Behavioral Effect of Terahertz Waves in Male Mice

N. P. Bondar, I. L. Kovalenko, D. F. Avgustinovich, A. G. Khamoyan*, and N. N. Kudryavtseva

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We studied the effect of terahertz waves (3.6 THz, 81.5 μ, 15 mW) on the behavior of mice. The mice perceived terahertz waves even after short-term exposure (15 min). The effect of terahertz waves was maximum in direct contact of the mice with the laser. Increased anxiety of experimental animals was observed on the next day after 30-min irradiation.

Key Words: *terahertz radiation; anxiety; mice; behavior*

The range of terahertz radiation (THz waves) varies from 0.1 to 10 THz (T rays). These waves probably propagate through some solid objects, do not have hazardous ionizing effect on biological objects, and due to these properties THz waves can be used in medical practice, security service, telecommunication, informational technology, and environmental protection [1,2,7-9,11]. However, terahertz radiation sources are of low power. Hence, it is difficult to detect and study THz waves (compared to infrared waves and microwaves). Little is known about the effect of THz waves on various biological objects.

This work was designed to evaluate whether terahertz waves are perceived by animals. The effect of terahertz radiation on living organisms was evaluated by the behavior of male mice under conditions of terahertz laser radiation. Mouse behavior in the post-irradiation period was also studied.

MATERIALS AND METHODS

Experiments were performed on adult male C57Bl/6J mice aging 3-4 months and weighing 28-30 g. The animals were bred and maintained in a vivarium of Institute of Cytology and Genetics (Novosibirsk) under standard conditions at 12:12-h light/dark regimen. The animals received water and pelleted food *ad libitum*. The mice were housed in cages (36×23×12 cm, 6-8 mice per cage). Five days before the study, each mouse was placed in 1 of 2 compartments of an experimental metal cage (28×14×10 cm). This cage was divided into 2 compartments with a transparent barrier with holes. A hole (diameter 9 mm, which corresponded to the laser beam diameter) was made in the wall of the metal cage at a distance of 3 cm from the barrier and at the level of mouse body (2.5 cm from the floor; Fig. 1, *a*). We assumed that waves passing through the hole lose their power, but are reflected from the opposite wall of the cage. On the day of the experiment, the animals were transferred to the room with the laser. After adaptation to novel conditions (not less than 1 h) and activation (5 min), the cage was placed near the laser. The hole was directed to the laser beam source. The laser was constructed and studied at the Novosibirsk Institute of Laser Physics (patent No. 2143162) [3,10]. The frequency of laser radiation was 3.6 THz (λ=81.5 μ, power 15 mW). The following parameters were recorded: number and total time of sniffing the hole, as well as the time spent near the hole; total time and number of approaches to the barrier (period when the animals were near the barrier or touched the barrier with the anterior part of the body and nose); total time spent in the area of laser radia-

Sector of Neurogenetics of Social Behavior, Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences; *Institute of Laser Physics, Siberian Division of Russian Academy of Sciences, Novosibirsk, Russia. ***Address for correspondence:*** natnik@bionet.ru. N. N. Kudryavtseva

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tion; and number and time of tearing/strewing the bedding in various areas (displaced activity). Behavioral parameters were recorded over the first and second 5-min periods of irradiation. The adjacent compartment was empty. Another male was placed in this free compartment during the third 5-min period [5]. Behavioral tests were performed by two investigators to exclude errors due to subjective evaluation. The animals subjected to similar manipulations under the same experimental conditions (except for laser irradiation) served as the control. The cage with mice of another group was illuminated through the hole using a 30-40-mW electricity supply device. This device was placed at a distance of 10 cm from the cage.

