DOI 10.26724/2079-8334-2020-1-71-232-235
UDC [613.2:615.24]:599.323.4

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A MEASURE OF THE EFFECT OF COMPLEX FOOD ADDITIVES ON RATS' ADAPTIVE RESPONSES

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In the paper was the investigation of the effect of consumed complex food additives on the rats' adaptive response. 88 outbred mature male rats, housed in the common conditions, were involved into the study. Control rats consumed drinking water and received saline per os. The rats of the experimental group were given ad libitum access to water and, supplementary, consumed sodium nitrite solution. Sodium glutamate was administered at a dose of 20 mg/kg, Ponceau 4R at a dose of 5 mg/kg once daily per os. Doses of food additives were half less the allowable normal rate in food. After being exposed to open field test the rats were killed within 1, 4, 8, 12 and 16 weeks under thiopentone anesthesia overdose. Consumption of complex food additives at acceptable doses affects the behavioral responses of experimental animals. It has been established that just from the first week of observation, rats experienced increased anxiety, fear, blunting of adaptive responses, decreased activity and disturbance of the emotional state, which were intensified up to week 16 of the experiment.

Key words: open field test, sodium nitrite solution, sodium glutamate, Ponceau 4R, adaptive-locomotor behavior of rats.

The study is a fragment of the research project "Morphogenesis patterns of organs, tissues and neurovascular formations in normal condition, pathology and under the influence of exogenous factors", state registration No. 0118U004457.

The widespread use of food additives for various purposes in the production, processing, packaging and storage of products that are associated with, sometimes, quite wide limits of acceptable dosage, leads to cases of allergic reactions and disorders of respiratory, digestive, endocrine and nervous systems [12, 15].

Current publications highlight the outcomes of the effects of various exogenous factors on organs and systems [2], including the use of separate food additives [5, 10, 13]; however, little data on the effect of several chemical agents, consumed simultaneously, on the body have been found to date.

Monosodium Glutamate (E 621) is used as a flavor enhancer for many foods and as a salt substitute. It has been reported that E 621 can cause headache and dyspeptic events known as Chinese restaurant syndrome [14].

Sodium Nitrite (E 250) is a preservative and color retainer. Its negative impact on the digestive and respiratory systems has been proved [3].

Ponceau 4r (food colorant) can cause mental disorders in children; it can be also associated with attention deficit hyperactivity disorder and asthma-like attacks [11]. The etiology and pathogenesis of attention deficit hyperactivity disorder are poorly understood to date; consequently, such risk factors as hereditary predisposition, adverse course of the perinatal period, psychosocial and environmental factors are more commonly discussed. Moreover, more than 10 years ago, the factor of use of food additives was added to the above risk factors. It has been established that disorders in morphogenesis and functional genesis, namely, dysfunction of neurotransmitter systems of the brain and delayed neurodevelopment are the major causes in pathogenesis of attention deficit hyperactivity disorder [9].

The purpose of the paper was to study the effect of consumed complex food additives on the rats' adaptive response.

Materials and methods. The total of 88 outbred mature male rats, housed in the common conditions, were involved into the study. The rodents were divided into two groups (the control and the experimental ones). The control rats consumed drinking water and received saline per os. The experimental group rats were given access to water ad libitum and supplementary consumed 10% sodium nitrite solution [6]. Sodium glutamate was administered at a dose of 20 mg / kg in 0.5 ml of distilled water [8], Ponceau 4r at a dose of 5 mg / kg in 0.5 ml of distilled water [3] once daily per os. Doses of food additives were half less the allowable normal rate in food.

After being exposed to open field test the rats were killed within 1, 4, 8, 12 and 16 weeks under thiopentone anesthesia overdose [7].

To evaluate adaptive behavior, the rat was placed into the corner of the box and its spontaneous locomotor behavior was recorded for 60 seconds. Parameters of motor and exploratory behavior of animals (number of outer edge square crossings and number of central square crossings), vertical activity (number of rearings), and vegetative activity (number of urinations/defecations) were measured.

The resulting data have been processed quantitatively by the variance statistics using Student's t-test and *Excel* software.

The experimental part of the research has been carried out in compliance with the requirements of the international principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) and Resolution of the First National Congress on Bioethics (Kyiv, 2007) [6].

