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The Effects of Ultrasound, Infrasound, and Electroconvulsive Stimulations on Depression-like Behavior in Mice

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Abstract

Introduction: In the present study, the effects of ultrasound, infrasound, and electroconvulsive stimulation on depression-like behavior was assessed in animal models.
Method: For carrying out this study, 60 male BALB/c mice (mean age of 60 days, weight range of 25 – 30 g) were randomly selected. Depression was induced using reserpine 0.1 mg/kg i.p. for 30 days. They were allocated to three groups of experimental (ultrasound, infrasound, and electroconvulsive stimulation) and one group of control animals, each including 15 mice. Experimental animals received ultra- or infrasound 0.5 hours or 1 electroconvulsive pulse, daily for 10 days. Finally the Forced Swim Test was carried out.
Results: There was a statistically significant difference between the groups regarding the duration of immobility posture [F (3,54) = 99.54]. Mean immobility time was significantly longer in the control group compared to the other groups. Also, immobility was significantly longer for electroconvulsive compared to the ultra- and infrasound groups. Group ultrasound showed longer immobility than group infrasound; however, the difference was not significant.
Conclusion: Ultrasound and infrasound stimulations are capable of decreasing depression-like behavior in mice. The results of this study were also compatible with the application of electroconvulsive therapy. However, a more successful response can be exploited with ultra- and infrasound stimulations.

Keywords: Brain Stimulation, Depression, Electroconvulsive Stimulation, Infrasound, Ultrasound

Introduction

Depression is the most frequent cause of illness and disability across the world and a leading contributor to the global burden of disease [1-3]. A large number of patients with depression do not respond to medical therapy favorably. Moreover, anti-depressant medications may cause a range of side effects. Therapists can select a variety of non-medical strategies to manage patients' manifestations. Therefore, alternative therapeutic options are increasingly attracting interest. Researchers are struggling to develop and establish non-invasive brain stimulation methods applicable in the treatment of mood and cognitive disorders [4]. Brain stimulation methods such as electroconvulsive therapy, transcranial magnetic stimulation, vagus nerve stimulation, and deep brain stimulation have been considered as possibilities for the treatment of depression [5, 6]. For instance, in transcranial direct current stimulation, desirable outcomes have been reported for the management of depressive disorders by modulating neuronal excitability.

Among the treatment modalities for depression, electroconvulsive therapy plays an important role [1]. Electroconvulsive therapy is mostly prescribed for elderly patients and is thought to be an effective, yet low-tolerated technique for the treatment of depression [7, 8].

The underlying mechanism of action remains unknown. Though, a neurotrophic action on the hippocampus and increase in neurogenesis, synaptogenesis, the proliferation of glial cells, and even reduction in dysfunction of the immune response have been suggested as the possible effects [1, 9]. The side-effects of the procedure are not fully described. Some patients experience amnesia or confusion, and individual differences in skull impedance and anatomy restrict spatial targeting of stimulations [10]. Moreover, the procedure requires specially trained staff, an anesthesiologist, and a well-equipped setting.

Transcranial ultrasound is a newer method of brain stimulation [6]. The procedure is noninvasive and provides exact spatial targeting. There is no report for the ultrasound technique suggesting serious threats to human life [11]. There are a few studies reported in the literature supporting the application of ultrasound for mood elevation in patients with chronic disease. For example, ultrasound is believed to have a favorable mood effect in patients with low back pain [12]. However, the effects of ultrasound on living organisms are still in preclinical testing and any possible anti-depressive application of the method should be assessed before carrying out clinical trials [13, 14].

Studies demonstrated that exposure to infrasound stimulation alters neural activity in brain regions including right superior temporal gyrus, anterior cingulate cortex, and right amygdala. The regions are associated with auditory or emotional processing, and autonomic control [15]. Preliminary evidence suggests that infrasound waves negatively impact mental health and cognitive functions. However, even favorable cognitive effects including potential improvement of working memory have been reported for infrasound waves [16].

Although each of the brain stimulation methods has potentials for the treatment of mood disorders, they convey limitations or weaknesses [5]. Few studies have compared the effects of different brain stimulation procedures in the treatment of depression. The exact pattern and magnitude of the effects are uncertain and further studies have been recommended to replicate the results. In addition, evaluation of the external validity is complicated by a lack of uniformity in these studies. We still need more beneficial alternative methods for the treatment of patients with depression. Until reliable information about different therapeutic methods is collected, it is difficult to design the best intervention strategy.

In the current study, it was aimed to provide enough evidence regarding the effects of ultrasound, infrasound, and electroconvulsive stimulation on depression-like behavior in animal models. The hypothesis of this research was that the three methods affect mood in mice differently.