In series II, the effect of 30-min irradiation on animal behavior was studied 1 day after exposure to THz waves. Male mice were housed in cages (28×14×10 cm, 4 specimens per cage) for 5 days before the study. On the day of the experiment, the animals were transferred (in home cages) to an experimental room. The laser beam passed through the cage (Fig. 1, *b*). Another hole with a mirror was made in the opposite wall to reflect the beam inside the cage. The mice were constantly exposed to direct and reflected laser beams. After irradiation, the animals were returned to the vivarium and placed in individual cages. Animal behavior was studied in an elevated plus-maze (EPM test for anxiety) on the next day [6]. This maze consisted of the central area and 2 open and 2 closed arms (closed from 3 sides) located opposite each other and was elevated by 1 m from the floor. We recorded the number of entries and exits and time spent in the closed arms, open arms, and central area. The data were presented as a the percent of the total test time (5 min) or total number of entries and exits. Other types of behavior were also studied (number of peepings under the maze, transitions between closed arms, and peepings from the closed arm). Control animals were subjected to similar manipulations, except for THz irradiation.

The results were analyzed by nonparametric Mann—Whitney test (comparison of the samples with non-Gaussian). Each group consisted of 10-12 animals.

RESULTS

The number of approaches to the barrier over the first 5-min period in experimental mice (laser irradiation of the cage through the hole) was lower than in controls (U=18.5, p<0.03). During other periods, this parameter and the total time spent near the barrier did not differ between treated and con-

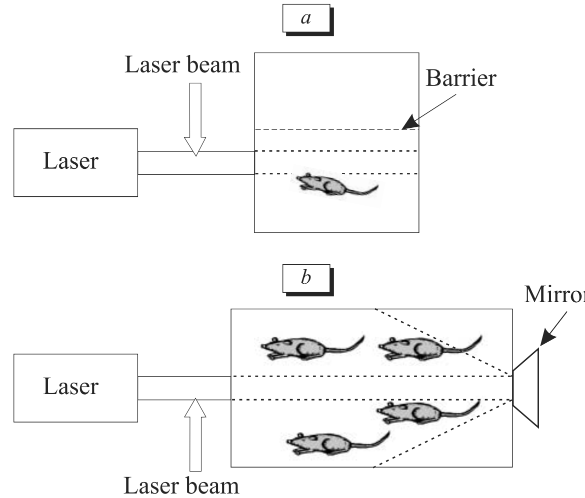

Fig. 1. Schemes of experiments.

trol specimens (Fig. 2, p>0.05). No intergroup differences were revealed in the number of entries and total time spent in the laser-irradiated area (Fig. 2, p>0.05). However, experimental group was characterized by a lower number of approaches to the laser entrance hole in the cage: the total time of exploration and sniffing the hole over the first, second, and third 5-min periods (U_1=13.5, p_1<0.010; U_2=18.0, p_2<0.027; and U_3=19.5, p_3<0.037, respectively), as well as the number of approaches to the hole (U_1=19.5, p_1<0.037; U_2=16.5, p_2<0.020; and U_3=15.0, p_3<0.014, respectively) in experimental group were lower than in controls. The number of approaches to the hole and the time spent near the hole were higher in controls (by 3 and 5 times, respectively). Laser-induced changes in behavioral parameters indicate that the animals perceive radiation. The decrease in exploratory activity of mice to a new stimulus (laser beam) attests to a negative effect of radiation (aversive properties).

The interpretation of strewing/tearing the bedding in the home cage depends on the situation. This type of behavior in intact animals sometimes reflects building of a nest or search for food. The increase in this behavior under stress conditions probably reflects displaced activity of animals. This form of activity manifests in the presence of several motivations, which cannot be realized due to certain circumstances. Our study revealed an increase in the frequency (U=15.0, p<0.010) and duration of this behavior (U=16.0, p<0.014). Such changes were particularly pronounced over the second 5-min period (Fig. 2). They reflect displaced activity in response to aversive stimulation, when the animal cannot escape radiation and reflected beam. Therefore, laser radiation produced a nega-

