High-frequency ultrasound exposure improves depressive-like behavior in an olfactory bulbectomized rat model of depression

Tsugumi Yamauchi[a,b], Toshinori Yoshioka[a], Daisuke Yamada[a], Takumi Hamano[a], Maika Ikeda[c], Masato Kamei[c], Takaya Otsuki[c], Yasuo Sato[c], Kyoko Nii[c], Masashi Suzuki[c], Satoshi Iriyama[d], Kazumi Yoshizawa[e], Shoichi Nishino[c], Hiroko Ichikawa[b], Satoru Miyazaki[f] and Akiyoshi Saitoh[a]

Objectives **According to previous studies, ultrasound exposure appears to be a noninvasive method for modulating brain activity related to cognition and consciousness; however, its effects on emotional states remain unclear. Therefore, an animal model is required in which the effects and effect mechanisms of ultrasound exposure can be investigated. Thus, we used olfactory bulbectomized rats as an animal model of depression and investigated their emotional state following ultrasound exposure.**

Methods **In male Wistar/ST olfactory bulbectomized rats, hyperemotionality was evaluated according to hyperemotionality scoring and the scores before and after 24-h ultrasound exposure were compared. Elevated plus maze (EPM) tests were also conducted after 24-h ultrasound exposure, and blood samples were collected in which plasma corticosterone concentrations were measured.**

Results **Following exposure to high-frequency (~50 kHz) ultrasound vocalizations (USVs) associated with the pleasant emotions of rats, the hyperemotionality scores of olfactory bulbectomized rats were significantly reduced. Additionally, the latency of the first entry into the open arm of the EPM was significantly decreased in USV-exposed olfactory bulbectomized rats, as were their plasma corticosterone levels. Furthermore, artificial ultrasound (50 kHz) at a similar frequency to that of USV also significantly decreased the hyperemotionality score of olfactory bulbectomized rats.**

Conclusions **Ultrasound exposure improved depressive-like behavior in olfactory bulbectomized rats and reduced their plasma corticosterone levels. Thus, we recommend the use of olfactory bulbectomized rats as an animal model for investigating the effects and effect mechanisms of ultrasound exposure.** ***NeuroReport*** **33: 445–449**

NeuroReport 2022, **33**:445–449

Keywords: animal model, antidepressant, anxiety, depression, hypersonic effect, Mozart effect, sonomedical, ultrasonic vocalizations, ultrasound, USV

[a]Laboratory of Pharmacology, Department of Pharmacy, Faculty of Pharmaceutical Sciences, Tokyo University of Science, [b]Laboratory of Psychology, Noda Division, Institute of Arts and Sciences, Tokyo University of Science, [c]FUJIMIC, Inc. Tokyo, [d]Laboratory of Quantum information dynamics, Department of Information Sciences, Faculty of Science and Technology, Tokyo University of Science, [e]Laboratory of Pharmacology and Therapeutics, Department of Pharmacy, Faculty of Pharmaceutical Sciences, Tokyo University of Science, and [f]Laboratory of Bioinformatics, Department of Pharmacy, Faculty of Pharmaceutical Sciences, Tokyo University of Science

Correspondence to Akiyoshi Saitoh, PhD, Department of Pharmacy, Faculty of Pharmaceutical Sciences, Tokyo University of Science. 2641 Yamazaki, Noda-shi, Chiba 278-8501, Japan
Tel/fax: +81 4 7121 3610; e-mail: akiyoshi_saitoh@rs.tus.ac.jp

Received 13 April 2022 **Accepted** 11 May 2022

Introduction

In recent years, the effects of ultrasound on brain function have been studied intensively. For example, several studies have shown that whole-body exposure to high-frequency ultrasound in humans significantly increases brain activity, including that in reward-related neural circuitry [1,2]. Indeed, ultrasonic exposure appears to be a noninvasive method for modulating brain activity related to consciousness. However, little is known about the effects and effect mechanisms of ultrasound on emotional states, such as depression. Furthermore, most studies on ultrasound exposure have been conducted on human subjects; therefore, molecular mechanisms have been difficult to elucidate.

In the present study, we report on the possibility of using an animal model to clarify the effects of ultrasound exposure on an emotional state. Specifically, we investigated the effects of ultrasound exposure in olfactory bulbectomized rats, which is a high validated animal model of depression [3]. We found that ultrasound had antidepressant-like

0959-4965 Copyright © 2022 The Author(s). Published by Wolters Kluwer Health, Inc. DOI: 10.1097/WNR.0000000000001804

effects in these animals, suggesting that olfactory bulbectomized rats might be an appropriate animal model to identify mechanisms underlying the effects of ultrasound exposure.