Method

In total, 60 male BALB/c mice (mean age of 60 days with the weight range of 25 – 30 g) were randomly selected from the animal breeding unit at Tehran University of Medical Sciences, Faculty of Veterinary Medicine. They were acclimatized for one week before the beginning of the experiment and were maintained at the temperature of 21 ± 2 ºC and the humidity of 45 ± 5%. The animals were housed per group and had free access to a standard pellet diet and water ad libitum except for the time of exposure. The light source in the animal house was set to provide a 12 h light/12 h dark cycle (07:00 – 19:00 h, light on). The mice were exposed to the stimulations within the light phase of the cycle. Three experimental groups and one control group of animals, each including 15 mice, were investigated in this study.

A reserpine was used to produce mice models of depression. Reserpine is a sympatholytic alkaloid and sedative medication and was a primary treatment for hypertension [17]. However, evidence from some trials indicated that it can cause depletion of monoamines in the brain and thereby induce major depression in prolonged use [18]. The mechanism of action for reserpine is to bind storage vesicles in monoaminergic neurons irreversibly and, therefore to decrease transmitter release at the synapse after depolarization [19]. Most commonly, reserpine is used in mice and rats to make animal models for depression. These models are used to assess the pathophysiology and the efficacy of treatment measures for depression.

For all mice, depression was induced by administering reserpine (0.1 mg/kg i.p., Reserpine, Daroupakhsh Pharmaceutical Co., Tehran, Iran) once per day for 30 days. Then, the mice were tested for depression using Forced Swim Test and the reserpine-treated mice were allocated to four groups of control (reserpine only), infrasound, ultrasound, and electroconvulsive therapy.

The mice in the ultrasound group were exposed to constant ultrasound waves of 3000 Hz with the intensity of 7 dB, 0.5 hour for 10 days. The ultrasound waves were generated in the laboratory room through a manufactured device (Touraj Sound Lab, Tehran, Iran). The even distribution of ultrasound waves was controlled by the use of a detector for the course of the research. The device was placed above the cage of the ultrasound group.

Experimental animals in the infrasound group were exposed to infrasound of 17 Hz at the intensity of 7 dB in an infrasonic chamber 0.5 hours for 10 days. The infrasound waves were generated with an infrasonic radiator.

Mice in the electroconvulsive stimulation group were anesthetized with isoflurane (3% in O_2 at 800 ml/min). The electroconvulsive shock was delivered with an instrument (Duo-Pulse, Ectron Ltd, England) using two saline-soaked skull electrodes, for two seconds (Figure 1).

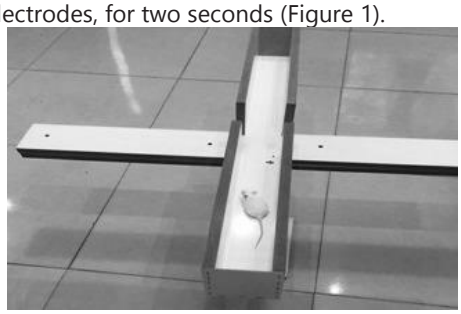

Figure 1. Delivering electroconvulsive stimulation to mice

The administered current generated tonic-clonic convulsions in the mice lasted for 10-20 seconds. A stopwatch was used to control the time taken for induction and the total duration of convulsions. One electroconvulsive pulse was administered to each mouse, daily over a period of 10 days.

The animals were randomly selected from a population of 2000 male BALB/c mice. A simple randomization was used to include 60 mice. At the end of the interval for induction of depression, reserpine-treated mice were randomly allocated to five study groups using block randomization. One assessor blinded to group assignments tested the mice for depression, before, and after the interventions.

The depressive state of the animals were compared in the exposed groups and the control by carrying out the Forced Swim Test. Each mouse was placed into a cylinder partially filled with water, and if it stopped swimming, this would be considered as a depression-like behavior. The exact duration of immobility posture was recorded in which the mouse would make only small movements to keep its head above the water. The cylindrical tank was 25 cm in height, 10 cm in diameter, and was filled with 25ºC water. The animal was not able to touch with its paws or tail to the bottom of the tank. The test lasted for 10 minutes, and the last five minutes were used for the assessment. Then, the test was stopped and the mouse was removed from the tank, dried, and returned to its cage. If a mouse failed to remain afloat, it was excluded from the study. An observer blinded to group assignment assessed all mice before, and after exposure to the interventions. Then, the results were compared and interpreted.

The study was carried out in accordance with the Guidelines for the Care and Use of Experimental Animals. The ethics review board of Azad University of Medical Sciences approved the research with the reference number: ir.iau.b.rec.1397.042.

