Article

Anhedonia, Decrease in Exploratory Activity, and Changes in the Level of Anxiety in Rats Under Chronic Ultrasound Exposure

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Abstract: In this work we investigated the effect of chronic ultrasonic exposure to variable frequencies in the range of 20-45 kHz for 1, 2 and 3 weeks on anxiety, exploratory activity and anhedonia in rats. The animals recorded the development of anhedonia and a decrease in exploratory activity in the Open Field, Elevated Cross Maze and Dark Light Chamber tests, indicating the formation of a depressive-like state in them. These behavioural changes were manifested simultaneously after 2 weeks of ultrasound exposure. At the same time, the rats did not show a decrease in horizontal activity, as well as the ratio of time spent in open and closed areas of the arena.

Keywords: rats, ultrasound, depression-like state, anxiety, exploratory activity, anhedonia.

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1. Introduction

More than 350 million people worldwide are currently diagnosed with a depressive disorder [Pehrson, Sanchez, 2015], so it is an urgent problem to study the development of a depressive-like state in experimental animals. Most of the existing models are based on direct physical effects on the experimental animal [Grigoryan, Gulyaeva, 2015; Duman, 2010], but the so-called emotional stress is closer to the causes of human depression development. One way to build such a model could be to use an unpredictable alternation of positive and negative stimuli, as was done in the experiments of I.P. Pavlov's associates when creating functional disorders of higher nervous activity.

On the basis of such alternation a new ultrasonic model of depressive-like state of animals was developed and tested [Morozova et al., 2016; Gorlova et al., 2017]. Ultrasound frequencies of 20-45 kHz, which fall within the range of normal rodent vocalization, are used as a stressor, with frequencies of 20-25 kHz being classified as so-called "negative", as they are emitted by animals during pain stimulation or defeat in a fight, and frequencies of the 25-45 kHz range being classified as "positive", as they are emitted by rats during food reinforcement or coitus [Brudzynski, 2007; Litvin et al., 2007]. In the model used, frequencies in the range of 20-45 kHz are randomly presented and produce a clash of opposing emotional and motivational stimuli. Thus, the stressor factor of this model can be considered one of the closest to the causes of stress-induced depressive disorder in humans, and the model of chronic ultrasonic exposure itself can be considered as a model of chronic information uncertainty [Ushakova et al., 2019; Morozova et al., 2007].

An increased level of anxiety often serves as a hallmark of rodent behavior with induced depression-like state [Zhang et al., 2017]. Typically, depression-like states and anxiety disorders are studied together by experimenters, as increased anxiety and depressive disorders can occur simultaneously [Estrela et al., 2015]. However, these conditions are not necessarily related to each other and have different etiologies (Knyazev et al., 2016). For example, some patients are found to have depression associated with anhedonia, an inability to experience pleasure, but not associated with increased anxiety. This raises the question of the relationship between anhedonia and anxiety in experimental animals in different models of depression-like states.

An interesting issue remains the analysis of stress-induced changes in research activity in animals, which is an important indicator of natural interest in novelty [Stepanichev et al., 2014]. In the available studies devoted to simulating the depressive-like state in rodents, exploratory activity has not been considered before in a whole battery of tests, although its close relationship, for example, with passive behaviour in stressful situations has been noted [Mällo et al., 2007].

The objectives of this work were to comprehensively study anhedonia, changes in anxiety levels and exploratory activity in rats during the development of a depressive-like state in an ultrasound stress model.

2. Patients and Methods

Experiments were performed on male Sprague-Dawley rats from the Central Laboratory Animal Breeding Facility of the Russian Academy of Sciences (Andreevka). At the beginning of the experiment, the animals were 2.5 months old. The animals were kept in individual polycarbonate cages of 30 × 20 × 15 cm at a constant temperature of 23°C, controlled direct light, 12:12 h, and free access to water and food. Rat housing and all experimental procedures were performed in accordance with international animal handling regulations (European Community Directive 2010/63 of 22 September 2010). Three experimental groups and four control groups were used. The experimental groups were subjected to continuous exposure to ultrasonic waves in the range 20-45 kHz for 1, 2 or 3 weeks; the control groups were not stressed. Three control groups were used to study the contribution of individual housing; the animals were kept in separate cages for 1, 2 or 3 weeks under conditions identical to those of the experimental groups except for ultrasound exposure. In the fourth control group, rats were kept 5 animals each in 55 × 35 × 20 cm cages. Each group consisted of 10 individuals. The animals were not exposed to any other potentially stressful conditions. All rats involved in the experiment were intact and had not previously participated in other experiments.