Methods

Animals

Male Wistar/ST rats (7 weeks old; purchased from Sankyo Labo Service Corporation, Inc., Tokyo, Japan) were used in our behavioral and biochemical experiments. All animals had free access to food and water and were kept in an animal room with stable temperature (23 ± 1 °C) and relative humidity (55% ± 5%) under a 12/12-h light-dark cycle (lights automatically switched on at 08:00). All experimental protocols were approved by the Institutional Animal Care and Use Committee of Tokyo University of Science (Approval No. Y21002) and were conducted in accordance with the guidelines of the National Institute of Health and Japan Neuroscience Society.

Ultrasound exposure

We recorded the high-frequency (~50 kHz) ultrasound vocalizations (USVs) associated with pleasant emotions using an ultrasound microphone (CM16/CAMPA; Avisoft Bioacoustics, Berlin, Germany) by tickling rats' abdomen [4,5]. In addition, we produced artificial 50-kHz ultrasound and white noise (35–100 kHz) using SASLab Pro software 5.2.09 (Avisoft Bioacoustics).

Evaluation of olfactory bulbectomy-induced hyperemotionality in rats

Rats were anesthetized using a solution containing medetomidine (2.4 mg/kg), midazolam (0.45 mg/kg) and butorphanol (3.0 mg/kg) dissolved in saline. They were then placed in a stereotactic apparatus, and an olfactory bulbectomy was performed according to the procedure reported in our previous study [6]. Sham operations were performed using the same method, but the olfactory bulbs were left intact. After surgery, all rats were housed individually for 14–18 days.

After surgery, rats were placed in a cage in a soundproof box (at 23 °C and 10 Lux) and exposed to high-frequency (~50 kHz) USVs or artificial 50-kHz ultrasound for 24 h. All ultrasounds were emitted directly into the rat cage using an UltraSoundGate Player 116 (Avisoft Bioacoustics). The control group was kept in a cage in a soundproof box for 24 h without exposure to ultrasound.

The hyperemotionality of olfactory bulbectomized rats was evaluated according to hyperemotionality scoring [6] before and after 24-h ultrasound exposure. Hyperemotionality of rats was measured by scoring their responses to the following stimuli: (1) attack response was scored by presenting a rod 4–5 cm in front of the snout, (2) startle response was scored by blowing air on the nose, (3) struggle response was scored by handling with a gloved hand and (4) fight response was scored by pinching the tail with forceps. The rat's tail was gently held from behind using mosquito forceps. Responses were graded as follows: 0, no reaction; 1, slight; 2, moderate; 3, marked or 4, extreme. During each emotional response, vocalization during the test was also scored and graded as follows: 0, no vocalization; 1, occasional vocalization or 2, marked vocalization. Vocal score was added to each emotional response score. The total emotional response score was the sum of these scores.

In addition, elevated plus maze (EPM) tests were conducted after 24-h ultrasound exposure, according to our previous study [7], and the latency of the first entry into the open arms of the EPM and time spent on these arms were observed using a video camera system and analyzed using Smart 3.0 (Harvard Apparatus, USA).

Plasma corticosterone assessment

Immediately after EPM tests, trunk blood samples were collected into tubes containing heparin. Plasma collection and measurement of plasma corticosterone levels were conducted according to methods reported in our previous study [8].

Data analysis

All data presented are means ± SEM. Data were analyzed using one-way analysis of variance (ANOVA) to compare three or more groups and *t*-tests to compare two groups. Post hoc individual group comparisons were made using Holm–Sidack multiple comparison tests. $P < 0.05$ was considered statistically significant. Smirnov–Grubb's tests were performed to detect outliers, which were rejected if found.

