UDC 57.084.1:[599.323.45+ 57.024+ 534.6]
DOI https://doi.org/10.32782/2226-2008-2024-6-1

O. V. Dienha[1] https://orcid.org/0000-0002-8630-9943
O. A. Makarenko[1] https://orcid.org/0000-0001-8029-4392
I. V. Khodakov[1] https://orcid.org/0000-0003-4352-4798
N. O. Nepriakhina[2] https://orcid.org/0009-0007-6182-1872
O. I. Demyd[2] https://orcid.org/0000-0001-6770-2929

MODELING CHRONIC STRESS IN LABORATORY RATS USING SOUNDS OF DIFFERENT RANGES

[1]State Establishment "The Institute of Stomatology and Maxillo-facial Surgery National Academy of Medical Sciences of Ukraine"
[2]Odesa National Medical University, Odesa, Ukraine

UDC 57.084.1:[599.323.45+ 57.024+ 534.6]

O. V. Dienha[1], O. A. Makarenko[1], I. V. Khodakov[1], N. O. Nepriakhina[2], O. I. Demyd[2]
MODELING CHRONIC STRESS IN LABORATORY RATS USING SOUNDS OF DIFFERENT RANGES
[1]State Establishment "The Institute of Stomatology and Maxillo-facial Surgery National Academy of Medical Sciences of Ukraine"
[2]Odesa National Medical University, Odesa, Ukraine

Under conditions of modern urbanized society, constant exposure to stressful situations is becoming a significant etiological factor in the development of many diseases. To study the efficacy of new anti-stress drugs, adequate models of chronic stress in laboratory animals are needed to avoid resistance to the stress factor and provide the natural progression of the adaptation syndrome.

The aim of the study was to evaluate the stages of adaptation syndrome in the modeling of sound stress by testing the behavior of laboratory rats in an open field system.

Materials and methods. To reproduce the stress, an ultrasonic pest repeller LS-912 "Leaven Enterprise" was used, which was turned on for 6 hours a day according to the following scheme: 2 days – ultrasound with a frequency of 30 kHz, the next 2 days – 40 kHz, the next 2 days – 50 kHz, the next 2 days – 65 kHz. Then the scheme was repeated. Testing in the "open field" was carried out for 3 minutes per rat on the 17th, 23rd, and 46th day of the experiment in terms of the "hole-board test", grooming, horizontal and vertical locomotor activity. The behavioral activity of stressed rats was compared with the corresponding indicators in intact animals. Comparison of mean values between the intact group and the group of rats exposed to sound stress was performed using Student's t-test. The results were considered significantly different at $p<0.05$.

Results. It was found that on the 17th day, sound stress caused an increase in animal anxiety with a 2.4-fold increase in the amount of grooming. On the 23rd day of the experiment, an increase in the overall hyperactivity of stressed animals by 46.3% was recorded, indicating their excessive excitement and fear. On the 46th day of observation, rats exposed to sound stress showed a significant decrease in locomotor, cognitive activity and general behavioral actions by almost 2 times, which corresponds to the stage of exhaustion. The obtained results allow the use of the LS-912 pest repeller with a change in the ultrasound range every 2–3 days to model chronic stress in laboratory rats in order to study the effectiveness of new anti-stress drugs.

Key words: experiment, stress, sounds, rats, biophysics.

УДК 57.084.1:[599.323.45+57.024+534.6]

О. В. Дєньга[1], О. А. Макаренко[1], І. В. Ходаков[1], Н. О. Непряхіна[2], О. І. Демид[2]
МОДЕЛЮВАННЯ ХРОНІЧНОГО СТРЕСУ У ЛАБОРАТОРНИХ ЩУРІВ ЗА ДОПОМОГОЮ ЗВУКІВ РІЗНИХ ДІАПАЗОНІВ
[1]Державна установа «Інститут стоматології та щелепно-лицевої хірургії Національної академії медичних наук України», Одеса, Україна
[2]Одеський національний медичний університет, Одеса, Україна

У дослідженні поведінки лабораторних щурів у системі «відкрите поле» оцінювали стадії адаптаційного синдрому під час звукового стресу. Для відтворення стресу використовували ультразвуковий відлякувач шкідників, який вмикали по 6 годин на добу. Тестування у «відкритому полі» проводили за показниками «норкового рефлексу», грумінгу, горизонтальної та вертикальної локомоторної активності. Встановлено, що стрес викликав збільшення тривожності тварин за підвищення кількості грумінгу, збільшення загальної гіперактивності стресованих тварин, а також показано суттєве зниження локомоторної, пізнавальної активності та загальних поведінкових дій, до стадії виснаження. Отримані результати дозволяють використовувати відлякувач шкідників LS-912 зі зміною діапазону ультразвуку кожні 2–3 дні для моделювання хронічного стресу у лабораторних щурів з метою дослідження ефективності нових антистресових препаратів.

