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STUDY OF EDUCATION AND MEMORY OF DESCENDANTS IN THE QUALITY OF ADDITIONAL LOADING OF AQUEOUS PLANT EXTRACTS

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Abstract. As the body ages, free radical processes intensify and the risk of various age-related diseases increases, including memory impairment, as exchange processes in nerve cells deteriorate. The internal antioxidant system, capable of neutralizing free radicals, is not always able to cope with the growing load, and then the supply of antioxidants from outside is necessary. A source of biologically active substances possessing antioxidant activity are plant extracts possessing a pronounced therapeutic effect combined with a minimum of side effects. The purpose of our study was to study the learning and memory of the offspring of rats that received aqueous plant extracts as an additional load. The study of the learning and memory of the offspring of rats that received aqueous plant extracts of Ginkgo biloba, Centella asiatica, Eleutherococcus and a mixture of aqueous extracts of Ginkgo biloba and Centella asiatica was performed on 4-month-old animals, obtained in an experiment from 15 males and 50 females, which in the course of 30 days water extracts were obtained in a dose of 30 mg/100 g of body weight in a volume of 1 ml. The study was carried out with the help of the test "Passive escape with negative (painful) reinforcement". Conclusions: the offspring of rats that received as an additional load intragastric mixture of aqueous extracts of Ginkgo biloba and Centella asiatica are characterized by the best indicators of learning and memory, compared to the offspring of animals of the control group, and rats that received aqueous extracts of Ginkgo biloba, Centella asiatica and Eleutherococcus separately.

Key words: memory, learning, rats, test "Passive escape with negative (painful) reinforcement", ginkgo biloba, Centella Asiatica, Eleutherococcus.

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ИЗУЧЕНИЕ ОБУЧАЕМОСТИ И ПАМЯТИ ПОТОМСТВА КРЫС, ПОЛУЧАВШИХ В КАЧЕСТВЕ ДОПОЛНИТЕЛЬНОЙ НАГРУЗКИ ВОДНЫЕ РАСТИТЕЛЬНЫЕ ЭКСТРАКТЫ

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Резюме. По мере старения организма интенсифицируются свободнорадикальные процессы и возрастает риск возникновения различных заболеваний, в том числе нарушений памяти, так как ухудшаются обменные процессы в нервных клетках. Внутренняя антиоксидантная система, способствующая нейтрализации свободных радикалов, не всегда способна справиться с нарастающей нагрузкой, и тогда необходимо поступление антиоксидантов из вне. Источником биологически активных веществ, обладающих антиоксидантной активностью являются растительные экстракты, обладающие выраженным терапевтическим действием в сочетании с минимумом побочных эффектов. Целью нашего исследование явилось изучение обучаемости и памяти потомства крыс, получавших в качестве дополнительной нагрузки водные растительные экстракты. Изучение обучаемости и память потомства крыс, получавших водные растительные экстракты гинкго билоба, центеллы азиатской, элеутерококка и смесь водных экстрактов гинкго билоба и центеллы азиатской, производили на 4-месячных животных, рождённых в эксперименте от 15 самцов и 50 самок, которым в течение 30 суток вводили водные экстракты в дозе 30 мг/100 г массы тела животного. Исследование проводили с помощью теста «Пассивное избегание с отрицательным (болевым) подкреплением». Выводы: потомство крыс, получавших в качестве дополнительной нагрузки внутрижелудочно смесь водных экстрактов гинкго билоба и центеллы азиатской характеризуется лучшими показателями обучаемости и памяти, по сравнению с потомством животных контрольной группы, и крыс, получавших водные экстракты гинкго билоба, центеллы азиатской и элеутерококка по отдельности.

Ключевые слова: память, обучаемость, крысы, тест «Пассивное избегание с отрицательным (болевым) подкреплением», гинкго билоба, центелла азиатская, элеутерококк.

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Many pathological processes in the organism, leading to various diseases and, eventually, aging, are based on the process of damage of cell membranes and other intracellular structures by free radicals [1]. As the organism ages, free-radical processes intensify and the risk of various age-related diseases, including memory disorders, increases, since exchange processes in nerve cells deteriorate [2, 3].

An internal antioxidant system normally functions in the body, contributing to neutralization of free radicals, but its activity may not be enough, and then additional supply of antioxidants from outside is necessary [4]. The source of biologically active substances with antioxidant activity are plant extracts that have a pronounced therapeutic effect combined with minimum side effects. Promising sources of antioxidants are aqueous extracts of Ginkgo biloba, Centella asiatica and Eleutherococcus, which have a pronounced nootropic effect and can modulate the psychotropic activity of the animal [5–7].

In this connection, the aim of our study was to investigate the learning ability and memory of the offspring of rats that received aqueous plant extracts as an additional load.