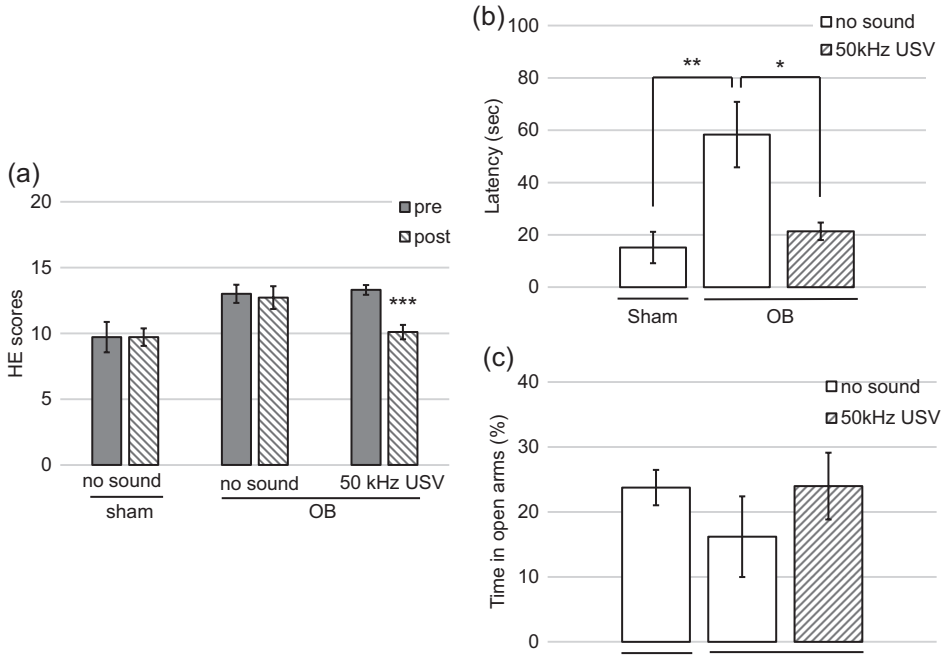
Results

Following 24 h of high-frequency (~50 kHz) USV exposure, the total hyperemotionality scores of olfactory bulbectomized rats were significantly decreased compared with the scores evaluated prior to exposure (paired *t*-test: $P < 0.01$; Fig. 1a). In contrast, hyperemotionality scores were unaffected in sham rats and control olfactory bulbectomized rats (without exposure to ultrasound) (paired *t*-test: $P = 1$, 0.5222, respectively; Fig. 1a). In EPM tests, the latency of the first entry of olfactory bulbectomized rats into the open arms was significantly increased relative to that of sham rats (one-way ANOVA, $F_{(2,19)} = 7.323$, $P < 0.01$; Fig. 1b). Additionally, the percentage of time spent by olfactory bulbectomized rats in the open arms was decreased relative to that spent by sham rats; this was recovered to some extent in 50-kHz-exposed olfactory bulbectomized rats, although not at a significant level (one-way ANOVA: $F_{(2,19)} = 0.7929$, $P = 0.07703$; Fig. 1c).

To determine the influence of the hypothalamic-pituitary-adrenocortical axis on the decrease in hyperemotionality induced by ultrasound exposure, we examined the plasma corticosterone levels of olfactory bulbectomized rats, which were found to be significantly decreased after exposure to 50-kHz USVs (Student's *t*-test: $P = 0.02288$; Fig. 2).

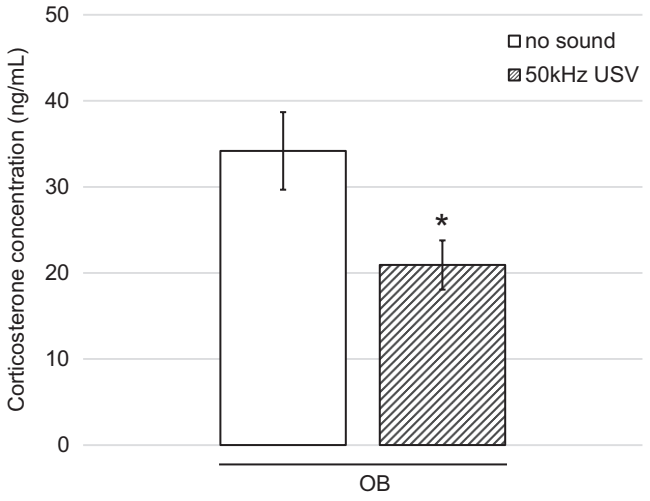
Moreover, we tested artificial 50-kHz ultrasound, which is similar frequency to USVs associated with pleasant

Fig. 1



Effects of recorded 50-kHz USV exposure on hyperemotionality in olfactory bulbectomized (OB) rats (a). Hyperemotionality scores were measured before and after ultrasound exposure. ***$P<0.001$: paired *t*-test (b,c). Elevated plus maze (EPM) tests conducted after ultrasound exposure. *$P<0.05$ and **$P<0.01$: one-way ANOVA with a post hoc Holm–Sidack's multiple comparison test. All data are means ± SEM. ANOVA, analysis of variance.

Fig. 2



Effects of recorded 50-kHz USV exposure on plasma corticosterone levels in olfactory bulbectomized (OB) rats. The plasma was collected immediately after elevated plus maze (EPM) tests. Data are means ± SEM. *$P<0.05$: unpaired *t*-test.

