Keywords: ischemic heart disease, anemia, heart rhythm disorder, treatment.

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INFLUENCE OF CHRONIC MILD STRESS ON RAT BEHAVIOR UNDER EXPERIMENTAL PHARMACOTHERAPY

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Vulnerability to the development of mood disorders, such as anxiety disorders and depression, depends on a combination of genetic and environmental factors, among which stress plays a significant role. Not everyone who experiences stress develops a mood disorder. Stress resistance is the ability to experience stressful events without the development of chronic stress (psychological and / or biological) and related changes in emotional behavior. Susceptibility to stress is associated with psychological factors such as passive coping skills and high emotional reactivity, but is also associated with biological factors such as hypo- or hypersensitivity to the stress response system, sex hormones, central and peripheral immune activation and glucocorticoid resistance. Interestingly, the level of biological response to stress and related neuroendocrine systems influences stress management, active stress management is enhanced by antidepressants. This suggests that drugs that affect the biological side of stress resistance may increase the effectiveness of existing treatments and preventive care. Intestinal microbiome is a biological factor that can affect resistance to stress. The widespread impact of gut microbiota on human health, including mental health, has come to be understood over the last decade. The gut-brain axis is a bidirectional link between the gut and the central nervous system, which plays an important role in maintaining nervous, hormonal, and immunological homeostasis. With the advent of evidence that gut microbiome can affect symptoms of depression and anxiety, it is now seen as a key component of this gut-brain cross-interaction, and the term has been extended to the gut-brain-brain axis. Stress also alters the intestinal microbiota, and the effects of early life stress on the microbiota can extend to adulthood.

This work covers the study of the effect of chronic mild stress on behavioral responses in experimental conditions.

Key words: *stress, microbiota, probiotics, vitamin D_3.*

Relationship of the publication with the planned research works. This work is a fragment of the planned scientific work of the Dnipro State Medical University «Pharmacological analysis of organ and endotheliprotection of experimental pathological conditions». State registration number 0118U006631.

Introduction. Today, no one denies the existence of the so-called intestinal nervous system, which is located between the esophagus and intestines and consists of 100 million nerve cells; there are more of them in it than in the spinal cord [1-4]. This is the second most difficult cluster of nerves in the human body after the brain, which is tentatively called «gut brain» [5-7]. Moreover, our brain with all its feelings, emotions and thoughts constantly communicates with the «gut brain». This process of communication is called the «gut-brain axis» [8, 9].

Recently, scientists have shown that the presence of intestinal microbiome at an early stage of development affects the topology of neurons (a set of properties of entire areas of the brain) associated with anxiety and depression [10]. Intestinal microbiome is associated with specific behavior, stress and disease. Changes in the intestinal microbiome can affect the development of

diseases, and the management of the microflora can become a new method of intervention in situations that have already reached the clinical stage (in the field of affective and anxiety disorders) [11, 12].

The aim of the study. To determine the effect of chronic mild stress (CMS) on the development of emotional and behavioral disorders in in rats under experimental pharmacotherapy.

Object and methods of research. The study design, all experimental protocols were approved by the Biomedical Ethics Committee of the Dnipro State Medical University (protocol No. 8 dated 15.12.2020). Experiments were performed in compliance with the Directive 86/609/EEC on the Protection of Animals Used for Experimental and Other Scientific Purposes and the order No. 3447-IV of 21.02.2006 new editorship of 08.08.2021 «On protection of animals against abuse» and the Provisions of the «European convention for the protection of animals used for experimental and other scientific purposes» (Ukraine).

Table 1 – Scheme of chronic mild stress

	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	Monday
The inclination of the cages at an angle of 30^0	10 am-5 pm			10 am-5 pm			
Lighting at night	5 pm-11 am						
Flashing light		11 am-5 pm					
«Dirty Cage»		5 pm- 10 am					
Drinking deprivation			10 am-5 pm				
Empty bottles			5 pm- 10 am				
Pungent odor and deprivation of food				5 pm- 10 am			
«A tight cage»						10 am-10 am	

Notes: Flashing light (frequency 5 Hz, in blackout conditions) – for 5 hours; "Dirty cage" – 500 ml of water was poured into the cage for 17 hours; Drinking deprivation – deprivation of water for 7 hours followed by placing empty bottles in the cage for 17 hours; Annoying new smell – placing wooden cubes with the smell of tar in the cage (41 hours, together with food deprivation); food deprivation – deprivation of food for 41 hours; "A tight cage" – moving each rat into an individual small cage for 24 hours, followed by putting bottles with water and 10% sugar solution into the cage.