TABLE 1. Effect of Electric Light on EPM Behavior of Male Mice

Parameter	Recording period, conditions					
	minutes 1-5, no partner		minutes 6-10, no partner		minutes 11-15, with partner	
	control	light	control	light	control	light
Position near the barrier						
number of approaches	5.5±1.4	8.5±2.0	5.5±1.2	7.1±1.1	9.5±1.6	10.6±1.2
time, sec	48.5±12.0	55.9±12.0	43.4±15.5	65.6±17.5	201.8±16.4	205.0±7.0
Position in the radiation area						
number	7.5±1.2	10.88±2.08	7.0±1.5	9.5±1.6	4.6±1.1	6.3±1.0
time, sec	191.0±22.1	168.5±14.8	163.4±24.0	144.6±21.8	266.3±7.6	260.1±5.8
Sniffing of the hole						
number	5.0±1.2	6.0±1.4	4.2±1.1	4.9±0.8	3.8±1.1	2.6±0.6
time, sec	24.8±8.0	28.5±6.7	16.5±5.9	18.4±5.1	9.6±2.6	8.1±2.9
Strewing of the bedding						
number	1.0±0.7	0.3±0.2	1.2±0.9	0.6±0.5	1.3±0.8	0.1±0.1
time, sec	5.5±5.1	2.0±1.3	3.7±3.3	0.5±0.4	3.8±2.4	0.40±0.4

tive effect on mice. Moreover, this exposure has a cumulative effect. The observed differences were much more significant during the second 5-min period. The appearance of another male in the adjacent compartment of the cage was followed by strong exploratory motivation. It was observed in control and treated mice, which is consistent with published data [5]. The number of approaches and the total time spent near the barrier were much higher in the absence of another animal in the adjacent compartment (Fig. 2, $p<0.01$). Even in another motivation, the time spent near the laser entrance hole and exploration frequency were lower in treated animals. Electric light had little effect on animal behavior under similar conditions. Behavioral parameters did not differ between experimental and control males (Table 1). Therefore, light exposure had a less pronounced effect and did not cause aversion.

TABLE 2. Delayed Effect of Irradiation on EPM Behavior of Male Mice (*M*±*m*, %)

Parameter	Control animals	Treated (irradiated) animals
In open arms		
number	2.9±1.7	0.0±0.0
time, sec	0.51±0.3	0.0±0.0
In central area		
number	49.2±0.7	46.4±1.0*
time, sec	12.1±1.5	9.5±1.3
In closed arms		
number	47.8±2.9	53.3±0.9**
time, sec	87.4±1.7	89.9±1.4

Note. Derived indexes are expressed as a percentage of the total number of entries into and exits from the arms and total time of the test (5 min). *$p<0.05$ and **$p<0.01$ compared to the control group.

Laser radiation for 30 min had a delayed effect on animal anxiety in the EPM test (1 day after exposure, Table 2). In the experimental group, the number of entries into the central area ($U=25.0$, $p<0.02$) was lower and the number of entries into closed arms was higher ($U=23.5$, $p<0.01$) than in the control group. These data illustrate higher anxiety of experimental mice. Similar results were obtained in studying the number of entries and time spent in open arms of the maze ($U=40.0$, $p<0.08$). Experimental animals did not enter open arms. The number of peepings from closed arm, peepings under the maze, and transitions between closed arms did not differ between experimental and control animals ($p>0.05$).

Our results indicate that short-term exposure to THz waves has a negative effect on mouse behavior. The mechanisms of these changes, as well as the level of physiological responses to THz radiation, remain unclear. Most significant changes were found in the number of approaches and time of exploring (sniffing) the laser entrance hole. It can be hypothesized that the effect of THz waves is maximum in direct contact with the laser. This assumption is confirmed by the absence of significant changes in the total time spent in the area of radia-

