Effects of Decabromodiphenyl Ether (BDE-209) on Ultrasonic Communication in Mating Behavior of Rats

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Introduction

Polybrominated diphenyl ethers (PBDEs) are commonly used as flame-retardants because they can render plastic and rubber goods nonflammable. However, PBDEs affect the thyroid hormone systems in animals [1, 3, 5, 8] as well as humans [19, 20]. Given that thyroid hormones are essential for the appropriate development of brain functioning, PBDEs may have developmental impacts on various brain functions in both animals and humans.

Recently, we introduced the concept of using ultrasonic vocalization (USV) of rats as a highly sensitive tool to study pup–dam relationships [15, 16]. Decabromodiphenyl ether (BDE-209), one of the PBDEs, was administered to pregnant rats and it was examined whether it affected the acoustic characteristics of USVs of the resultant pups [16]. The BDE-209-treated pups exhibited reductions of USV durations and percentages of frequency-modulated USVs compared with the control pups. USVs with shorter durations and lower frequency modulations are less attractive and noticeable to dams. The survival of pups may thus be threatened when acoustically altered USVs go unnoticed by nearby dams.

Adult rats also produce USVs as a mating behavior [2, 4, 9, 12, 18]. Male and female rats call by emitting 30–100 kHz USVs and then mate with each other. After ejaculation, male rats produce 20–30 kHz USVs and ignore the attentions of the female partner. USVs have communicative function in both pups and adult rats.

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

Materials and methods

Seven pregnant Wistar rats at gestational day (GD) 13 were purchased from Japan SLC Inc. (Hamamatsu, Japan). These rats were housed in individual cages 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 amount 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, on PND 4, the pups were culled to four males and four females per litter. All pups were weaned on PND 22, individually housed, and served as subjects for study in adulthood.

On PNDs 90–92, the adult rats were individually put into a plastic cage for 10 min/day for habituation. The size of the cage was 34 cm in length, 29 cm in width, and 18 cm in height. On PNDs 93–98, one male rat and one female rat from the same dose group but different litters were put into the cage and paired for 15 min/day. The numbers of pairs were as follows: control (n = 8), low-dose (n = 6), and high-dose pairs (n = 8). Female rats accept male rats every 4–5th day because of the estrous cycle of rats. Therefore, there was a mating opportunity of at least one day. The recording of USVs was performed daily in 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, with the microphone 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 throughout the mating period.

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. 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 of the experimental conditions complied with the Guide for the Care and Use of Laboratory Animals (Hokkaido University).

The collected USV data recordings were processed via automatic analyses in the Sonotrack system. USV data were statistically analyzed using the Kruskal–Wallis test. When a main effect was found to be significant, multiple pair-wise comparisons of groups were performed using Siegel and Castellan's method [14].

Results and discussion

Figure 1 exhibits the scattergrams of USVs in mating behavior. Each USV is plotted on the scattergram as a closed circle in two dimensions of frequency and duration. Striking differences were revealed in USV durations at frequencies between 20 and 40 kHz. Both the low-dose and control pairs emitted USVs with much longer durations than 400 ms, whereas the high-dose pairs never produced USVs with durations longer than 400 ms. The mean duration of total USVs was significantly different among the three dose pairs [$H = 6.071$, $p < 0.05$]. The high-dose pairs exhibited shorter durations than the control pairs [$p < 0.05$].

The USVs with longer durations were observed in frequencies between 20 and 40 kHz. The durations of USVs produced by the low-dose and control pairs reached approximately 1600 ms. The durations of USVs within this bandwidth were significantly different among the three dose pairs [$H = 5.893$, $p < 0.05$]. The high-dose pairs exhibited a reduction of USV durations compared with the control pairs [$p < 0.05$]. Furthermore, the percentage of USVs in frequencies between 20 and 40 kHz was also significantly different among the three dose pairs [$H = 6.941$, $p < 0.05$]. A decreased percentage of USVs within this bandwidth was obtained in the high-dose pairs compared with that in the control pairs [$p < 0.05$]. There were no significant differences in the number and frequency of total USVs among the three dose pairs.

Adult rats produce USVs in mating behavior [2, 4, 9, 12, 18]. A male rat produces 30–100 kHz USVs to a female rat. In response to these signals, the female rat also produces USVs with the same frequencies. They then pair and copulate, emitting 30–100 kHz USVs. These USVs are observed when rats encounter food, perform tickling and playing, or take addictive drugs [4, 9, 12, 17, 18]. The USVs in this frequency band are considered to reflect positive (P) emotionality. We thus name these USVs the type P call. In contrast, male rats produce 20–30 kHz USVs following ejaculation and refuse the female partner. The durations of these USVs are often over 300–3000 ms [13]. This type of USV is emitted when male rats are defeated by male conspecifics or encounter predators [4, 9, 12, 13, 18]. The USVs in frequencies between 20 and 30 kHz are considered to reflect negative (N) emotionality, such as anxiety, fear, or distress. We thus name these USVs the type N call.

Our low-dose and control pairs produced USVs in frequencies between 20 and 40 kHz. The USV bandwidth is slightly wider than that reflecting negative emotionality. However, the durations of USVs in the low-dose and control pairs reached 1600 ms nearby and have a characteristic of the type N call. This suggests that the low-dose and control pairs mated successfully and, consequently, the male rats produced type N calls after ejaculation and refused the female partner.

In contrast, type N calls completely disappeared in the high-dose pairs. One explanation for this is that the male rats were not able to emit type N calls. The generation of type N calls is regulated by vocalization-related central organs. Lesions of amygdala, hypothalamus, or thalamus are reported to decrease or abolish type N calls [13]. Moreover, 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. 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 [6]. Laryngeal muscles are particularly important to align USV durations in lower frequencies such as type N calls [10, 11]. The male rats in the high-dose pairs might be suffering from dysfunctions of type N call-related central organs or neural systems that regulate laryngeal muscles.