Results of the study and their discussion. The measure of numbers of outer edge square crossings showed 7.54 ± 0.35 crossings made by the animals of control group, indicating no anxiety and fear. During the observation, the parameter ranged from 7.61 ± 0.29 times within 1 week to 7.31 ± 0.29 times on week 16, with insignificant difference (Table 1).

With the increase in the period of consuming the complex food additives, the number of outer edge square crossings was tending to increase relative to the control group. The average number of outer edge square crossings in the experimental group was 12.46 ± 1.78 times, that was by 1.67 times more often compared to controls.

After 1 week of consuming the complex food additives the animals walked by 1.31 times more often on the outer edge, after 4 weeks by 1.51 times, after 8 weeks by 1.72 times, after 12 weeks by 1.88 times, after 16 weeks by 1.94 times, which indicated the presence of anxiety and fear, and thus, the increase in their manifestations in proportion to the period of consuming the complex food additives (table 1).

Table 1

Orientation and exploratory behavior of the rats on the outer edge squares

	Control group	Experimental group
Control	7.54±0.35	-
Week 1	7.61±0.29	10±0.23**
Week 4	7.46±0.35	11.3±0.37*. **
Week 8	7.46±0.31	12.9±0.24 *. **
Week 12	7.38±0.31	13.9±0.28 *. **
Week 16	7.31±0.29	14.2±0.29 **

Notes: hereinafter *p<0,05, compared to the previous time period of observation; ** p<0,05, compared to control group of animals.

The number of the central square crossings in the rodents of the control group, regardless of the time period, had no significant changes, accounting on the average of 2.46 ± 0.11 times, indicating no disorder of adaptive responses (table 2).

In the experimental group, there was a tendency to drastic reduce in the number of central square crossings during the observation. Rats who consumed the complex food additives for 1 week showed the parameter by 1.68 times lower than in the control group, and after 4 weeks it was by 4.62 times lower. Groups of animals that consumed additives for 8 weeks, 12 weeks, and 16 weeks avoided walking in the open field (table 2).

On the average, the animals of the experimental group crossed the central squares 0.39 ± 0.63 times, which is by 6.3 times less than in the control group, which showed the development of anxiety and blunting of the adaptive responses in rats, with increasing manifestations proportional to the duration of the consuming of food additives.

Table 2

Orientation and exploratory behavior of the rats on the central squares

	Control group	Experimental group
Control	2.38±0.24	-
Week 1	2.46±0.24	1.46±0.21**
Week 4	2.31±0.24	0.5±0.17*. **
Week 8	2.46±0.22	0**
Week 12	2.54±0.18	0**
Week 16	2.62±0.14	0**

Vertical activity of the animals, regardless of time period, had no significant changes, accounted on the average of 2.07 ± 0.07 times in the control group (table 3), indicating that the animals had no disturbance of activity and manifestations of fear.

In rats of the experimental group, we observed a tendency to decrease the number of erected postures with an average parameter of 0.85 ± 0.8 times. In animals who consumed food additives for 1 week it was by 1.27 times lower than in the control group of rats, for 4 weeks by 1.54 times lower, for 8 weeks by 1.74 times lower, and at 12 and 16 weeks the parameter was zero (table 3).

On the average, animals of the experimental group performed erected postures by 2.4 times less frequently, indicating activity disorder and appearance of fear with worsening of the health state that correlated with the duration of consuming the complex food additives.

Table 3

Vertical activity of the animals

	Control group	Experimental group
Control	2,08±0,24	-
Week 1	2,15±0,22	1,69±0,13**
Week 4	2,15±0,19	1,4±0,16**
Week 8	2±0,23	1,15±0,15**
Week 12	2,08±0,18	0*, **
Week 16	2±0,16	0*, **

Increased number of defecations and urinations indicated changes in the emotional state of animals. The rats of the control group had insignificant differences in the number of boluses depending on the observation period; the average value was 1.64 ± 0.08 times, which indicated an adequate emotional background.

Animals who consumed food additives tended to increase the number of boluses depending on the time period and their average number was 2.87 ± 0.5 times, which was by 1.75 times more than in the control group. Within a week of taking food additives animals had by 1.18 times more boluses than in the control group, after 4 weeks by 1.79 times, after 8 weeks by 1.72 times, after 12 weeks by 2.08 times, after 16 weeks by 2.1 times, which indicated changes and disorders of emotional state (table 4).