Data are presented as mean (Standard Deviation) for continuous variables and absolute number (percent) for categories. The data were tested on normality with the help of the Kolmogorov-Smirnov test. Mean immobility times for mice were compared using one-way ANOVA and for the post-hoc tests, the Duncan test was used. Point estimates, 95% confidence intervals, and p-values were calculated. P-values less than 0.05 were considered significant. Statistical analyses were done with SPSS Version 20 (IBM Corp., Armonk, N.Y., USA).

Results

In total, 58 mice were included in the analytical sample (n = 13 for electroconvulsive therapy, and n = 15 for other groups). Two mice died in the electroconvulsive stimulation group. Table 1 and Figure 2 show the duration of immobility posture for the study groups after the interventions. The Kolmogorov-Smirnov test showed that data for the duration of immobility posture were normal for all four groups (all P > 0.05). The Levene's test also showed that the groups were homogenous in variances of the data [F(3,54) = 1.70, P = 0.180].

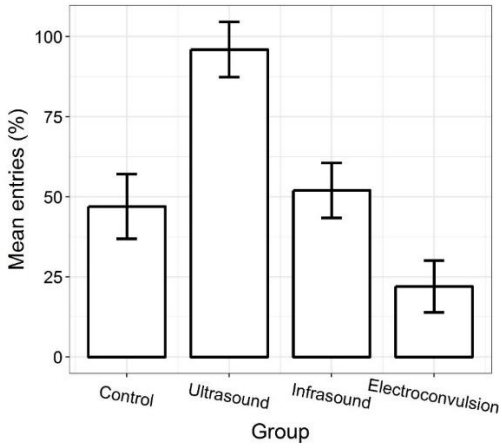

Figure 2. Entries into the open arms of elevated plus maze (error bars represent 95% confidence interval)

Table 1. Between-group analysis for the duration (second) of immobility posture

Group	n	Mean (SD)	Minimum	Maximum	*P
Control	15	237.0 (54.2)	160.0	305.4	
Ultrasound	15	10.0 (19.2)	0.01	56.2	
Infrasound	15	32.1 (35.4)	0.01	96.4	0.001
Electroconvulsive stimulation	13	82.8 (42.1)	0.01	133.6	

*One-way ANOVA

There was a statistically significant difference between the groups regarding the duration of immobility posture [F(3,54) = 99.54, P = 0.001]. Duncan's test showed that mean immobility time was significantly longer in group control compared with other groups (Table 2). Also, immobility was significantly longer for group electroconvulsive compared to ultra- and infrasound. The mice in group ultrasound showed longer immobility than group infrasound; however, the difference was not statistically significant.

Table 2. Duncan's new multiple range test for mean immobility time between study groups

Group	n	Subset for alpha = 0.05		
		1	2	3
Ultrasound (n=15)	15	10.0		
Infrasound (n=15)	15	32.1		
Electroconvulsive Stimulation (n=13)	13		82.8	
Control (n=15)	15			237.0
Significance		0.14	1.00	1.00

Discussion

In the present study, we tried to provide evidence to assess the effects of ultrasound, infrasound, and electroconvulsive stimulations, on reserpine-treated animal models. Evidence rejected the null hypothesis and the study showed that the treatments are different in efficacy. The immobility time of mice was used in the Forced Swim Test as the surrogate for depression. Immobility time was significantly different in group control compared with other groups. A plausible explanation would be that the interventions are effective in decreasing depression-like behavior in mice. Moreover, mice in group electroconvulsive stimulations showed longer immobility compared with the mice exposed to sound waves. In other words, ultrasound, and infrasound waves were more successful in lowering depression-like behavior than electroconvulsive stimulation. Overall, the results of this study were consistent with some previous findings in the literature.

Delivering acoustic energy is thought to stimulate revascularization, to modulate cortical function, and to cause neurotherapeutic effects [5, 20]. Favorable outcomes have been reported in the application of ultrasound waves for the treatment of neuropathic pain, essential tremor, and Parkinson's disease. While the mechanism of action is not yet exactly known, ultrasound has been demonstrated to inhibit monoamine and increases brain-derived neurotrophic factor especially in the hippocampus [21, 22]. This may explain the anti-depressive activity of ultrasound waves. Moreover, ultrasound can open the blood-brain barrier and thereby increase the permeability to peripheral neurotrophic factor and improve depression symptoms [23]. Ultrasound increases neurogenesis in the dentate gyrus of the dorsal hippocampus in mice and augments the anti-depressive effects of ultrasound waves [23].

