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Effects of Bioresonance Application in Mice with Depressive-Like Behavior

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We studied the effects of bioresonance application on mice with depressive-like behavior induced by stress. A chronic mild stress model was developed in mice to monitor the effects of bioresonance application. After that, behavioral tests were performed. In the forced swimming test, the animals of the long bioresonance therapy demonstrated shorter group immobility time in comparison with mice of the stress group and stress group without therapy (animals of this group were sacrificed at the same time point as therapy groups in order to reveal a possibility of spontaneously recover in animals after stress without therapy). In the tail suspension test, a decrease in immobility time was observed in the long bioresonance therapy group, stress group, and stress without therapy group. These changes in behavioral test results can indicate that the application of bioresonance in mice can be an effective method of treating depressive-like behavior, but these conclusions should be supported by additional experimental studies and the use of different frequencies.

Key Words: *bioresonance; stress; depression; mouse; Trikombin device*

Treatment method with bioresonance applications is a method of complementary medicine in which electromagnetic waves emitted from healthy tissues of the body and pathological tissues or substances are used in the diagnosis and treatment [1]. Every structure or every system has its own natural frequency of vibration. If the resonant vibrations emitted from biological structures are considered to be unique, the natural frequencies of each organ or structure are determined. In bioresonance applications, electric fields or electric currents are generally used [2]. Bioresonance applications can be used as a supportive treatment in the treatment of addiction such as smoking cessation, in allergic rhinitis and asthma [3,4], infectious diseases, especially in the treatment of parasites such as *Entamoeba histolytica* and *Trichomonas vaginalis* [4], or diseases with pain syndrome [4]; this method can also be applied as a supportive treatment in oncological patients [5-8].

Our aim was to study the effects of long and short bioresonance treatment on the behavior of mice with stress-induced depressive-like behavior.

MATERIALS AND METHODS

Animals and experimental groups. Male BALB/c mice (n=50) weighing 25-30 g obrained from Kobay D.H.L. were used. The study was carried out after obtaining the necessary permission (23.09.2019/397) and approved by the Animal Experiments Local Ethics Committee of Kobay D.H.L. The control group mice (n=10; without stress) were kept in 42×26×19 cm cages at 23-24°C and 12/12-h dark/light cycle and received water and standard laboratory food *ad libitum*. Chronic mild

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stress conditions were applied to mice in the following 4 groups (10 animals per each group): stress group (group 2), stress+short bioresonance therapy (SBT; group 3), stress+long bioresonance therapy (LBT; group 4), and stress group without therapy (group 5). The mice were brought to the experimental laboratory about 1 h before the study and kept to adapt to the ambient conditions. At the end of the 21-day experimental intervention, the animals of the control and stress groups were sacrificed on day 22, while other animals (SBT, LBT, and stress without therapy groups) were sacrificed in 10 days, *i.e.* on day 32 after behavioral tests.

Chronic mild stress protocol. For chronic mild stress, the animals were exposed to different stress factors daily for 3 weeks. These stress factors include: 12-24 h of fasting; 18-24 h of dehydration; exposure to an empty water bottle for 1 h; 7 h of cage 45° tilted; 12 h of overnight illumination for, 24 h wet sawdust; 2 h of immobilization; 3 h in the dark; tail suspension for 5 min and 3 h of 2000 lx white light [9].

Bioresonance treatment protocol. Trikombin branded bioresonance device (serial No. 019003580043) was used. SBT and LBT protocols were applied every 2 days after stress, a total of 10 days as follows. For the frequency selection, Trikombin User Manual (Software version 5.0.1#3) and the seminars (J. Baklayan and A. E. Vollmer, 2018) and webinar documents of the Akademie der Harmonikalische Frequenzanwendung were used as well as Frequenz-Therapie (2014). Special cages have been prepared to transmit frequencies to animals in a homogeneous way. Trikombin device has 3 frequency generators and 3 different electrodes connected to them. Holes were cut at the floor of Eurostandard Type-III Cage by laser cutting technique with 0.7 cm intervals, and 2 mm metal rods were placed in them (Fig. 1). Metal bars are left empty on one side of the cage and fixed on the other side by welding in accordance with the scheme of 1,4,7... for the 1st frequency generator, 2,5,8... for the 2nd frequency generator, 3,6,9... for the 3rd frequency generator. As a result, the welded rods were converted into a single electrode for connection to the Trikombin bioresonance device. The contact of all 3 frequency generators with the electrodes was ensured even if the mice were in a position to move in the cage.

