Effects of Decabromodiphenyl Ether (BDE-209) on Ultrasonic Communication in Fighting of Male Adult Rats

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Introduction

Decabromodiphenyl ether (BDE-209) is one of brominated flame retardants and worldwidely used in various consumer products, such as plastic and textile goods, construction materials, and electronic equipments. BDE-209 makes inflammable consumer products nonflammable and protects us from burning. However, animal model studies have demonstrated that BDE-209 disrupts thyroid hormone system [4, 7, 8]. Because thyroid hormones (THs) are essential for development of brain functions, BDE-209 has potentials to cause brain dysfunctions [6].

Recently, we introduced ultrasonic vocalization (USV) of rats because USVs have communicative functions and are a highly sensitive tool to study the pup-dam relationships. BDE-209 was administered to pregnant rats and examined whether BDE-209 affects acoustic characteristics of USVs of the resultant pups [14]. The BDE-209-treated pups indicated the reduction of USV durations and percentages of frequency-modulated USVs compared with the control pups. The USVs with shorter durations and lower frequency-modulations are less attractive and noticeable to dams. The survival of pups may be subsequently threatened when the acoustically altered USVs go unnoticed by nearby dams.

Adult rats also produce USVs as a communicative tool when they fight each other for territory and female partners. During fighting, male rats produce two different types of USVs. One is 50–80 kHz USVs with shorter durations and the other is 20–30 kHz USVs with longer durations. The 50-80 kHz USVs are considered to express positive emotionality, whereas the 20-30 kHz USVs are considered to express negative emotionality [2, 13].

In the present study, we examined effects of BDE-209 on USVs in fighting behavior of adult rats. Pregnant rats were administered BDE-209 and their pups were served as subjects in adulthood. We hypothesized that BDE-209 causes acoustic alterations of USVs in fighting behavior.

Materials and methods

Pregnant Wistar rats at gestational day (GD) 13 were purchased from Japan SLC Inc. (Hamamatsu, Japan). They were individually housed and randomly assigned to the control (n = 2), low-dose (n = 2), or high-dose group (n = 3). BDE-209 (purity ≥ 97.0%; Merck, Darmstadt, Germany) was administered daily to the pregnant rats from GD 15 to postnatal day (PND) 21 at the following doses: 0 mg/kg (control group), 500 mg/kg (low-dose group), or 1000 mg/kg (high-dose group). BDE-209 was mixed with sweetened condensed milk (Megmilk Snow Brand Co., Ltd., Tokyo, Japan). The pregnant rats freely and voluntarily ate the sweetened condensed milk containing BDE-209. The volume of sweetened condensed milk used was 10 g/kg. The control group ate sweetened condensed milk only.

The birth date of the litter was designated PND 0. Then the pups were culled to four males and four females per litter on PND 4. All pups were weaned on PND 22, individually housed, and served as subjects in

adulthood.

On PNDs 100–102, two male rats from the same dose group but different litters were put into a plastic cage and paired for 15min/day. The size of the cage was 34 cm in length, 29 cm in width, and 18 cm in height. The recording of USVs was daily done on this period using an ultrasonic microphone and the Sonotrack system v.2.4.0. (Metris, Hoofddorp, The Netherlands). The ultrasonic microphone and the plastic cage were set in a sound-insulated box. The microphone was located 5 cm above the cage. USVs were recorded for 10 min after a 5-min period of habituation. The paired rats were returned to their individual cages after USV recording. The pair was fixed through the fighting period. The numbers of pairs were 4 (control), 3 (low-dose), and 4 (high-dose).

The temperature in the sound-insulated boxes was maintained at 19–22°C, and the relative humidity was kept within 40–60%. All rats were subjected to a 12-h light/dark cycle (light, 20:00–08:00 h; dark, 08:00–20:00 h), and the USV was recorded during the dark period of 13:00–16:00. The rats were supplied with rat chow and tap water *ad libitum*. The experimental protocol was approved by the Animal Ethics Committee of Hokkaido University, and all the experimental conditions complied with the Guide for the Care and Use of Laboratory Animals (Hokkaido University).

All USV data were processed via automatic analyses in the Sonotrack system. A two-factor ANOVA tested the main and interactive effects of BDE-209 and age on the USV parameters such as the number, fundamental frequency, and duration. If a main effect was found to be significant, multiple pair-wise comparisons of group means were performed using Ryan's method. The above statistical analyses were conducted by the ANOVA 4 software (http://www.hju.ac.jp/~kiriki/anova4/about.html).

Results and discussion

Figure 1 displays fundamental frequencies of USVs. Effects of BDE-209 were significant [$F(2, 8)= 12.538$, $p < 0.005$]. Both the low- and high-dose groups had significantly higher fundamental frequencies than the control group (ps < 0.05). Figure 2 exhibits durations of USVs. BDE-209 was a significant factor [$F(2, 8) = 5.950$, $p < 0.05$]. The durations of both the low- and high-dose groups were shorter than those of the control group (ps < 0.05). However, BDE-209 had no significant effects on the number of USVs.