In order to achieve this objective we had to solve the following tasks: to obtain offspring from rats which daily during 30 days were given Ginkgo biloba, Asiatic Centella, Eleuterococcus extracts and a mixture of Ginkgo biloba and Asiatic Centella extracts as an additional load and to analyze their influence on learning and memory processes in the postponed period.

Materials and methods of research

Experimental investigations were performed in accordance with "Regulations on work with the use of experimental animals" and with observance of the principles of humanity stated in the European Community directives (86/609/EC). Study of learning ability and memory of offspring of rats treated with aqueous herbal extracts of Ginkgo biloba, Asiatic Centella and Eleuterococcus and a mixture of aqueous extracts of Ginkgo

biloba and Asiatic Centella was performed on four-month old animals obtained in the experiment from 15 male and 50 females, which were injected with aqueous extracts in a dose of 30 mg/100 g of animal body weight. There were 5 groups of animals in the experiment, each group had:

group 1 (control) – rats received daily intragastric distilled water in a volume of 1 ml;

group 2 – animals received aqueous extract of Ginkgo biloba;

group 3 – animals treated with aqueous extract of Centella asiatica;

group 4 – animals treated with aqueous extract of Eleuterococcus;

group 5 – animals treated with a mixture of aqueous extracts of Ginkgo biloba and Centella asiatica in a 1:1 ratio.

The study was conducted with the help of the test "Passive avoidance with negative (painful) reinforcement". The test was carried out in a chamber consisting of two compartments: an illuminated one and a darkened one with an electrically conducting floor. Rats were placed in the illuminated compartment and the latent period of transition to the dark compartment was measured. After transfer to the dark compartment, the animals were subjected to electrodermal irritation.

The test assessed the following: the relative number of animals not avoiding the light chamber; the latent period of entering the dark chamber at the first presentation; the latent period of entering the dark chamber 24 hours and 48 hours after the irradiation. The duration of testing was was 180 seconds [8].

We also performed statistical processing of the results using nonparametric statistical methods.

The results of the experiment

The results of the experiment are presented in Table 1.

The relative number of animals not avoiding the light chamber in the first series of experiments in the group of animals receiving aqueous extract of eleutherococcus corresponded to the control, but in other experimental groups it was higher: in the group receiving the extract of Centella asiatica – more by 31.3% (Manna-Whitney: U = 103,500, Z = 3.245542 at p = 0.000000), in the group receiving gingko biloba extract, 37.5 % more (Manna-Whitney: U = 92,000, Z = 3.241147 at p = 0.002221), and in the group of rats receiving a mixture of plant extracts, 50.0 % more (Manna-Whitney: U = 95,6000, Z = 4.525261 at p = 0.000001).

The latent period of transition to the dark compartment in the first series of experiments in the offspring of rats of the control group did not last too long and was longer in the experimental groups: in the offspring of rats receiving the extract of Centella asiatica – longer by 50.0%, (Manna-Whitney: U = 137.8000, Z = 4.245874 at p = 0.000111), the offspring of rats receiving ginkgo biloba extract – longer by 62.2% (Manna-Whitney: U = 168.900, Z = 2.589850 at p = 0.000196), the offspring of rats receiving eleuterococcus extract – longer by 60.6% (Manna-Whitney: U = 147.500, Z = 4.325352, at p = 0.000001), and longer by 67.3% (Manna-Whitney: U = 101.400, Z = 4.698686 at p = 0.002458) in offspring of rats receiving a mixture of aqueous plant extracts. The study found that the latent period of transition to the dark compartment lengthened in all groups of animals with each subsequent test.

In the second series of experiments (24 hours later), the relative number of animals not avoiding the light chamber in the control group of animals was higher than in the first series, and in other experimental groups it was even higher: in the group receiving the extract of Centella asiatica – more by 183.1% (Manna-Whitney: U = 114.800, Z = 4.566252 at p = 0.000111), in the group receiving gingko biloba extract – more by 101.7% (Manna-Whitney: U = 187,900, Z = 3.336323 at p = 0.003336), in the group of rats receiving eleuterococcus extract – more by 71.2% (Manna-Whitney: U = 147.5000, Z = 2.855457 at p = 0.000000), and in the group of rats receiving a mixture of plant extracts, 281.4% more (Manna-Whitney: U = 163.4000, Z = 5.477414 at p = 0.000245).

The latent period of transition to the dark compartment 24 hours after the first test significantly increased in animals of the control group compared to the first presentation and comparable to the control in animals receiving extracts of Ginkgo biloba and eleuterococcus, and increased by 14.2 % in the progeny of rats receiving extracts of Centella asiatica and a mixture of water extracts (Manna-Whitney: U = 114.400, Z = 5.487874 at p = 0.00000) and 26.2% (Manna-Whitney: U = 167,8000, Z = 4.565252 at p = 0.003965), respectively.