SBT and LBT protocols using Trikombin bioresonance device are presented in Tables 1 and 2.

Behavioral tests. After stress exposure, the emotional states, stool qualities, and appetite of the

TABLE 1. SBT Protocol for the Therapy of Depressive-Like Behavior with Trikombin Device-I

Name	Duration	Description	Wobble direction	Frequency
Hormonal Balancing Serotonin-6215	3 min	Wobble 3, Sweep, Modulation	Right	112.59084-130.68492 kHz
Hormonal Balancing Serotonin-4403				15.95275-19.3275 Hz
Hormonal Balancing Serotonin-6137				49.258496-58.640896 kHz
Hormonal Balancing Serotonin-5215				879.616-1.020976 kHz

TABLE 2. LBT Protocol for the Therapy of Depressive-Like Behavior with Trikombin Device-I

Name	Duration	Description	Wobble direction	Frequency
Basic Therapy-3500	5 min	Wobble 3, Sweep, Modulation	Duplex	0.1342187-1.07375 Hz
Basic Therapy-4500				17.18-137.44 Hz
Emotional blockage-11330		Wobble, Low, Increase	Right	130.68492-140.73856 kHz
Brain Detox-4400		Wobble 3, Sweep, Modulation	Duplex	8.59-68.72 Hz
Brain Detox-4417			Duplex	20.616-24.052 Hz
Brain Detox-4423			Duplex	30.924-37.003 Hz
Brain Detox-4403				15.95275-19.3275 Hz
Brain Detox-4421				19.3275-22.905 Hz
Brain Detox-4437				24.052-28.63325 Hz
Hormonal Balancing Serotonin-6215	3 min	Wobble 3, Sweep, Modulation	Right	112.59084-130,68492 kHz
Hormonal Balancing Serotonin-4403				15.95275-19.3275 Hz
Hormonal Balancing Serotonin-6137				49.258496-58.640896 kHz
Hormonal Balancing Serotonin-5215				879.616 Hz-1.020976 kHz

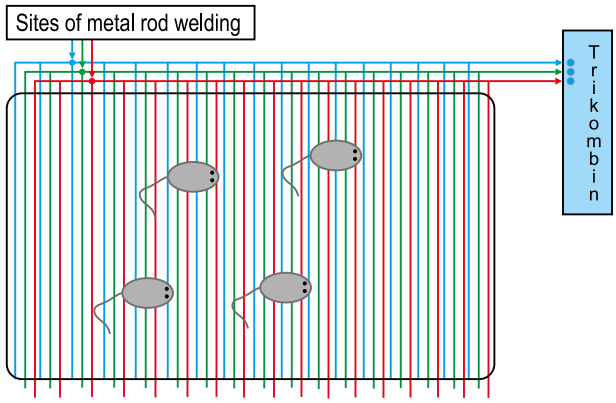

Fig. 1. Modified Eurostandard Type-III cage.

animals were observed and recorded. After the stress, anxiety-like behavior and locomotor activity were evaluated in the open-field test (data not shown), depressive-like behavior was assessed using the tail suspension and forced swimming test [10,11].

For the forced swimming test, the mice were placed in a glass cylinder (height 25 cm, diameter 10 cm) filled to a height of 10 cm with water (22-23°C) and their movements were observed and recorded over 6 min. Depressive-like behavior was assessed by immobility time. The very small movements of mice to keep their heads above the water and their sedentary stances were considered as immobility.

During the tail suspension test, the duration of mouse inactivated while hanging was recorded in seconds. Depressive-like behavior was assessed by the immobility time [10].

Statistical analysis. SPSS Statistics 22.0 (IBM) software was used for statistical analysis. Descriptive statistical methods were used while evaluating the study data. Normality of distribution was evaluated using the Shapiro–Wilk test. Normally distributed data were evaluated by ANOVA analysis and multiple comparisons were made using the Tukey test in groups with significant differences. The results were evaluated at 95% confidence interval and $p<0.05$.