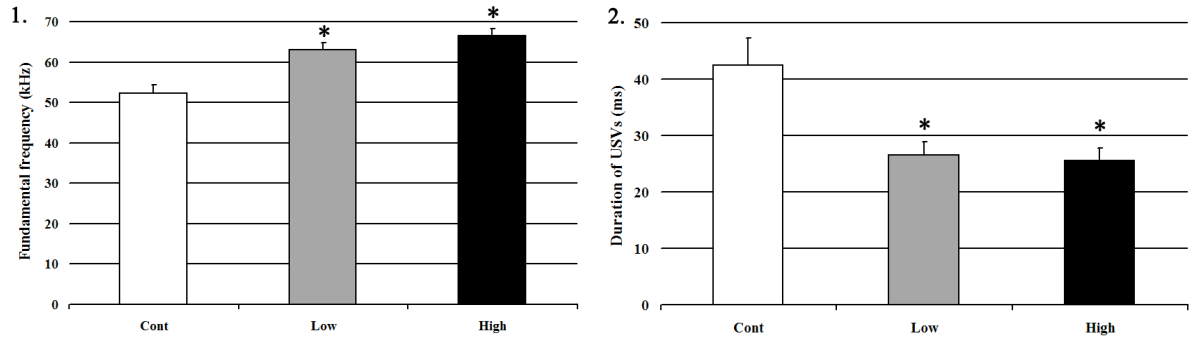

Fig. 1. Effects of BDE-209 on the fundamental frequencies of USVs.

Fig. 2. The effects of BDE-209 on the durations of USVs.

Data represent the mean and SEM. (*, $p < 0.05$ vs Cont)

Figure 3 exhibits the scatter grams of USVs in fighting behavior. Each USV is plotted on the scatter gram as a closed circle in two-dimensions of frequency and duration. The striking differences were revealed in USV

durations in frequencies between 20–40 kHz. The control group emitted USVs with much longer durations than 300ms, whereas the low- and high-dose groups decreased USVs with durations longer than 300ms.

We calculated the percentages of 20–40 kHz USVs (20 kHz ≤ USVs < 40 kHz) and 50–80 kHz USVs (50 kHz ≤ USVs < 80 kHz) and revealed significant effects of BDE-209 on the percentages of 20–40 kHz and 50–80 kHz USVs [$F(2, 8) = 10.358$, $p < 0.01$; $F(2, 8) = 13.130$, $p < 0.005$], respectively. The percentages of 20–40 kHz USVs were reduced in both the low- and high-dose groups compared with the control group (ps < 0.05) (Figure 4A). In contrast, the percentages of 50-80 kHz USVs were elevated in both the low- and high-dose groups compared with the control group (ps < 0.05) (Figure 4B). The durations of USVs in frequencies of 20-40 kHz and 50-80 kHz were not affected by BDE-209. Effects of age were not significant in any USV parameters.

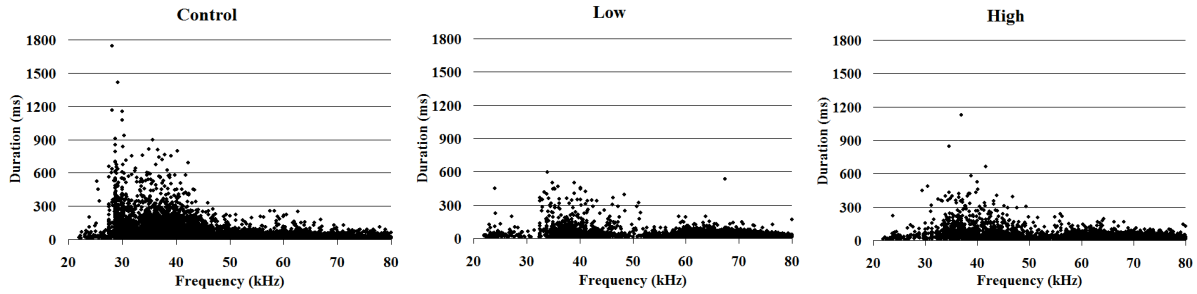

Fig. 3. USV scatter diagrams of the control, low-dose and high-dose groups in fighting behavior.
Each closed circle represents one USV in two-dimensions of frequency and duration.