The study was carried out on 24 male Wistar rats weighing 230–250 g. The basis of the modeling of chronic muscle stress was based on the classic method of Willner [13], adapted to the conditions and purpose of our experiment. The animals of the experimental groups were subjected to daily exposure to weak stressors alternating with a 168-hour (7-day) cycle of changes in these stimuli for 4 weeks **(table 1)**.

Animals were randomly divided into five groups (n=8 in each). I – negative control, II – positive control (CMS). Group III rats for 28 days of the experiment received intragastric combination therapy – a symbiotic combining the prebiotic Lactulose at a dose of 2680 mg/kg («Normaze®», Delta Medical Promotions AG, Lac) and a probiotic containing 4 billion active cells (CFU)): *Lactobacillus acidophilus, Lactobacillus rhamnosus, Streptococcus thermophilus, Lactobacillus delbrueckii subsp. Bulgaricus* at a dose of 1 billion CFU/kg («Yogurt», Pharmascience, Yo). Group IV animals also received intragastric vitamin D_3 (Aquadetrim Vitamin D_3, Pharmaceutical Works POLPHARMA S. A., vit. D_3) at a dose of 2500 IU/kg. Group V received triple combination pharmacotherapy, which included yogurt / lactulose + vitamin D_3 in the above doses. Doses were calculated based on the higher therapeutic dose for humans using the interspecific human / rat dose conversion factor.

To assess the behavioral responses of animals, the open field test was used, which allows to relatively quickly determine the physiological response to the new environment and to obtain multifaceted information about the motor, research and emotional activity of animals. This model is based on the conflict of two motivations – the instinctive tendency to explore a new environment and the tendency to minimize the possible danger from him.

The field (1 m^2) is divided into 25 squares and surrounded by walls approximately 40 cm high. On the entire floor plane of this installation evenly placed 16 holes («holes») with a diameter of 6 cm. The test animal is placed in the center surface and for 4 minutes monitored their behavior. The main variables recorded during the next 4 minutes are: latency (the time taken to leave the starting square), crossings (number of times the line of a square is crossed with all 4 legs), rearings (number of times the animal stands on its hind legs), peeping into holes (stick more than half of the head with whiskers into the hole) grooming (frequency of grooming activity), and number of defecation boli [14, 15]. The response to novelty is determined by the ratio of the number of crossed squares in the open field for 4 minutes of observation under red light to their number for 1 minute in bright light.

Statistical data analysis was performed by AnalystSoft, StatPlus (version 2006) and AtteStat (version 13.1). All results are expressed as x ± SD (standard deviation). Statistical significance (P<0.05)

Research results and their discussion. The results of the study showed significant changes in the positive control group. According to the data in **table 2**, on the 28th day of the experiment, the rate of crossing in rats was 12.3% (p<0.05) lower than the group of negative control. In addition, these rats showed a statistically significant decrease in the number of peeping into holes by 44.4% (p<0.05). It was also characterized by a pronounced suppression of rearing, which was characterized by a decrease in the number of vertical rises by 35.3% (p<0.05) compared with the negative control group **(table 2)**. At the same time, there was an impact on emotional activity – there was a decrease in grooming by 37.5% and an increase in defecation by 100.0%, but the values obtained had no statistical significance for the group of negative control **(table 2)**. The latency of the exit from the center of the open field in positive control group rats was 22.2% (p<0.05) lower than in negative control animals **(table 2)**. The reaction to novelty in CMS rats tended to decrease.