Ключові слова: експеримент, стрес, звуки, щури, біофізика.

© O. V. Dienha, O. A. Makarenko, I. V. Khodakov et al., 2024

Стаття поширюється на умовах ліцензії

Relevance. The current state of human health in an urbanized society is associated with the constant impact of stressful situations, which is an important etiological factor in the development of "diseases of civilization" [1; 2]. According to Hans Selye, when exposed to stressors of any genesis, the adrenal cortex produces the same hormones, which help the body adapt to the stressor and provide a nonspecific response. Prolonged exposure to a stressor and insufficient adaptive potential of the body results in functional exhaustion, which is accompanied by a significant decrease in resistance. This condition causes the development of many diseases that require medical intervention [3; 4].

To develop effective and affordable anti-stress prevention schemes, convenient stress models in laboratory animals are needed that should mimic the natural progression of the adaptation syndrome and can be extrapolated to humans. There are a number of different methods, including creating hyperdynamics in a water tank for forced swimming [5], chronic immobilization of rats in special cages, the use of suprathreshold pain, light, electrical stimuli, hypothermia, pharmacological agents, etc. [6; 7; 8].

The main disadvantage of the known stress models is the development of resistance to chronic exposure to the factor. Therefore, the idea to model chronic stress in laboratory rats was to use the LS-912 pest repeller (Leaven Enterprise, Taiwan) as a stressful stimulus, which allows changing the ultrasound range from 30 to 65 kHz. Thus, resistance to the stressful sound factor will not develop.

In general, the systemic response to stress is aimed at eliminating or reducing stress, which is accompanied by changes in behavioral, motor, autonomic, cognitive and other functions of the body [9; 10]. It is known that the shift in behavioral response under stress in animals occurs towards the extreme states of "excitation – inhibition" of the central nervous system, which fits into a single scale of etiological stress activity "fear – anxiety – exhaustion". To study qualitative and quantitative indicators of stress, general and special behavioral tests are used. One of these tests, which is used mainly in a number of studies, is the "open field" test, which allows detecting significant disorders in the nervous, muscular and vegetative systems of the body when assessing the impact of stress factors [11].

The aim of the study was to model chronic stress in laboratory rats by exposing them to sounds of different ranges.

Materials and methods of the study. The experiment was performed on 20 male Wistar rats aged 4.5 months at the beginning of the experiment. The duration of the experiment was 70 days. The rats were fed with standard combined complete food with free access to drinking water, in accordance with the rules for the maintenance of experimental animals established by Directive 2010/63/EU of the European Parliament and the Council. The procedure for conducting experimental trials was approved by the Bioethical Review Committee of the State Establishment "The Institute of Stomatology and Maxillo-facial Surgery National Academy of Medical Sciences of Ukraine" (Protocol No. 1020 of 04/30/2024).

The animals were divided into two experimental groups by 10 animals each.

The first group was intact, the second group was subjected to sound stress.

Sound stress was induced in rats by turning on the sound in the audible and ultrasonic ranges. An ultrasonic pest repeller LS-912 manufactured by Leaven Enterprise (Taiwan) was used as a sound source. The device has an ultrasound emission range from 30 to 65 kHz with a sound pressure of 130 dB, a power of 1.5 W and an area of action of up to 232 m^2. According to the manufacturer, the repeller is effective in killing mice, rats, cockroaches, fleas, crickets, ants and other insects.

Rats were treated with ultrasound for 5 consecutive days, excluding weekends, for 6 hours per day (from 10.00 to 16.00) according to the following scheme: 2 days – ultrasound at a frequency of 30 kHz, the next 2 days – 40 kHz, the next 2 days – 50 kHz, the next 2 days – 65 kHz. Then the scheme was repeated. If a new frequency was used on the last day of the week, the same frequency was used on the first day of the following week. Sound of the audible range was also added to the ultrasound for 1 hour each day (from 10.00 to 11.00) by fixing the sound control button on the device body. The repeller was installed at a distance of 3 m from the cages with rats at the same level as them.