Таблица 1. Показатели обучаемости и памяти потомства крыс, получавших водные растительные экстракты
Table 1. Indicators of learning and memory of the offspring of rats that received aqueous plant extracts

Indicator	Control group	Group 1 (Centella asiatica)	Group 2 (Gingko biloba)	Group 3 (Eleutherococcus)	Group 4 (Centella + Gingko)
Relative number of animals not avoiding the light chamber at 1 presentation, %	1,6 ± 0,09	2,1 ± 0,07[1]	2,2 ± 0,08[1]	1,6 ± 0,07	3,2 ± 0,05[1]
Latent period of transition to the dark compartment at 1 presentation, s	25,4 ± 0,81	38,1 ± 1,45[1]	41,2 ± 1,40	40,8 ± 1,31	42,5 ± 1,36[1]
Relative number of animals not avoiding the light chamber after 24 hours, %	5,9 ± 0,18	16,7 ± 0,63[1]	11,9 ± 0,38[1]	10,1 ± 0,39[1]	22,5 ± 0,97[1]
Latent period of transition to the dark compartment after 24 hours, s	109,8 ± 3,73	125,4 ± 4,38[1]	115,7 ± 3,70	112,6 ± 4,17	138,6 ± 4,43[1]
Relative number of animals not avoiding the light chamber after 48 hours, %	12,8 ± 0,46	25,1 ± 0,93[1]	20,6 ± 0,72[1]	18,9 ± 0,64[1]	28,5 ± 0,97[1]
Latent period of transition to the dark compartment after 48 hours, s	135,6 ± 4,33	159,3 ± 5,73[1]	148,9 ± 4,52[1]	145,7 ± 5,21	178,8 ± 5,72[1]

Note: In this table the differences are significant at $p < 0.05$: 1 – in comparison with the control group animals.

In the third series of experiments (after 48 hours), the relative number of animals not avoiding the light chamber in the control group of animals became even higher than in the first and second series, and in other experimental groups it was even higher: in the group receiving the extract of Centella asiatica – more by 96.1% (Manna-Whitney: U = 122.500, Z = 4.142147 at p = 0.000000), in the group receiving gingko biloba extract – more by 60.9% (Manna-Whitney: U = 181.700, Z = 4.488857 at p = 0.004556), in the group of rats receiving eleutherococcus extract – more by 47.7% (Manna-Whitney: U = 105.6000, Z = 3.455252 at p = 0.000125), and the group of rats receiving a mixture of plant extracts was 122.7% higher (Manna-Whitney: U = 157.5000, Z = 5.142441 at p = 0.004225).

The latent period of transition to the dark compartment 48 hours after the first test also increased in animals of the control group in comparison with the first and second presentation, and increased even more in the progeny of rats receiving aqueous plant extracts: in the progeny of rats receiving the extract of Centella asiatica – more by 17.5 % (Manna-Whitney: U = 112,500, Z = 4.121411 at p = 0.003458), progeny of rats receiving ginkgo biloba extract increased by 9.6% (Manna-Whitney: U = 103,6000, Z = 5.262322 at p = 0.000001), progeny of rats receiving eleuterococcus extract increased by 7.4% (Manna-Whitney: U = 97.800, Z = 2.874744 at p = 0.000000), and in offspring of rats receiving a mixture of aqueous plant extracts, 31.9% more (Manna-Whitney: U = 125.800, Z = 4.157512 at p = 0.000012).

Discussion of results

During the transition to the dark compartment from the light compartment, rats satisfy the biological motivation of protection in the dark, confined space from aversive stimuli of bright light and open space. It can be assumed that the latent period of transition to the dark compartment during the first and subsequent series of experiments reflects the expression of the rat's motivation to avoid open space and bright light, an innate rodent behavior. However, the duration of the latent period of transition to the dark compartment can also be influenced by the animal's exploratory motivation. Accordingly, animals not avoiding the bright chamber showed high exploratory activity, and although there were not many of them in each group (not more than 3.2% of the total number of rats), the greatest number of animals with high exploratory activity was among the offspring of rats receiving the mixture of plant extracts. This is also confirmed by the longest latent period of transition to the dark compartment in the first series of the experiment.

In the second and third series of the experiment, rat behavior is based on the interaction of two motivations: on the one hand, avoidance of open space and bright light, and, on the other hand, anticipation of painful irritation in the dark compartment. In our experiment, the number of animals avoiding the dark chamber in each subsequent series of optics increased in each group, and their highest number was among the offspring of rats receiving the mixture of plant extracts. The latent period of transition to the dark chamber also increased.

Conclusions

In the distant periods of postnatal ontogenesis the offspring of the rats born after intragastric feeding of the mixture of aqueous extracts of Ginkgo biloba and Asiatic Centella are characterized by better parameters of learning and memory, Compared to the control group animals and rats receiving aqueous extracts of Ginkgo biloba, Asiatic Centella and EleutheroCocus separately, which confirms a pronounced total nootropic effect of the studied plant extracts.

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