In animals treated with vit. D_3, compared with the group of animals with CMS, on the 28th day of the study

Table 2 – Study of behavioral reactions in rats in the open field test under the conditions of administration of the studied drugs for 28 days, M±m

Experimental groups, n	I. Locomotion	II. Exploration		III. Emotionality			
	Crossing	Rearing	Holes	Grooming	Defecation of boli/ urination	Latency, (c)	Reaction to novelty
Positive control, (n=8)	17,8±1,30#	2,8±0,54#	1,3±0,16#	1,3±0,40	1,0±0,37	3,5±0,41#	0,5±0,18
Negative control, saline, n=8	20,3±3,09	4,3±0,79	2,3±0,47	2,0±0,26	0,5±0,18	4,5±0,18	0,8±0,30
Yo + Lac, (n=8)	23,8±2,64	6,3±1,22	5,5±1,47	1,5±0,32	0,5±0,18	5,8±0,40*	4,3±0,47*
Vit. D_3, (n=8)	23,0±2,91	9,5±0,61*	4,5±1,28*	2,3±0,40	0*	7,5±0,41*	3,9±0,56*
Vit. D_3 Yo/Lac, (n=8)	35,0±5,96*	8,5±1,43*	3,3±1,14*	2,3±0,30	0,3±0,16	5,0±0,89	1,8±0,65

Notes: * – p<0.05, (versus Negative control); # – p<0.05, (versus Positive control).

there was a statistically significant increase of 245.5% and 260.0% (p<0.05), respectively, rearing and research activity **(table 2)**. Either, the growth of crossing by 29.6% (p=0.35) was not characterized by statistical significance. It should be noted that under these experimental conditions, administration of the study drug led to a decrease of 100.0% (p<0.05) acts of defecation, which indicates a decrease in animal anxiety. The number of grooming acts tended to increase, which indicated a positive effect of the drug on the emotional state of animals. The latency of the exit from the center of the open field in rats treated with vit. D_3 was 114.3% (p<0.05) higher than in animals with CMS **(table 2)**. The reaction to novelty in rats of this group tended to increase.

In animals treated with a combination of vit. D_3 with Yo + Lac, compared with the group of animals with CMS, on the 28th day of the study there was a statistically significant increase by 209.1% (p<0.05) of rearing and crossing by 97.2% (p<0.05). It should be noted that under these experimental conditions, the administration of the study combination led to a decrease of 75.0% (p<0.05) acts of defecation, which indicates a decrease in animal anxiety. The number of grooming acts tended to increase, which indicated a positive effect of the combination on the emotional state of the animals.

Thus, based on the studies, it can be concluded that chronic mild stress changes the behavior of rats in the open field test, which is characterized by significant inhibition of motor and research activity of experimental animals.

It should be noted that the administration of probiotic and prebiotic, both alone and in combination with vitamin D_3 changed the rates of behavioral responses in the open field test in rats on the background of chronic mild stress.

Conclusions. Chronic stress changes the behavior of rats in the open field test, which is characterized by significant inhibition of motor and research activity of experimental animals and symbiotic drugs with vitamin D_3 can be a significant lever in the correction of this condition.

Prospects for further research. This direction is promising and justified for various reasons. However, its widespread introduction requires additional *in vitro* studies in animals and humans, which can explain the relationship between stress and intestinal microbiom.

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ВПЛИВ ХРОНІЧНОГО М'ЯКОГО СТРЕСУ НА ПОВЕДІНКУ ЩУРІВ ЗА УМОВ ЕКСПЕРИМЕНТАЛЬНОЇ ФАРМАКОТЕРАПІЇ

Харченко Ю. В., Абрамов С. В., Крижановський Д. Г., Федченко М. П., Черненко Г. П., Філіпенко В. В., Терещенко Н. М.

Резюме. Уразливість до розвитку розладів настрою, таких як тривожні розлади та депресія, залежить від поєднання генетичних факторів та факторів навколишнього середовища, серед яких стрес відіграє значну роль. Цікаво, що на подолання стресу впливає рівень біологічної реакції на стрес і пов'язаних з ним нейроендокринних систем, активне подолання стресу підвищується за допомогою антидепресантів. Це свідчить

про те, що препарати, які впливають на біологічну сторону стресостійкості, можуть підвищувати ефективність наявних методів лікування та профілактичну допомогу. Мікробіом кишечника є біологічним фактором, який також може впливати на стійкість до стресу. Широкий вплив мікробіоти кишечника на здоров'я людини, включаючи психічне здоров'я, почав усвідомлюватися і розумітися протягом останнього десятиліття.

Метою дослідження було визначити вплив хронічного м'якого стресу (ХМС) на розвиток емоційно-поведінкових розладів у щурів за умов експериментальної фармакотерапії вітаміном D та пробіотиком.