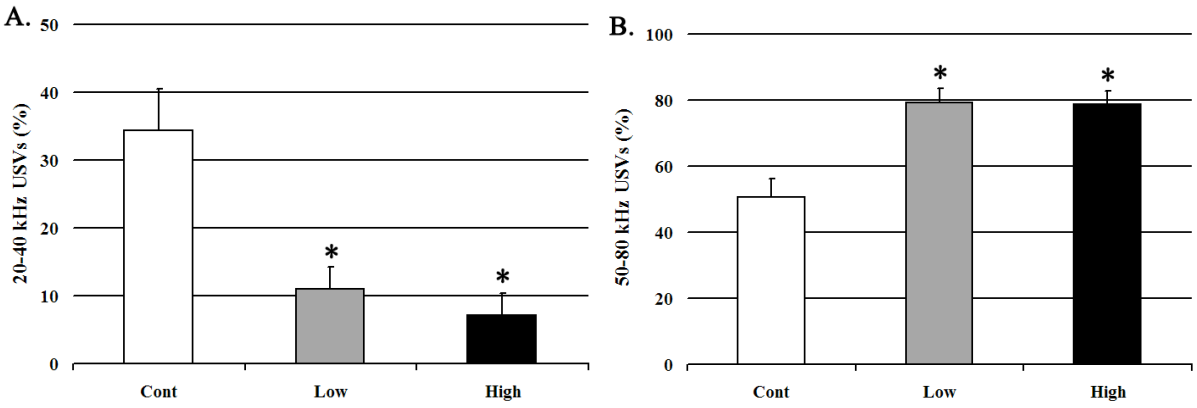

Fig. 4. Effects of BDE-209 on the percentage of USVs in frequencies of 20–40 kHz (A) and 50–80 kHz (B).
Data represent the mean and SEM. (*, $p < 0.05$ vs Cont)

Male adult rats fight each other for territory and female partners. During fighting behavior, male rats produce both 50–80 kHz USVs with shorter durations and 20–30 kHz USVs with longer durations. The 50-80 kHz USVs reflect positive emotionality and the 20-30 kHz USVs reflect negative emotionality [2, 13].

In our study, both the low- and high-dose groups revealed lower percentages of USVs in frequencies 20–40 kHz. The generation of USVs is regulated by both central and peripheral vocalization-related organs. Rat USVs are primarily regulated by the brainstem vocal circuit comprising the superior colliculus, periaqueductal gray, lateral parabrachial region, and paralemniscal area [6]. This brainstem vocal circuit connects to the vocal pattern generator, including the nucleus retroambiguus and lateral reticular formation [2, 13]. The vocal pattern

generator regulates the muscles of the larynx, abdominal intercostal diaphragm, jaw, lip, tongue, and face via motor neurons and ultimately produces USVs [15]. Laryngeal muscles are particularly important to align USV durations in lower frequencies. The low- and high-dose groups in our study might be suffering from dysfunctions of laryngeal muscles or the motor neurons that regulate laryngeal muscles.

BDE-209 affects thyroid hormone systems and reduces triiodothyronine and thyroxine [4, 5, 7, 8]. Because these thyroid hormones regulate neural development, hypothyroidism may induce dysfunctions of vocalization-related neural systems.

Another interpretation is that the low- and high-dose groups were less aggressive. BDE-209 reduces thyroid hormones [4, 7, 8] and consequently, results in lower testosterone levels in male animals [5, 11, 12]. The correlations between testosterone levels and aggressive behavior have been extensively investigated in rat models [10]. The lack of testosterone makes male rats less aggressive. Therefore, the reduction of testosterone may alter aggressive behavior and accordingly, reduce the percentages of USVs in frequencies of 20–40 kHz.

The 20–40 kHz USV is a submissive signal to the superior rat in fighting behavior and consequently, the fighting is ceased [1]. Defeated male rats that emit few submissive signals may be fatally injured. Furthermore, the 20–40 kHz USVs are emitted as a warning signal to notice a nearby predator to conspecifics [1, 9]. Therefore, 20–40 kHz USVs have biologically crucial roles for survival.

This is the first study to evaluate long-term effects of BDE-209 in fighting behavior of male adult rats by using USV analyses. USV-based analyses are expected to be a new sensitive method to characterize the impacts of polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), dioxins, and other neurotoxicants.

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References

1. Blanchard RJ, Blanchard DC, Agullana R, et al. (1991); *Physiology & Behavior*. 50: 967-972.
2. Brudzynski SM, (2013); *Neurobiology*. 23: 310-317.
3. Burgdorf J, Kroes RA, Moskal JR, Pfaus JG, et al. (2008); *Comparative Psychology*. 122: 357-367.
4. Ćurčić M, Janković S, Jaćević V, et al. (2012); *Arh Hig Rada Toksikol*. 63: 255–262.
5. Flood DEK, Fernandino JI, Langlois VS, (2013); *General and Comparative Endocrinology*. 192: 2-14.
6. Howdeshell KL, (2002); *Environmental Health Perspectives*. 110: 337–348.
7. Kim TH, Lee YJ, Lee E, Kim MS, et al. (2009); *Toxicology and Environmental Health, Part A*. 72: 1296–1303.
8. Lee E, Kim TH, Choi JS, et al. (2010); *Toxicological Sciences*. 35: 535–545.
9. Litvin Y, Blanchard DC, Blanchard RJ. (2007); *Behavioural Brain Research*. 182: 166-172.
10. Montoya ER, Terburg D, Bos PA, Honk JV, (2011); Motivation and Emotion. 36: 65-73.
11. Sarkar D, Chowdhury JP, Singh SK, (2016); *General and Comparative Endocrinology*. 239; 50–61.
12. Saekar D, Singh SK, (2018); *Toxicology letters*. 290: 83-96.
13. Schwarting RKW, Wöhr M, (2012); *Medical and Biological Research*. 45:337-348.
14. Wada H, Qi Y. (2017); *Organohalogen Compounds*. (in press)