Ultrasonic exposure in the 20-45 kHz range was performed using an ultrasonic generator (Wietech, Belgium); the sound pressure level at the experiment distance of 1.5 m to the animal cage was 80 dB.

One day after the end of the ultrasound exposure, behavioural testing was carried out. Animal behavior in all tests was recorded by digital video camera and analyzed using computer program RealTimer (Open Science, Russia). The intervals between the tests for all groups were one day. The following behavioral tests were used:

- Sucrose solution preference test. For 24 h, rats were simultaneously given access to choose between two identical drinkers, one containing 1% sucrose solution and the other containing plain water. The location of the drinkers was changed after 12 h to eliminate the effect of site preference. Sucrose solution preference was calculated using the following formula: Preference = (weight of sucrose solution/total weight of fluid consumed) × 100%. Consumption of water and sucrose solution was estimated by weighing the drinkers before and after the experiment.

- Open field test. The rat was placed in a 45 × 45 × 40 cm grey plastic chamber, divided into peripheral and central sectors. The total time the rat spent in the central and peripheral sectors and the number of perfect racks, as well as the number of crossed sectors, were recorded for 5 min.

- Elevated cross maze test. The rat was placed in a setup consisting of two closed (29 cm high walls) and two open (0.5 cm high sides) arms measuring 52 × 14 cm, with a central platform measuring 14 × 14 cm, placed 100 cm above the floor. The test animal was placed in the centre of the unit and the time spent in closed and open arms, the number of perfect stances, peeks from closed arms into the open arms of the maze and peeks from the open arms were recorded for 5 min.

- Dark Light Chamber Test. The rat was placed in a unit consisting of two compartments measuring 30 × 30 × 32 cm. One compartment was darkened, the other was brightly illuminated. For 5 min, the total time the animal spent in the bright compartment, the number of stoops made in the bright compartment, and the number of peeks from the dark compartment were recorded.

- Statistics. Data on measured behaviors were expressed as Mean±SEM. Statistical analyses were performed using two-factor ANOVA for significance of single and ultrasonic factors, followed by post hoc analysis with Fisher's LSD test. Differences were considered statistically significant at $p < 0.05$. A Kolmogorov-Smirnov test was performed beforehand, the results of which did not negate the normal distribution, except for the number of hangings from the open arm of the elevated cruciform labyrinth. For this case, the Kruskal-Wallis test was used, followed by a Dunn's multiple comparison test. A one-way ANOVA was used for statistical analysis of sucrose solution preference prior to the experiment. GraphPadPrism version 6.0 software was used for statistical analysis.

3. Results

There was no statistically significant difference in sucrose preference between the groups before the experiment ($F6.63 = 0.12$, $p = 0.96$, one-way ANOVA, Figure 1(a)). There was no statistically significant interaction between the single content and ultrasound factors ($F9.54 = 0.36$, $p = 0.699$, two-way ANOVA), and no statistically significant effect of the individual content factor on sucrose solution preference ($F3.54 = 2.43$, $p = 0.097$, two-way ANOVA). However, the factor of chronic ultrasound exposure was statistically significant ($F3.54 = 8.42$, $p = 0.0054$, two-way ANOVA). Reduced preference for sucrose solution relative to plain water was evident after 2

weeks of stress exposure (p = 0.0313, post hoc Fisher's LSD test) and persisted after 3 weeks of exposure (p = 0.0046, post hoc Fisher's LSD test; Fig. 1(b).