Об'єкт і методи дослідження. Тварин піддослідних груп протягом 4 тижнів піддавали щоденному впливу слабких стрессорних факторів. За основу було взято класичну методику (ХМС) Вілнера, адаптовану до умов та завдань нашого експерименту. Протягом певного проміжку часу щурів щодня піддавали дії м'яких стресогенних чинників, що чергувалися зі 168-годинним (7-денним) циклом зміни подразників для тварин. Тварин було розподілено на п'ять груп (n=8 у кожній). I – негативний контроль, II – позитивний контроль (ХМС). Щури III-ї групи протягом 28 діб експерименту інтрагастрально отримували комбіновану терапію – симбіотик, що поєднував пребіотик Лактулозу (Lac) та пробіотик, що містить 4 млрд активних клітин (КОЕ): *Lactobacillus acidophilus, Lactobacillus rhamnosus, Streptococcus thermophilus, Lactobacillus delbrueckii subsp. Bulgaricus* (Yo). Тварини IV групи також отримували інтрагастрально вітамін D_3 (vit. D_3). Група тварин V отримувала потрійну комбіновану фармакотерапію, яка включала йогурт/лактулозу + вітамін D у вищезазначених дозах.

Оцінку проводили на 29 добу експерименту. Кожного щура поміщали в експериментальну установку «відкрите поле». Тварин по черзі поміщали в центр і протягом 4 хвилин відстежували їх поведінку. Цілісну оцінку проводили по кількості перетнутих квадратів (горизонтальна активність), вертикальних підйомів та заглядань у нірки (дослідницька активність), латентності виходу щурів із чотирьох центральних квадратів «відкритого поля». Реакцію на новизну визначали відношенням числа перетнутих квадратів за 4 хвилини спостереження при червоному світлі до їх числа за 1 хвилину при яскравій освітленості.

Результати. Встановлено значне зниження поведінкових реакцій у тварин з ХМС. Так, на 28 добу експерименту показник горизонтальної рухової активності у щурів групи позитивного контролю був на 12,3% нижчим групи негативного контролю. Крім цього, у щурів спостерігалося зменшення кількості заглядань у нірки на 44,4%. Характерним було зниження вертикальної рухової активності на 35,3% у порівнянні з групою негативного контролю. При цьому, був помітний вплив на емоційну діяльність – спостерігалося зниження актів грумінгу на 37,5% та підвищення актів дефекацій на 100,0% відносно групи негативного контролю. Латентний час виходу з центру площадки «відкритого поля» в групі позитивного контролю була на 22,2% нижчою відносно негативного контролю. У тварин, які отримували vit. D_3 у порівнянні з групою позитивного контролю, на 28 добу дослідження спостерігалося зростання вертикальної рухової і дослідницької активності; призводило до зниження на 100,0% актів дефекацій, що свідчить про зменшення тривожності тварин. Латентність виходу з центру «відкритого поля» у щурів, які отримували vit. D_3, була на 114,3% вищою, ніж у тварин групи позитивного контролю. У тварин, які отримували комбінацію vit. D_3 з Yo + Lac, у порівнянні з групою тварин позитивного контролю, на 28 добу дослідження спостерігалося зростання на 209,1% вертикальної рухової активності. Введення досліджуваної комбінації призводило до зниження на 75,0% актів дефекацій, що свідчить про зменшення тривожності тварин. Показник кількості актів грумінгу мав тенденцію до зростання, що свідчило про позитивний вплив комбінації на емоційний стан тварин.

Отже, на основі проведених досліджень можна зробити висновок, що хронічний м'який стрес змінює поведінку щурів в тесті «відкрите поле», яка характеризується істотним пригніченням рухової та орієнтовно-дослідницької активності експериментальних тварин. Введення йогурту та лактулози як самостійно, так і при сумісному використанні з вітаміном D_3, покращує показники поведінкових реакцій в тесті «відкрите поле» у щурів на тлі порушень емоційної сфери.

Висновки. Хронічний стрес змінює поведінку щурів в тесті «відкрите поле», яка характеризується істотним пригніченням рухової та орієнтовно-дослідницької активності експериментальних тварин, а комбінована терапія з використанням симбіотичних препаратів та вітаміну D_3 може бути вагомим важелем в корекції цього стану.