Table 4

Vegetative activity of the animals

	Control group	Experimental group
Control	1,69±0,24	-
Week 1	1,77±0,23	2,08±0,18**
Week 4	1,62±0,24	2,9±0,18*, **
Week 8	1,61±0,18	2,97±0,20**
Week 12	1,54±0,14	3,2±0,25**
Week 16	1,62±0,14	3,4±0,22*, **

The study findings have established a direct correlation between the changes in the parameters of the open field test and the duration of consuming the complex food additives, which is consistent with the data of other researchers [7] regarding the influence of ethanol and tincture of canuper on the central nervous system and emotional state of rats.

The average number of outer edge square crossings in animals who consumed food additives for 16 weeks was by 1.92 times greater than in the control group; the number of central square crossings was zero in the experimental group and 2.62 ± 0.14 times in the controls; the vertical activity of animals in the experimental group also was zero at 16 weeks, and in the controls it was 2 ± 0,16. Similar behavioral changes have been described by other authors in chronic alcohol intoxication in rats. [1]

Conclusion

Consumption of complex food additives at acceptable doses affects the behavioral responses of experimental animals. It has been established that just from the first week of observation, rats experienced increased anxiety, fear, blunting of adaptive responses, decreased activity and disturbance of the emotional state, which were intensified up to week 16 of the experiment.

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Реферати

ВИЗНАЧЕННЯ ВПЛИВУ КОМПЛЕКСУ ХАРЧОВИХ ДОБАВОК НА АДАПТИВНІ РЕАКЦІЇ ЩУРІВ

Ячмінь А.І., Кононов Б.С., Єрошенко Г.А., Білаш С.М., Білаш В.П.

В статті вивчений вплив вживання комплексу харчових добавок на адаптивні реакції щурів. Дослідження проведено на 88 статевозрілих безпорідних щурах-самцях. Щурам експериментальної групи, за умов безперешкодного доступу до рідини, давали пити розчин нітриту натрію. Глутамат натрію вводили в дозі 20 мг/кг, Понсо 4R – в дозі 5 мг/кг 1 раз на добу перорально. Дози харчових добавок вдвічі були меншими за допустиму норму у харчових продуктах. Тварин виводили з експерименту через 1, 4, 8 та 16 тижнів шляхом передозування тіопенталового наркозу. Перед цим проводили тест «відкрите поле». Встановлено, що вживання комплексу харчових добавок у допустимих дозах впливає на поведінкові реакції експериментальних тварин. З першого тижня спостереження у щурів посилюється тривога, страх, спостерігається притуплення адаптивних реакцій, зниження активності та порушення емоційного стану, які посилюються до 16 тижня експерименту.

Ключові слова. тест «відкрите поле», глутамат натрію, нітрит натрію, понсо 4R, адаптивно-дослідницька поведінка щурів.

Стаття надійшла 16.04.2019 р.

ОПРЕДЕЛЕНИЕ ВЛИЯНИЯ КОМПЛЕКСА ПИЩЕВЫХ ДОБАВОК НА АДАПТИВНЫЕ РЕАКЦИИ КРЫС

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В статье изучено влияние употребления комплекса пищевых добавок на адаптивные реакции крыс. Исследование проведено на 88 половозрелых беспородных крысах-самцах. Крысам экспериментальной группы, в условиях беспрепятственного доступа к жидкости, давали пить раствор нитрита натрия. Глутамат натрия вводили в дозе 20 мг/кг, Понсо 4R - в дозе 5 мг/кг 1 раз в сутки перорально. Дозы пищевых добавок вдвое были меньше допустимой нормы в пищевых продуктах. Животных выводили из эксперимента через 1, 4, 8 и 16 недель путем передозировки тиопенталового наркоза. Перед этим проводили тест «открытое поле». Установлено, что применение комплекса пищевых добавок в допустимых дозах влияет на поведенческие реакции экспериментальных животных. С первой недели наблюдения у крыс усиливается тревога, страх, наблюдается притупление адаптивных реакций, снижение активности и нарушение эмоционального состояния, которые усиливаются до 16 недели эксперимента.

Ключевые слова: тест «открытое поле», глутамат натрия, нитрит натрия, понсо 4R, адаптивно-исследовательское поведение крыс.

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