There was no statistically significant interaction between the single content and ultrasound exposure factors in the Open Field test (F9.54 = 0.31, p = 0.733, two-way ANOVA), no statistically significant effect of the individual content factor (F3. 54 = 0.07, p = 0.927, two-way ANOVA) and a statistically significant effect of the ultrasound exposure factor (F3.54 = 0.13, p = 0.725, two-way ANOVA) on the ratio of time spent by animals in the central sector to time spent in the periphery (Figure 2 (a)).

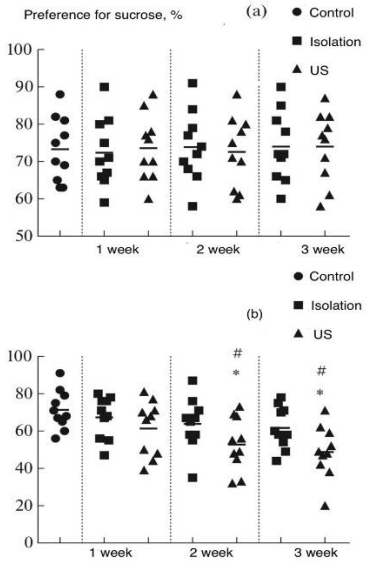

Figure 1. Effect of ultrasound on the preference of sucrose in rats. 2 and 3 weeks of ultrasound stress resulted in a decrease of the sucrose preference in comparison with the control group (* – p ‹ 0.05, Fisher's LSD test) and relevant groups in which rats were housed individually for 2 or 3 weeks (# – p ‹ 0.05, Fisher's LSD test). (a) – Sucrose preference at the beginning of the experiment. The group names correspond to the way they were subsequently distributed. (b) – Sucrose preference of the experimental groups.

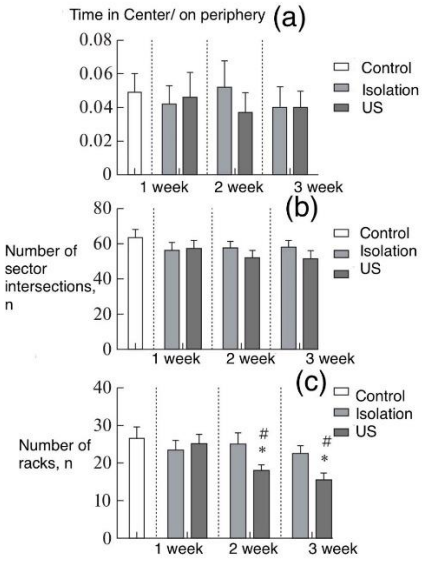

Figure 2. Effect of ultrasound on the behavior of rats in the Open Field test. (a) – Chronic ultrasound stress did not change the proportion between time spent in the central area and time spent in the periphery. (b) – Chronic ultrasound stress did not change the number of crossed sectors. (c) – 2 and 3 weeks of ultrasound stress resulted in a decrease of the number of rearings in comparison with the control group (* – p ‹ 0.05, Fisher's LSD test) and relevant groups in which rats were housed individually for 2 or 3 weeks (# – p ‹ 0.05, Fisher's LSD test).

There was also no statistically significant interaction between the single confinement and ultrasonic exposure factors (F9.54 = 0.48, p = 0.622, two-way ANOVA), no statistically significant effect of the individual confinement factor (F3.54 = 0.15, p = 0.865, two-way ANOVA) and no statistically significant effect of the ultrasonic exposure factor (F3.54 = 1.12, p = 0.295, two-way ANOVA) on the number of sectors crossed (Fig. 2 (b)). Thus, the effect of the ultrasound on the level of anxiety and horizontal activity in the Open Field test was found to be insignificant. Regarding the number of perfect racks, there was no statistically significant interaction between the single content and ultrasound factors (F9.54 = 2.33, p = 0.106, two-way ANOVA) and no statistically significant effect of the individual content factor (F3.54 = 2.05, p = 0.09, two-way ANOVA) on this parameter. However, the effect of the ultrasound exposure factor was statistically significant (F3.54 = 4.66 p = 0.035, two-way ANOVA). Namely, a decrease in the number of perfect racks was registered after 2 weeks (p = 0.0471, post hoc Fisher's LSD test) and after 3 weeks of stress exposure (p = 0.0256, post hoc Fisher's LSD test; Fig. 2(c).