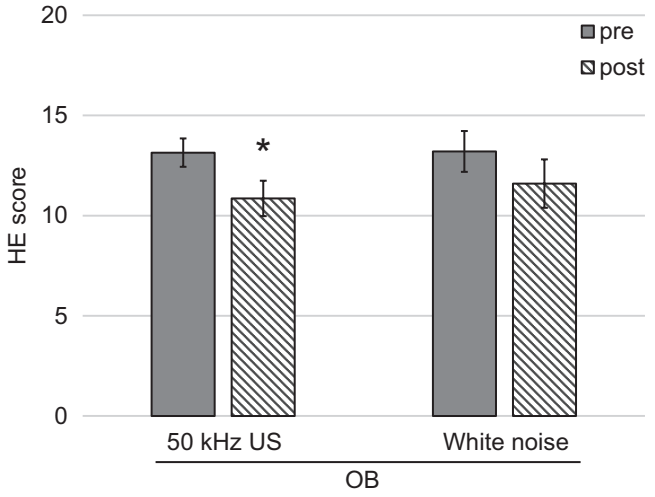
emotions in rats. This artificial ultrasound also significantly reduced the hyperemotionality scores of olfactory bulbectomized rats; however, white noise exposure had no significant effect on these scores (paired *t*-test: $P = 0.01860$, 0.2454, respectively; Fig. 3).

Discussion and conclusion

In olfactory bulbectomized rats, exposure to 50-kHz USVs associated with pleasant emotions in rats significantly reduced hyperemotionality scores and the latency of first entry into the open arms of EPMs. In addition, the levels of plasma corticosterone, which is an endogenous stress biomarker, in USV-exposed olfactory bulbectomized rats were significantly decreased. Olfactory bulbectomized rats represent a valid animal model of depression, especially in terms of predictive validity, because both the hyperemotionality scores and anxiety-like behavior in EPM tests observed in these rats are reversed with treatments of many types of antidepressants [9,10]. Thus, our results suggest that high-frequency (~50 kHz) USVs produced antidepressant-like effects in olfactory bulbectomized rats. Moreover, artificial 50-kHz ultrasound with a similar frequency to that of the USVs also significantly reduced the hyperemotionality score of olfactory bulbectomized rats. This suggests that the specific frequency of ultrasound exposure plays an important role in the antidepressant-like effects observed in olfactory bulbectomized rats. To the best of our knowledge, this is the first study to show that ultrasound exposure produces such antidepressant-like effects in rats, and we suggest that ultrasonic exposure could be used as a potential therapy to treat psychiatric disorders.

The high-frequency (~50 kHz) USVs used in this study, which reduced hyperemotionality scores, anxiety-like

Fig. 3



Effects of artificial 50-kHz ultrasound exposure on the hyperemotionality scores of olfactory bulbectomized (OB) rats. Hyperemotionality scores were measured before and after ultrasound exposure. Data are means ± SEM. *P<0.05: paired t-test.

behavior and plasma corticosterone levels in olfactory bulbectomized rats, were emitted by rats in response to tickling. Indeed, tickling stimulation by human handling is known to induce high-frequency USVs that are believed to indicate positive emotions in rats [11]. Furthermore, rats have been reported to make 50-kHz ultrasonic vocalizations to communicate with conspecifics while in positive emotional states [12]. In contrast, previous studies have shown that playback of low-frequency (~20 kHz) USVs emitted by rats showing fear or anxiety produced anxiety-like behavior in rats, and these results were supported by changes in c-FOS-expressing cells within the basolateral amygdala [13]. Overall, our results suggest that the playback of high-frequency (~50 kHz) USVs emitted by rats in a pleasant emotional state is capable of reversing depressive-like behaviors in olfactory bulbectomized rats.

Interestingly, transcranial ultrasound exposure has been suggested as a noninvasive method for modulating brain activity related to cognition, consciousness and mental state. In a double-blind placebo control study, Harneroff *et al*. [14] found that transcranial ultrasound exposure over the frontotemporal cortex in subjects with chronic pain significantly improved mood and slightly reduced pain levels within 40 min of the exposure. Thus, ultrasound exposure therapy could be useful for treating a variety of mood and neurologic disorders, including depression. Some studies have shown that ultrasound exposure applied to the body surface of humans activates reward system-related brain regions; this is known as the 'hypersonic effect', and it could be applied to treat depression during cognitive behavioral therapy [2,15].

In conclusion, we showed that ultrasound exposure in olfactory bulbectomized rats improved depressive-like behavior and reduced plasma corticosterone levels. Moreover, our results suggest that olfactory bulbectomized rats are an appropriate animal model for investigating the effects and effect mechanisms of ultrasound exposure.

Acknowledgements

The authors would like to thank Enago (www.enago.jp) for the English language review.

Conflicts of interest

The study was supported by a grant from the FUJIMIC, Inc. Tokyo.

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