Ключові слова: стрес, мікробіота, пробіотик, вітамін D_3.

INFLUENCE OF CHRONIC MILD STRESS ON RATH BEHAVIOR UNDER EXPERIMENTAL PHARMACOTHERAPY

Kharchenko Y. V., Abramov S. V., Kryzhanovskyi D. H., Fedchenko M. P., Chernenko H. P., Filipenko V. V., Tereshchenko N. M.

Abstract. *Introduction.* Vulnerability to developing mood disorders, such as anxiety disorders and depression, depends on a combination of genetic and environmental factors, among which stress plays a significant role. It is interesting that coping with stress is influenced by the level of the biological response to stress and related neuroendocrine systems, active coping with stress is increased with the help of antidepressants. This indicates that drugs that affect the biological side of stress resistance can increase the effectiveness of existing treatment methods and preventive care. The gut microbiome is a biological factor that may also influence resistance to stress. The broad impact of the gut microbiota on human health, including mental health, has begun to be recognized and understood over the past decade.

The *aim* of the study was to determine the effect of chronic mild stress (CMS) on the development of emotional and behavioral disorders under the conditions of experimental pharmacotherapy in rats.

Materials and methods. The study was carried out on 24 male Wistar rats weighing 230–250 g. The basis of the modeling of chronic muscle stress was based on the classic method of Willner, adapted to the conditions and purpose of our experiment. The animals of the experimental groups were subjected to daily exposure to weak

stressors alternating with a 168-hour (7-day) cycle of changes in these stimuli for 4 weeks. Animals were randomly divided into five groups (n=8 in each). I – negative control, II – positive control (CMS). Group III rats for 28 days of the experiment received intragastric combination therapy – a symbiotic combining the prebiotic Lactulose (Lac) and a probiotic containing 4 billion active cells (CFU)): *Lactobacillus acidophilus, Lactobacillus rhamnosus, Streptococcus thermophilus, Lactobacillus delbrueckii subsp. Bulgaricus* (Yo). Group IV animals also received intragastric vitamin D_3 (vit. D_3). Group V received triple combination pharmacotherapy, which included yogurt / lactulose + vitamin D_3 in the above doses.

The results of the study showed significant changes in the positive control group. The rate of crossing in rats was 12.3% lower than the group of negative control. These rats showed a statistically significant decrease in the number of peeping into holes by 44.4%. It was also characterized by a decrease in the number of vertical rises by 35.3% compared with the negative control group. The latency of the exit from the center of the open field in positive control group rats was 22.2% lower than in negative control animals. The reaction to novelty in CMS rats tended to decrease. In animals treated with vit. D_3, compared with the group of animals with CMS, on the 28th day of the study there was a statistically significant increase of 245.5% and 260.0%, respectively, rearing and research activity. It should be noted that under these experimental conditions, administration of the study drug led to a decrease of 100.0% acts of defecation, which indicates a decrease in animal anxiety. The number of grooming acts tended to increase, which indicated a positive effect of the drug on the emotional state of animals. The latency of the exit from the center of the open field in rats treated with vit. D_3 was 114.3% higher than in animals with CMS. The reaction to novelty in rats of this group increased.

In animals treated with a combination of vit. D_3 with Yo + Lac, compared with the group of animals with CMS there was a statistically significant increase by 209.1% of rearing and crossing by 97.2%. It should be noted that under these experimental conditions, the administration of the study combination led to a decrease of 75.0% acts of defecation, which indicates a decrease in animal anxiety. The number of grooming acts tended to increase, which indicated a positive effect of the combination on the emotional state of the animals.

Results. Therefore, based on the studies, it can be concluded that chronic mild stress changes the behavior of rats in the open field test, which is characterized by significant inhibition of motor and research activity of experimental animals. Administration of probiotic and prebiotic, both alone and in combination with vitamin D_3, changed the rates of behavioral responses in the open field test in rats on the background of emotional disorders.

Conclusion. Chronic stress changes the behavior of rats in the open field test, which is characterized by significant inhibition of motor and research activity of experimental animals and symbiotic drugs with vitamin D_3 can be a significant lever in the correction of this condition.

Key words: stress, microbiota, probiotics, vitamin D_3.

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The Authors declare no conflict of interest.

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