The degree of stress of the animals was assessed by the behavioral test "open field", which was conducted on the 17th, 23rd and 46th day from the beginning of the experiment. For the test, a square plastic arena with a side of 60 cm and 30 cm high boundary walls was used. The arena surface had 16 holes with a diameter of 3.6 cm and was divided by painted lines into 25 equal square segments with holes placed at the corners of the segments.

The test duration for each rat was 3 minutes. The area around the field was not illuminated during the test; a single 50 W illuminator (incandescent lamp) was installed 1 m above the field and illuminated only the field surface. The test was conducted in complete silence with no outside sounds. The test rat was removed from the cage, placed in the central segment of the arena and observed for 3 minutes of the test. Before testing the next animal, the arena was thoroughly cleaned with wet and dry cloths. The number of the following actions of the rats was recorded: crossing lines (locomotor horizontal activity), examination of holes (immersion of the head to the level of the eyes or attempts to penetrate the holes – "hole-board test"), performing hind legs stands (locomotor vertical activity), grooming – washing the face and cleaning the fur (anxious grooming), the total number of actions.

A line crossing was considered to be the movement of the rat through the nearest line of the square segment or the passage through the burrow if such a crossing was made with both hind limbs.

The results of the study are presented as arithmetic means with standard errors. Comparison of mean values between the intact group and the group of rats exposed to sound stress was performed using Student's t-test. The results were considered significantly different at $p<0.05$.

Results and discussion. Studying horizontal locomotion of rats (number of line crossings) on day 17 of sound stress modeling, no significant changes in this parameter of animal behavior were observed, as well as in vertical motor activity by the number of upright postures (Table 1).

Table 1

Behavioral parameters of rats in the "Open field" test on the 17th day of exposure to sound stress

Group	Number of actions				The sum of behavioral actions
	Intersection of lines	Inspection of holes	Upright postures	Grooming	
Intact	31.9 ± 6.46	10.4 ± 1.90	5.7 ± 0.70	0.5 ± 0.07	48.5 ± 5.92
Stress	30.8 ± 6.11	9.4 ± 0.82	6.4 ± 0.94	1.2 ± 0.09 $p<0.05$	47.8 ± 6.99

Note: p – probability of difference from the control group.

A type of orientation and exploratory behavior of rats is the "hole-board test" indicator – the number of holes examined, which indicates the ability of animals to explore the "open field" and show cognitive activity, in particular, to look into the holes. The total number of holes examined by rats exposed to sound exposure also did not change on day 17 of observation (see Table 1).

When studying the activity of rats on day 17 of stress modeling using a combination of sounds, only a significant increase in the number of grooming 2.4 times ($p<0.05$) was observed, indicating an increase in animal anxiety (see Table 1). It is known that frequent and short "washing" is anxiety grooming, since emotional stress during the experiment correlates with the frequency of grooming [12]. Manifestations of autonomic reactions in the form of grooming frequency are considered to be markers of the emotional state of animals. Therefore, it is believed that grooming reflects the attempts of animals to overcome emotional stress or a conflict situation [13; 14].

The total sum of all behavioral actions of rats stressed by sound did not change on day 17, and a significant increase in the number of anxious grooming at this stage can be said to correspond to the anxiety stage of the Selye adaptation syndrome.

Testing of animals on the 23rd day of exposure to sound revealed a significant increase in the "hole-board test", i.e. the number of holes examined by 130.9% ($p<0.001$) and the level of grooming frequency by 150.0% ($p<0.05$).

That is, we can talk about the activation of exploratory behavior and increased anxiety. In general, the sum of all behavioral actions on day 23 of sound stress in rats showed hyperactivity of animals – by 46.3 % ($p<0.05$), indicating their excessive excitement and fear (Table 2). According to Selye, this is the stage of resistance.

On the 46th day of observation, a significant decrease in the number of line intersections was recorded in rats exposed to sound stress, i.e., a decrease in horizontal motor activity by 57.9% ($p<0.05$) (Table 3). They also showed a decrease in the "hole-board test" – the number of holes examined by 40.0% ($p<0.05$), and inhibition of cognitive activity. The frequency of grooming slightly increased against the background of its absence in intact animals at this stage of observation. When summarizing the number of all behavioral actions, they decreased almost 2 times ($p<0.05$).