RESULTS

Animal weights. Animal weights were measured at the beginning of the study and before sacrification, chronic mild stress exposure caused body weight loss. An increase in body weight was observed in the LBT group in comparison with the stress group. A decrease in appetite, hair loss, and a decrease in the brightness of hairs and skin injuries (*e.g.* scraches) were observed in the stress group.

Forced swimming test. In this test, the duration of immobility was shortened in LBT, SBT, and group stress without therapy, but the difference was observed between the LBT and stress without therapy groups (Fig. 2). The duration of immobility in the LBT group was significantly lower ($p\leqslant0.001$) than in the stress without therapy group (Fig. 2).

Tail suspension test. In this test, a decrease in immobility time was observed in the LBT group in comparison with stress and stress without therapy groups ($p=0.028$, $p=0.009$) (Fig. 3).

According to WHO data, depression is one of the most serious health problems of modern society [12]. Bioresonance therapy, which is one of the complementary medicine methods, has been used in the diagnosis and treatment of various pathologies for many years [4]. One of the advantages of clinical use of electromagnetic field is that it can be performed from the outer surface of the body, it is painless and does not result in injury or fatigue [13]. It was demonstrated that exposure to very low frequency electromagnetic fields changed the behavior, physiology, and stress protein

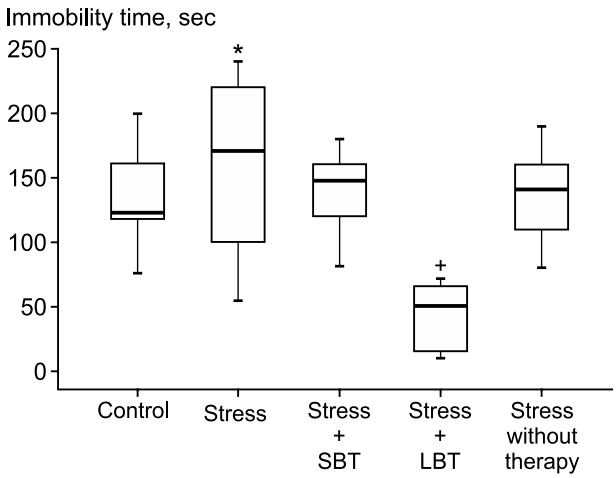

Fig. 2. Immobility time in the forced swimming test. $p<0.001$ in comparison with *control, +stress without therapy.

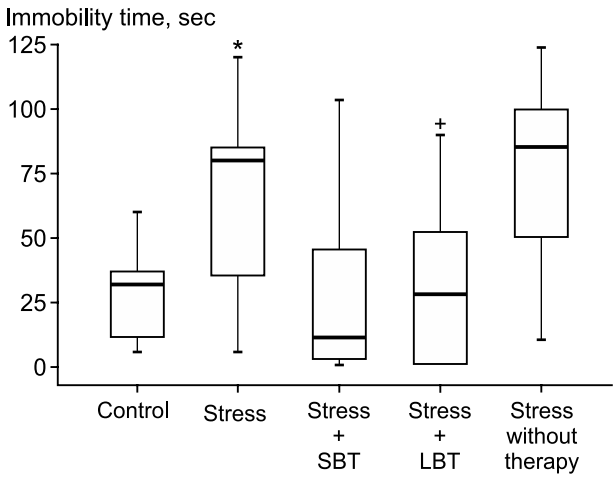

Fig. 3. Immobility time in the tail suspension test. $p<0.01$ in comparison with *control, +stress without therapy.

levels of flying insects in arid regions [14]. In a clinical study, patients with depression resistant to treatment received low-voltage electromagnetic field therapy that passed through the skull. After this treatment, patients showed a better clinical and significant result in the first weeks of treatment compared to their initial condition [15]. In our study, the effects of LBT and SBT on the symptoms of depressive-like behavior were demonstrated using commonly used tail suspension and forced swimming tests [10,11]. In both tests, a decrease in immobility time was observed in the LBT group in comparison with the stress group and stress without therapy group. Thus, LBT increases mobility and reduces the duration of immobility in stressed mice. In this regard, it can be concluded that manifestations of depressive-like behavior decreased in mice of the LBT group. Thus, application of bioresonance can be an effective method of treating depression given the changes in behavioral test results, however, it should be supported by further experimental studies, as well as the use of different frequencies.

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