BDE-209 affects thyroid hormone systems and reduces triiodothyronine and thyroxine [5, 8]. Given that these thyroid hormones regulate neural development, hypothyroidism might induce dysfunctions of vocalization-related central organs and neural systems.

Another explanation is that the female rats in the high-dose pairs did not accept the male partner and, accordingly, the high-dose pairs resulted in unsuccessful mating. Given that BDE-209 is reported to reduce estradiol [8], the estrous cycles of the female rats might be disrupted.

Rats emit type N calls not only in mating behavior but also in fighting behavior. Defeated male rats produce type N calls that express subordinate to the superior rat and, consequently, the fighting ceases [7, 9, 13]. Furthermore, type N calls are emitted as a warning signal to draw the attention of conspecifics to a nearby predator [9, 13, 18]. This signal induces behavioral inhibition in conspecifics [13, 18]. Therefore, type N calls play crucial roles in social contexts. The lack of type N calls may thus be a threat to survival.

BDE-209 is manufactured and marketed in some countries and BDE-209 pollution has been spreading in the ecosystem. Our results reveal the impacts of BDE-209 on social behavior in animals. Legal regulation of the manufacturing and marketing of BDE-209 is thus required.

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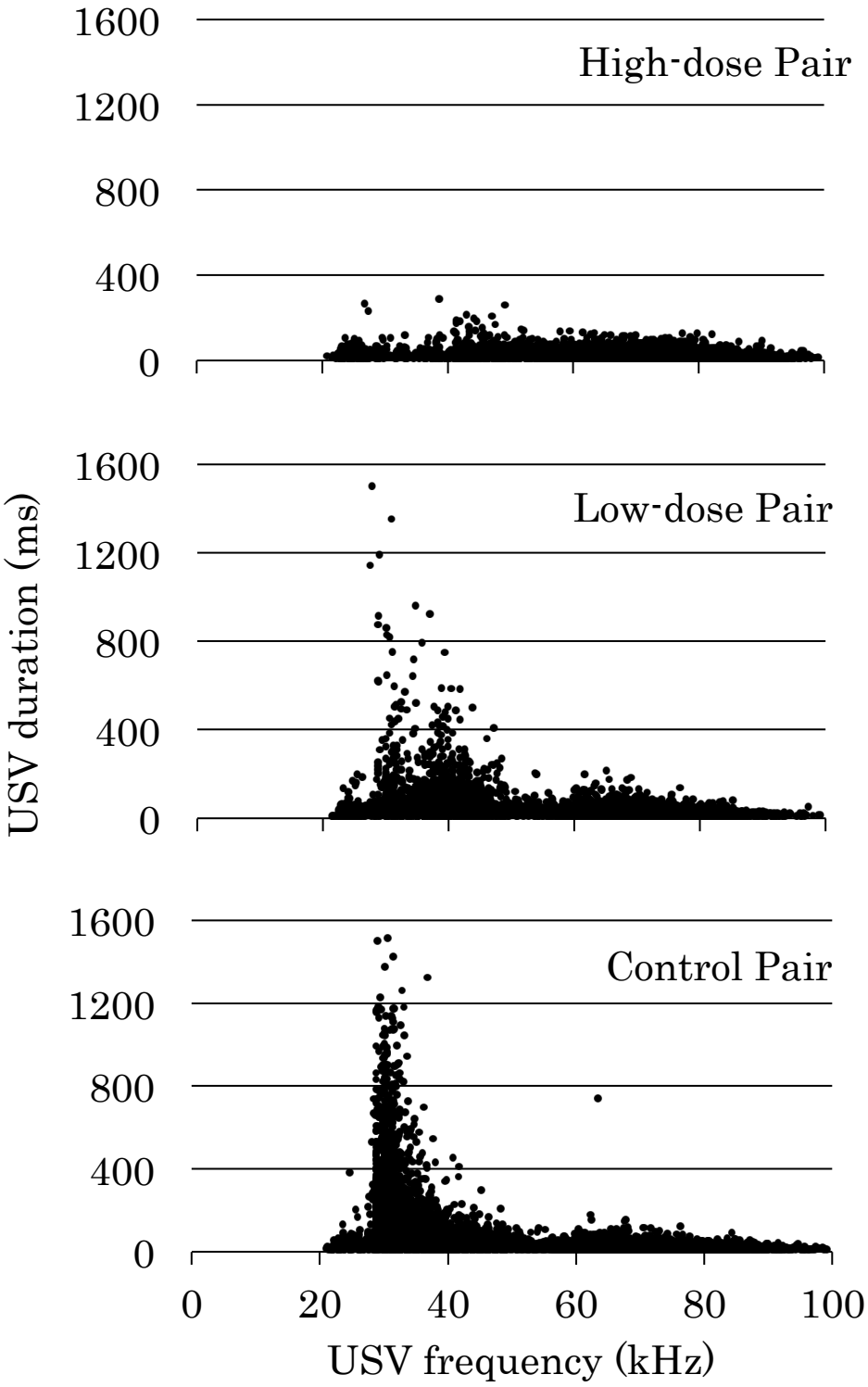
1600
1200
800
400
0
High-dose Pair
1600
1200
800
400
0
Low-dose Pair
1600
1200
800
400
0
Control Pair
USV duration (ms)
0
20
40
60
80
100
USV frequency (kHz)

Figure 1. The USV scattergrams of three dose pairs.
Each closed circle represents one USV in two dimensions of frequency and duration.