On day 46, animals exposed to regular ultrasound in combination with audible sound showed a significant decrease in motor and exploratory activity. Researchers associate low motor activity with increased emotional reactivity. This condition can be characterized as inhibition of the nervous system, increased stress and emotionality. According to Selye, this is the stage of exhaustion.

As a result, during 46 days of exposure to combined sound stress in laboratory rats, the LS-912 pest repeller was able to model all stages of the adaptation syndrome according to Selye, who proposed to distinguish three stages of stress: anxiety reaction, resistance (adaptation) stage, and exhaustion. The anxiety stage was recorded on the 17th day of exposure to sound stress, the resistance stage on the 26th day, and the exhaustion stage on the 46th day of stress modeling.

Table 2

Behavioral parameters of rats in the "Open field" test on the 23rd day of exposure to sound stress

Group	Number of actions				The sum of behavioral actions
	Intersection of lines	Inspection of holes	Upright postures	Grooming	
Intact	22.9 ± 2.29	5.5 ± 0.76	4.0 ± 0.78	0.4 ± 0.09	32.8 ± 5.50
Stress	28.8 ± 4.45	12.7 ± 0.76 $p<0.001$	5.5 ± 0.86	1.0 ± 0.41 $p<0.05$	48.0 ± 4.22 $p<0.05$

Note: p – probability of difference from the control group.

Table 3

Behavioral parameters of rats in the "Open field" test on the 46th day of exposure to sound stress

Group	Number of actions				The sum of behavioral actions
	Intersection of lines	Inspection of holes	Upright postures	Grooming	
Intact	16.4 ± 2.46	6.5 ± 0.91	1.4 ± 0.35	0	24.3 ± 4.33
Stress	6.9 ± 1.19 $p<0.05$	3.9 ± 0.53 $p<0.05$	1.6 ± 0.43	0.3 ± 0.05	12.7 ± 2.55 $p<0.05$

Note: p – probability of difference from the control group.

Thus, the present study proves that the use of the LS-912 pest repeller with a change in the ultrasound range every 2–3 days can be used to model chronic stress in laboratory rats. The device is very convenient and its operation does not require manipulation of each animal like in the case when modeling immobilization, hyperdynamics or administration of pharmacological agents. Therefore, we can recommend the use of the LS-912 pest repeller to study the effectiveness of new anti-stress drugs and regimens.

Conclusions. On the 17th day of exposure to the combination of sounds, a significant increase in the number of grooming was observed in rats 2.4 times, which indicates an increase in animal anxiety and corresponds to the anxiety stage of the Selye adaptation syndrome.

On the 23rd day of exposure to sound, an increase in the "hole-board test" was found, i.e., the number of holes examined by 130.9%, the level of grooming frequency by 150.0%, and the general hyperactivity of the animals by 46.3%, indicating their excessive excitement and fear. According to Selye, this is the stage of resistance.

On the 46th day of observation, rats exposed to sound stress showed a significant decrease in horizontal motor activity by 57.9%, the number of holes examined by 40.0%, and the number of all behavioral actions almost 2 times. This condition can be characterized as inhibition of the nervous system, according to Selye – the stage of exhaustion.