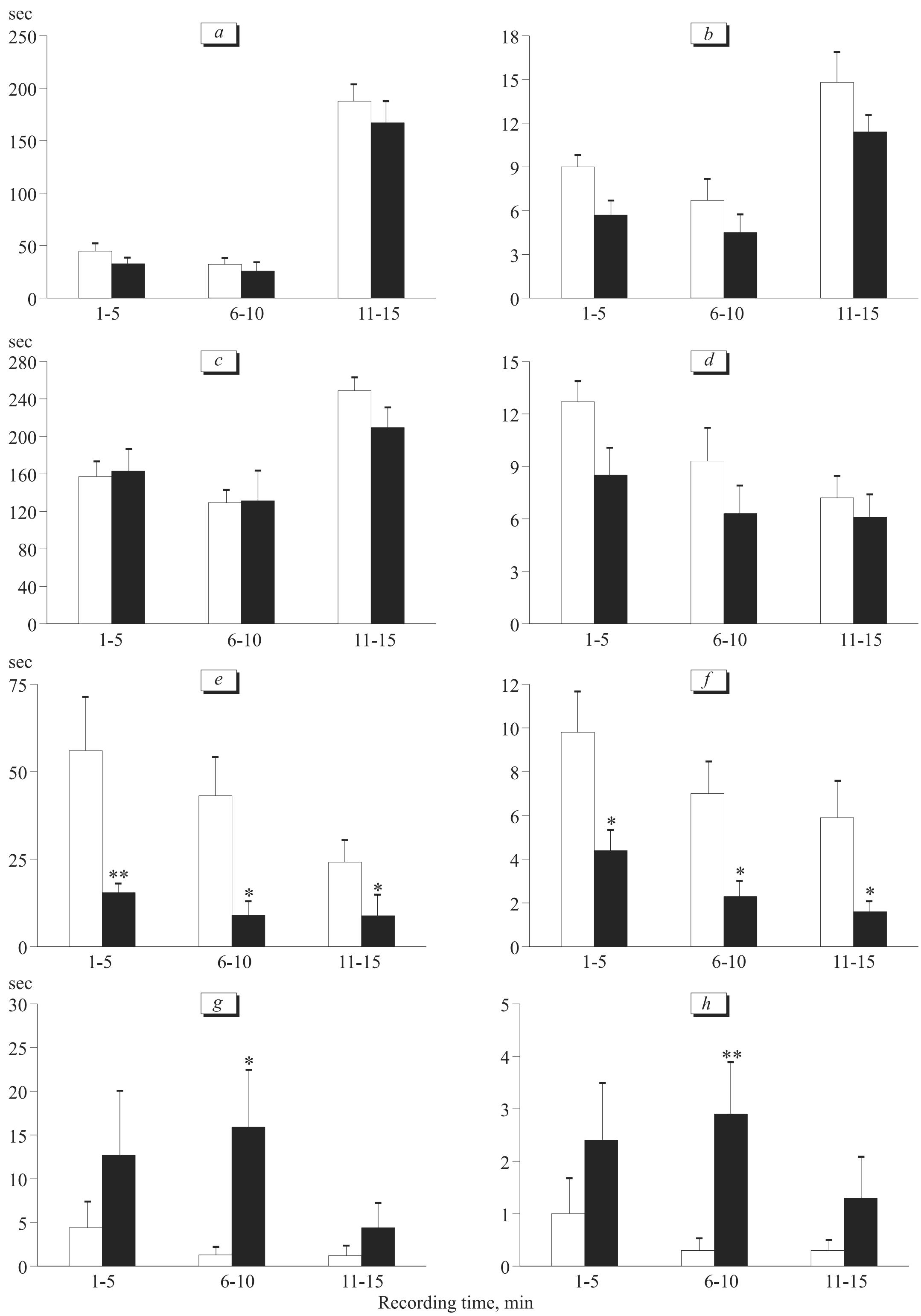

Fig. 2. Behavior of THz-irradiated male mice (dark bars) and untreated animals (light bars, control). *$p<0.05$ and **$p<0.01$ compared to the control. Time spent near the barrier (*a*); number of approaches to the barrier (*b*); time spent in the radiation zone (*c*); number of entries into the radiation zone (*d*); time of sniffing the hole (*e*); frequency of sniffing the hole (*f*); time of strewing the bedding (*g*); and frequency of strewing the bedding (*h*).

tion. The exposure to a distant source of THz waves probably has little effect and directly modulates the tissues, cells, and biological molecules, which is consistent with published data [4,8,9]. Irradiation for 30 min had a strong effect on the state of experimental animals. On the next day after irradiation, the level of anxiety in irradiated mice was higher than in controls.

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