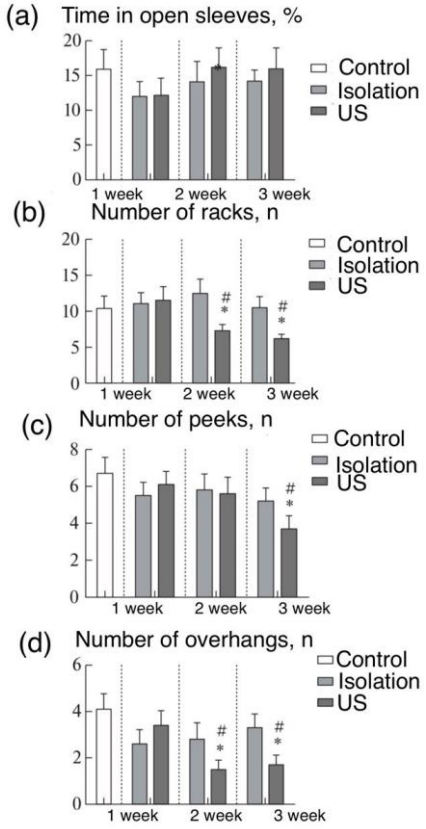

Figure 3. Effect of ultrasound on the behavior of rats in the "Elevated plus-maze" test. (a) – Chronic ultrasound stress did not change the percent of time spent by animals in the open arm of the maze. b) – 2 and 3 weeks of ultrasound stress led to a decrease in the number of rearings compared with the control group (* – p ‹ 0.05, Fisher's LSD test) and relevant groups in which rats were housed individually for 2 or 3 weeks (# – p ‹ 0.05, Fisher's LSD test). (c) – 3 weeks of ultrasound stress led to a decrease in the number of looking-outs compared with the control group (* – p ‹ 0.05, Fisher's LSD test) and relevant groups in which rats were housed individually for 3 weeks (# – p ‹ 0.05, Fisher's LSD test). (d) – 2 and 3 weeks of ultrasound stress led to a decrease in the number of hanging outs compared with the control group (* – p ‹ 0.05, Fisher's LSD test) and relevant groups in which rats were housed individually for 2 or 3 weeks (# – p ‹ 0.05, Fisher's LSD test).

In the Elevated Cross Labyrinth test, there was no statistically significant interaction between the single content and ultrasound exposure factors (F9.54 = 0.08, p = 0.922, two-way ANOVA), no statistically significant effect of the individual content factor (F3. 54 = 0.92, p = 0.404, two-way ANOVA) and a statistically significant effect of the ultrasonic exposure factor (F3.54 = 0.43, p = 0.516, two-way ANOVA) on the percentage of time rats spent in the open arm (Figure 3 (a)). When the number of racks performed by the animals was considered, there was no statistically significant interaction between the factors single confinement and ultrasonic exposure (F9.54 = 2.03, p = 0.141, two-way ANOVA), no statistically significant effect of the factor individual confinement (F3. 54 = 1.96, p = 0.152, two-way ANOVA), but there was a statistically significant effect of the ultrasound exposure factor (F3.54 = 6.2, p = 0.0159, two-way ANOVA): rats committed

fewer stoops after 2 weeks (p = 0.0455, post hoc Fisher's LSD test) and after 3 weeks exposure to ultrasound (p = 0.0394, post hoc Fisher's LSD test; Fig. 3 (b). There was also no statistically significant effect of the individual confinement factor on the number of peeks performed by the rats (F3.54 = 2.19, p = 0.145, two-way ANOVA), but there was a significant interaction of confinement and exposure to ultrasound (F9.54 = 3.41, p = 0.0402, two-way ANOVA) and a statistically significant effect of the ultrasound exposure factor (F3.54 = 5.31, p = 0.079, two-way ANOVA). Rats made fewer peeks after 3 weeks of stress exposure (p = 0.0247, post hoc Fisher's LSD test; Fig. 3 (c). There was a difference between groups in the number of hopping out of open maze arms (H = 19.82, p = 0.003, Kruskall-Wallis test). The rats made fewer hopping after 2 weeks (p = 0.0337, Dunn's test), and after 3 weeks of stress exposure (p = 0.0135, Dunn's test; Fig. 3 (d).