BIBLIOGRAPHY

1. Hill RJ, McKernan LC, Wang L, Coronado RA. Changes in psychosocial well-being after mindfulness-based stress reduction: a prospective cohort study. *J Man Manip Ther*. 2017; 25(3): 128–136. doi: 10.1080/10669817.2017.1323608.
2. Wang C, Wang XR, Song DD, et al. The establishment of a rat model in myocardial ischemia with psychological stress. *Ann Transl Med*. 2020; 8(6): 322. doi: 10.21037/atm.2020.02.128.
3. Plotnikova LM, Berezovskyi VY, Levashov MI, Chaka OG, Yanko RV. Vplyv hipoksiyi ta melatoninu na povedinkovu reaktsiyu shchuriv z riznym rivnem energetychnoho metabolizmu [The influence of hypoxia and melatonin on the behavioral response of rats with different levels of energy metabolism]. *Fiziologichnyi Zhurnal*. 2017; 63(6): 66–71. (In Ukrainian). Available from: https://fz.kiev.ua/journals/2017_V.63/2017_6/6-66-71.pdf.
4. Pimple P, Lima BB, Hammadah M, et al. Psychological Distress and Subsequent Cardiovascular Events in Individuals With Coronary Artery Disease. *J Am Heart Assoc.* 2019; 8(9): e011866. doi: 10.1161/JAHA.118.
5. Pshychenko VV, Cherno VS. Vplyv khronichnoho stresu na morfometrychni pokaznyky pinealnykh klityn shchuriv [Effect of chronic stress on morphometric parameters of rat pineal cells]. *Visnyk problem biolohii ta* medytsyny. 2018; 2(147): 298–300. (In Ukrainian). doi: 10.29254/2077-4214-2018- 4-2-147-298-300.
6. Savotchenko AV, Semenikhina MO, Krasnianchuk IV, Bogovyk RI, Honcharova AE, Isaeva EV. Behavioral reaktsiyi shchuriv pry enterobiozi [Behavioral reactions of rats with enterobiosis]. *Fiziologichnyi Zhurnal*. 2019; 65(1): 20–25. (In Ukrainian). Available from: https://fz.kiev.ua/journals/2019_V.65/2019-1/2019-1-20-25.pdf.
7. Bilets MV, Omelchenko OY, Vesnina LE, Mamontova TV, inventors; Ukrainian Medical Dental Academy, assignee. Sposib modelyuvannya pidvyshchenoyi chutlyvosti shchuriv do khronichnoho stresu [A method for modeling the increased sensitivity of rats to chronic stress]. Ukrainian patent 127604. 2018 March 19.
8. Miskiv VA, Zhurakivska OY, Paliichuk IV, et al., inventors; Ivano- Frankivsk National Medical University, assignee. Sposib modelyuvannya khronichnoho stresu u eksperymental'nykh tvaryn riznykh vikovykh hrup [Method for modeling chronic stress in experimental animals of different age groups]. Ukrainian patent 125623. 2022 May 4.
9. Hrabovska SV. Neyrofiziolohichni reaktsiyi na diyu nyz'kykh doz khlorpiryfosu u shchuriv i yikh potomstva [Neurophysiological reactions to low doses of chlorpyrifos in rats and their offspring]. Dissertation of Candidate of Biological Sciences (Doctor of Philosophy). Lviv: Ivan Franko National University of Lviv; 2018. 132 p. https://uacademic.info/ua/document/0418U005423#!
10. Sturman O, Germain PL, Bohacek J. Exploratory rearing: a context- and stress-sensitive behavior recorded in the open-field test. *Stress*. 2018; 21(5): 443–452. doi: 10.1080/10253890.2018.1438405.
11. Hrabovska SV, Salyha YT. Vplyv khronichnoyi intoksykatsiyi nyz'kymy dozamy khlorpiryfosu na povedinku samtsiv shchuriv [The effect of chronic intoxication with low doses of chlorpyrifos on the behavioral parameters of female rats]. *Fiziologichnyi Zhurnal*. 2015; 61(2): 94–101. (In Ukrainian). doi: 10.15407/fz61.02.094.
12. Kozlova YV. Behavior of Rats in the Open Field within the Early Period after Light-Degree Blast-Induced Neurotrauma. *Neurophysiology*. 2021; 53(2): 101–108. https://doi.org/10.1007/s11062-022-09921-z.
13. Zinovieva ML, Karpezo NO, Lynchak OV. Osoblyvosti povedinkovykh reaktsiy samok i samtsiv shchuriv za subkhronichnoyi diyi 7- hidroksykumarynu [Peculiarity of the Behavioral Reactions of Rats Males and Females Exposed Subchronically to 7-Hydroxicoumarin]. *Ukrainian journal suchasnykh problem toksykolohii*. 2014; 5: 45–49. (In Ukrainian). Available from: http://protox.medved.kiev.ua/index.php/ua/issues/2014/5/item/431-peculiarity-of-the-behavior-reactions-of-rats-males-and-females-exposed-subchronically-to-7-hydroxicoumarin.
14. Antipova RV, Komisova TE, Sak AY. Osoblyvosti povedinkovykh reaktsiy samtsiv shchuriv u testi "vidkryte pole" pry alimintarnomu nadkhodzhenni zhyriv riznoho pokhodzhennya [Peculiarities of behavioral reactions of male rats in the open field test in case of alimentary intake of fats of different origins]. *Bioriznomanittya, ekolohiya ta eksperymental'na biolohiya*. 2020; 22(1): 9–20 (In Ukrainian). https://doi.org/10.34142/2708-5848.2020.22.1.01.

Надійшла до редакції 17.11.2024 р.
Прийнята до друку 30.01.2025 р.

Електронна адреса для листування oksanadenga@gmail.com