maze.
***Notes:* * – p<0.05 compared to the control group, ** – p<0.05 compared to the previous term of the experimental study**

group. The number of returns to the previous compartment was 12±0.52, which was higher than the control group by 63.3%.

The analysis of the data in Figures 1-3 makes it possible to assert that the use of a complex of food additives (monosodium glutamate, sodium nitrite, Ponceau 4R) has a negative effect on memory functions, the ability to learn, and the speed of conditioned reflex formation.

One of the causes of cognitive disorders can be the influence of exogenous and endogenous factors [6, 7]. Cognitive dysfunction is manifested by a violation of the processes of memorization, reasoning, concentration of attention, the ability to make decisions, and a deficit of visual-spatial perception.

The analysis of literature sources showed that the food additives that we used in our study are quite often the cause of cognitive dysfunction.

It was established that using monosodium glutamate (MSG, E621) in newborn mice causes obesity and memory deficits at 16-17 weeks of E621 use [8]. E621 causes a deficiency of neurotransmitters, in particular, γ-aminobutyric acid (GABA), and memory loss in rats [9]. When studying the short-term memory of rats in the T-maze test, it was found that MSG significantly reduced the number of cases of entering the starting and other compartments of the maze, both during their training and during testing, compared to the control group. In addition, MSG significantly increased the time rats spent in the start compartment while significantly decreasing the time spent in the new maze corridors. This behavior of rats confirmed that MSG led to severe impairment of short-term spatial memory in rats [10].

Sodium nitrite leads to impairment of cognitive functions and also contributes to a decrease in the level of GABA and its precursor glutamine. When evaluating the spatial memory of rats in a water maze administered sodium nitrite in combination with D-galactose for 60 days, signs of spatial memory impairment were revealed, which were manifested by the lengthening of the swimming path and the increase in cases of non-exit during the latent period and the number errors compared to the control group [11]. Another study evaluated the short-term memory performance of rats in a Y-maze using the same combination of substances. It has been established that sodium nitrite, combined with D-galactose, leads to typical signs of aging, manifested by impaired learning and memory due to necrotic neuron changes and excessive oxidative stress [12].

Prenatal use of food dyes, to which Ponceau 4R belongs, leads to increased mobility,

decreased motivation, and manifestations of anxiety in offspring [13].

Thus, the data described above confirm that each of these food additives, when used separately, could cause the cognitive dysfunction found in experimental animals in our study. Unfortunately, the number of works describing the complex effects of these substances is limited.

Although, Ukrainian scientists studied rats' adaptive function when using monosodium glutamate, sodium nitrite and Ponceau 4R in the complex. It was established that from the first week of using this complex of food additives, anxiety, and fear increased in rats, blunting of adaptive reactions, decreased activity, and signs of emotional disturbance appeared, which increased until the 16th week of the experiment [14].

Conclusion. Despite some signs of activation of brain activity in rats at the initial stages of using a complex of food additives, from the 12th week of the experiment, cognitive function disorders were detected, which intensified until the 20th week. It was established that the use of a complex of food additives leads to cognitive dysfunction in rats, which was characterized by impaired memory function and learning ability. Thus, this research can be a basis for a more detailed study of this problem and finding ways to solve it.

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ВЛИЯНИЕ КОМПЛЕКСА ПИЩЕВЫХ ДОБАВОК НА КОГНИТИВНЫЕ ФУНКЦИИ И СКОРОСТЬ ФОРМИРОВАНИЯ УСЛОВНОГО РЕФЛЕКСА

С.М.Билаш[1], Я.А.Олийниченко[1], Е.Н.Пронина[1], С.В.Донченко[1], М.М.Коптев[1], А.В.Пирог-Заказникова[1], О.В.Давыдова[2], М.А.Олийниченко[1], Е.В.Безега[1], О.В.Мамай[1], Н.С.Копытько[1]

[1]Полтавский государственный медицинский университет, г. Полтава, Украина

[2]Кременчугский национальный университет имени Михаила Остроградского, г. Кременчуг, Украина

Резюме. Пищевые добавки относятся к экзогенным факторам, которые могут быть причиной возникновения когнитивной дисфункции, что может проявляться нарушениями процессов запоминания и формирования условных рефлексов. Целью исследования было установить изменения поведенческих реакций крыс на разных сроках употребления комплекса химических пищевых добавок (глутамат натрия, нитрит натрия, Понсо 4R).

Исследование базировалось на введении экспериментальным подгруппам лабораторных крыс в течение 1, 4, 8, 12, 16 и 20 недель комплекса пищевых добавок ежедневно однократно перорально. Определение когнитивных функций было проведено путем оценки скорости формирования условного пищедобывающего рефлекса в сложном лабиринте.

Оценка поведения подопытных животных в сложном лабиринте показала, что на начальных сроках введения комплекса пищевых добавок (до 8 недель) полученные результаты имели положительную динамику, при значениях $p<0,05$. Начиная с 12 недели экспериментального исследования все исследуемые параметры имели отрицательную тенденцию. Достоверно ($p<0,05$) худшие показатели были зарегистрированы после 20 недели экспериментального исследования.

Было установлено, что употребление комплекса пищевых добавок приводит к когнитивной дисфункции у крыс, которая характеризовалась нарушением функции памяти, а также способности к обучению.

Address for correspondence:
Oliinichenko Yaryna, Department of Anatomy with Clinical Anatomy and Operative Surgery, Poltava State Medical University, Poltava, Ukraine
E-mail: jarinaoliinichenko93@gmail.com