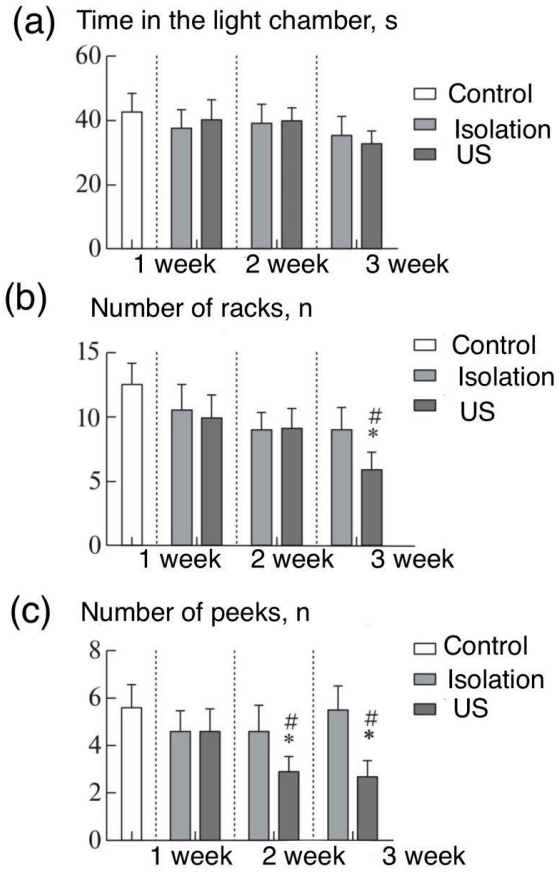

Figure 4. The influence of ultrasound on the behavior of rats in the "Dark-Light box" test. (a) – Chronic ultrasonic stress did not lead to a change in the time spent by the animals in the light box of the arena. (b) – 3 weeks of ultrasound stress led to a decrease in the number of rearings compared with the control group (* – $p < 0.05$, Fisher's LSD test) and relevant groups in which rats were housed individually for 3weeks (# – $p < 0.05$, Fisher's LSD test). (c) – 2 and 3 weeks of ultrasound stress led to a decrease in the number of looking-outs compared with the control group (* – $p < 0.05$, Fisher's LSD test) and relevant groups in which rats were housed individually for 2 or 3 weeks (# – $p < 0.05$, Fisher's LSD test).

There was no statistically significant interaction between the single content and ultrasound exposure factors (F9.54 = 0.136, p = 0.873, two-way ANOVA), a statistically significant effect of the individual content factor (F3. 54 = 0.0144, p = 0.969, two-way ANOVA) and a statistically significant effect of the ultrasound exposure factor (F3.54 = 0.617, p = 0.543, two-way ANOVA) on the time the rats spent in the light compartment (Figure 3 (a)). There was also no statistically significant interaction between the factors single housing and ultrasonic exposure (F9.54 = 1.79, p = 0.176, two-way ANOVA) and no statistically significant effect of the factor individual housing (F3.54 = 2.32, p = 0.134, two-way ANOVA) on the number of stances the animals performed. However, the effect of exposure to ultrasound was significant (F3.54 = 3.19, p = 0.049, two-way ANOVA). Rats performed fewer stoops after 3 weeks of stress exposure (p = 0.036, post hoc Fisher's LSD test; Fig. 4 (b). In addition, there was no statistically significant interaction between the single confinement and ultrasonic exposure factors (F9.54 = 1.26, p = 0.292, two-way ANOVA) and no statistically significant effect of the individual confinement factor (F3.54 = 0.46, p = 0.632, two-way ANOVA) on the number of times rats looked out of the dark compartment, but the effect of the ultrasonic

exposure factor was significant ($F_{3.54} = 4.27$, $p = 0.0436$, two-way ANOVA). The rats committed fewer racks after 2 weeks ($p = 0.047$, post hoc Fisher's LSD test) and after 3 weeks of stress exposure ($p = 0.044$, post hoc Fisher's LSD test; Fig. 4 (d).