In a placebo-controlled trial on patients with chronic pain, researchers assessed possible effects of ultrasound waves on mental states [11]. Participants completed two visual analog scales for pain and mood. The results showed that patients felt significant improvement in their mood 10 minutes ($P = 0.03$) and 40 minutes ($P = 0.04$) after transcranial ultrasound compared with placebo. Participants also experienced slight pain palliation following the intervention ($P = 0.07$) at 40 minutes. It was concluded that ultrasound leads to safe and favorable neurophysiological and mental effects through resonating intra-neuronal microtubules. Histochemical properties were not assessed on neuronal tissue in the study animals in the present study. However, the behavioral evaluations of this study are compatible with the positive effects reported for ultrasound waves.

A study indicated that near-threshold infrasound produces physiological effects and stimulates brain areas involved in auditory processing and emotional and autonomic control [15]. Researchers evaluated the response of the brain for near- and supra-threshold infrasound waves under resting-state fMRI. In a first session, 14 healthy participants underwent threshold and loudness measurement with different sound pressure levels. In the second session, they underwent three resting-state acquisitions. Analysis of data for regional homogeneity and inter-regional connectivity showed that there was higher local connectivity in right superior temporal gyrus near to primary auditory cortex, in the anterior cingulate cortex, and in the right amygdala during the near-threshold, compared to both the supra-threshold and the no-tone condition. Also, changes in functional connectivity were recognized in the right amygdale with no-tone more than near-threshold condition and in the right superior frontal gyrus during the near-threshold state. As a conclusion, their findings indicated that infrasound has the potential to alter physiology and connectivity in neural tissues.

In another study,13 healthy participants exposed to short sinusoidal tone bursts of 12 Hz administered monaurally underwent fMRI and cognitive evaluations using the n-back working memory task [16]. It was shown that executing the task was associated with significant activation of the prefrontal, parietal, and bilateral primary auditory cortices, and also of the striatum, and the cerebellum. It was concluded that working memory function improves with exposure to infrasound waves. There is a definite lack of information in the literature on the effects of infrasound on depression and anxiety.

Electroconvulsive shock is given to depressed patients a few times a week under anesthesia and muscle relaxant. The electrical stimulation triggers a tonic-clonic seizure. There is a good deal of research regarding the effects of electroconvulsive therapy on depression symptoms. However, the underlying mechanism of action is still controversial. Animal studies have demonstrated that electroconvulsive seizures induce cellular and molecular changes in the brain and that the seizures are associated with alterations in central and peripheral neurotrophin level and immune signaling. The concepts of immune dysregulation and neurotrophic deficits might explain the pathophysiology of depression and the favorable effects of electroconvulsive shock. A low-grade inflammatory response can be recognized in the peripheral blood of patients with major depression. Electroconvulsive stimulation is also associated with an increased hematogenous and neuroinflammatory immune response [24]. It has been suggested that inflammatory stimulation augments neurotrophin expression and mediate antidepressant-like effects [24].

In a study on MAP6 KO mice, the behavioral and biological consequences of electroconvulsive stimulations were assessed with two different protocols: 10 stimulations over a 2-week period and 2 stimulations per week for 5 weeks [25]. That study showed that behavioral improvement is associated with an obvious increase in the survival and integration of neurons born before the interventions. The stimulations in the first program reinforced synaptogenesis in the cortex of the brain and sustained neuron survival rate for almost 40 days. However, the second stimulation protocol caused persistent behavioral improvement and neuron survival. It was concluded that synaptic connectivity and neuronal survival are key to the short and long-term efficacy of

electroconvulsive stimulations.

There are a few reported studies on biological effects of ultrasound and infrasound waves; however, little information is available about their behavioral effects on human or animals. To our knowledge, there was no recent study in the literature comparable to ours regarding the comparison of ultrasound, infrasound, and electroconvulsive shock for the treatment of depression-like behavior. The sample of this study was sufficiently large to detect important differences. However, the effects of combined interventions were not assessed. Studies with factorial designs should determine the combined effect of brain stimulation methods with other known modalities for the treatment of depression. Also, further dose-response studies are needed to delineate the exact therapeutic pattern of the treatment methods.

Conclusion

Brain stimulation has the potential of being considered as an effective measure against depression as a widespread illness. This study showed that ultrasound and infrasound stimulations are capable of decreasing depression-like behavior. These noninvasive techniques neither necessitate surgery nor require anesthesia or genetic alteration. Moreover, the beneficial neuromodulation can be attained through implementing easy to setup infrastructures. The results of the present study were compatible with the application of electroconvulsive therapy; however, the authors were able to elicit a more successful response with ultra- and infrasound waves in the sample of reserpine-treated mice.

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