4. Discussion

One of the classic signs of clinical depression is a decreased capacity for pleasure. Normally, rodents prefer to consume a sweetened solution, while in the presence of a depressive-like state this preference is absent, which is considered as anhedonia (Overstreet, 2013). The test for the development of anhedonia is the most common criterion for determining a depressive-like state in rodents [Moreau, 2012]. According to our data, chronic exposure to variable frequency ultrasound for 2 weeks or more can induce anhedonia in rats, which confirms the validity of this model.

It is known from the literature that most theoretical and experimental studies emphasize the close relationship between anxiety and the development of both human clinical depression [Rallis et al., 2014; Fan et al., 2015] and rodent depression-like state [Beerya, Kauferb, 2015; Huang et al., 2017; Neumann et al., 2011; Frazer, Morilak, 2005]. However, increased anxiety levels in rodents do not always manifest together with the development of depressive-like disorders, similar to what is observed in patients with clinical depression [Ju et al., 2018; Knyazev et al., 2016]. Therefore, it was of interest for us to assess the possible change in the level of anxiety accompanying the development of depression-like state in rats in a new model of chronic ultrasound exposure.

The data on the effect of chronic ultrasound exposure on anxiety levels in rats were contradictory. On the one hand, similar results were observed in all tests aimed at examination of anxiety: namely, experimental groups of rats did not spend statistically significantly less time in the central sector in the "Open Field" test, open arms in the "Elevated Cross Labyrinth" test or light chamber in the "Dark Light Chamber" test, which may be regarded as a sign of absence of increased anxiety. On the other hand, a number of findings confirm the possibility of an increased level of anxiety under the influence of ultrasound, which is expressed in a decrease in the number of peeks into open arms of the maze and peeks out of open arms in the Elevated Cruciform Maze test, and a decrease in the number of peeks into the light chamber in the Dark Light Chamber test.

It is known that assessing the level of anxiety of an experimental animal is a complex task, since in different experimental models the same animals may exhibit different behaviour [Bourin et al., 2007; Ramos, 2008], and therefore to determine the level of individual anxiety or predisposition to the effects of emotional stress it is recommended to use several tests for the same animals [Sudakov et al., 2013]. The similarity of changes in the behavior of experimental animals traced in this work suggests the absence of development of obvious signs of anxiety associated with the depressive-like state of mice induced by chronic ultrasound exposure, however, the influence of this type of stress on the anxiety level of experimental animals cannot be completely excluded.

Thus, the data obtained in our model rather support the possibility of an anxiety-independent development of a depressive-like state, already described in the literature [Overstreet, 2012]. However, the presence of this relationship manifested at the behavioural level and the mechanisms responsible for the presence or absence of increased anxiety as a comorbidity of depressive disorder need to be further explored.

Also in the present study, research activity in experimental animals was examined in detail. It was found that rats exposed to ultrasound develop a significant decrease in exploratory activity in all of the tests used. Therefore, along with assessment of other depressive-like state parameters, this index may be considered important and meaningful for consideration in this type of research. The change in exploratory activity observed under stress in experimental animals is considered to replicate the symptoms characteristic of human depression - loss of interest in novelty and any activity [Garibova et al., 2017]. In view of this, the decrease in rat activity index during chronic ultrasound exposure once again confirms the validity of the model.

5. Conclusions

The ultrasonic stress model results in the development of anhedonia and reduced research activity in experimental animals.

Application of artificial intelligence:

The article is written without the use of artificial intelligence technologies.

Conflicts of Interest: The authors declare no conflict of interest.

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