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The potential relationship between neurobehavioral toxicity and visual dysfunction of BDE-209 on zebrafish larvae: a pilot study

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Abstract

Background: Although listed in the Stockholm Convention, commercial Decabromodiphenyl ether (c-DecaBDE) is still being produced in many factories and used as a kind of flame retardants primarily in plastic polymers and textiles. Widespread use offered many exposure ways of its major ingredient, BDE-209, to humans and the environment. Most current studies of BDE-209 focused on the health effects and toxicity of thyroid disruption, oxidative stress, neurotoxicity, and reproductive function, but seldom spread light on the relationship between neurobehavioral toxicity and visual dysfunction. Using zebrafish larvae model, we hope to uncover the potential relationship between the neurobehavioral and visual effects after exposure to BDE-209.

Results: BDE-209 exposure could not induce the changes of locomotion and path angle in 5 days post fertilization (dpf) larvae; however, 5 μg/L BDE-209 exposure caused locomotor hyperactivity and more responsive turns at 7 dpf. The social activity of 50 μg/L exposure group was significantly higher than the control group at 6 dpf. Besides, 5 and 50 μg/L exposure caused the upregulation and downregulation of four cone opsin genes, respectively. The expression of rhodopsin gene was not influenced by both concentration exposures.

Conclusion: The neurobehavioral effects induced by 5 μg/L BDE-209 exposure were consistent with the upregulation of four cone opsins in 7 dpf larvae. The low concentration of BDE-209 exposure caused the hyperactivity and more responsive turns of larvae possibly contributing to the disruption on the cone opsin expressions of larvae. Our results would provide the mechanism cue of neurobehavioral toxicity after BDE-209 exposure and call for more attention on the ecotoxicology studies of BDE-209.

Keywords: BDE-209, Neurobehavior, Vision, Zebrafish

Background

Commercial Decabromodiphenyl ether (commercial mixture, c-decaBDE) was listed as a new member of persistent organic pollutants (POPs) under the Stockholm Convention in 2017 amendment because of its environmental persistence, high potential for bioaccumulation and biomagnification, long-distance transport capacity, and adverse effects; after commercial Pentabromodiphenyl ether (c-PentaBDE) and commercial Octabromodiphenyl ether (c-OctaBDE) have been banned in 2009 amendment (http://chm.pops.int/TheConvention/ThePOPs). However, c-decaBDE is still being produced in many factories and used as a kind of flame retardants primarily in plastic polymers and textiles [1]. Widespread use and slow degradation offered many exposure ways of BDE-209, the main constituent in c-decaBDE and

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also one of the predominant environmental pollutants in China, to humans and the environment [2, 3]. In sediments collected from Tai Lake and Dianshan Lake, two freshwater lakes in Yangtze delta area of China, BDE-209 was the most abundant congeners of poly brominated diphenyl ethers (PBDEs) accounting for 46% (1.3 ng/g dw) and 99% (5.8 ng/g dw), respectively [4]. One study detected seven kinds of PBDE congeners in both air and dust in the TV and the refrigerator recycling workshop, finding that the workers were the most substantially exposed to BDE-209, which accounted for more than 85% of the seven PBDE congeners [5]. In animal-based foodstuffs, BDE-209 was also recognized as the most abundant congener, comprising a proportion of at least 85% of total PBDEs [6].

Studies on the health effects and toxicity of BDE-209, the highest brominated PBDEs, were not commonly seen than of other congeners. Besides limited reports of acute toxicity on invertebrates [7], the researches focused on the effects of thyroid disruption, oxidative stress, and reproductive function. Thyroid disruption of rats was observed after exposure to BDE-209, including histological abnormities in structure and ultrastructure of the thyroid gland, oxidative damages of thyroid gland, and the changes of thyroglobulin contents [8]. One study showed that reproductive toxicity in peripubertal mice offspring was induced by BDE-209 during lactation exposure [9]. However, even 400 μg/L of BDE-209 did not show genotoxicity, while 50 μg/L BDE-47 affected the cell viability in mutant cells and BDE-99 and BDE-138 began to perform effects at 200 μg/L or higher [10]. Recently, the neurotoxicity of BDE-209 has also been getting more attention. Sun et al. showed that the learning and memory abilities of rats were damaged after BDE-209 exposure during pregnancy [11]. Similarly, another study revealed that a delayed learning in a spatial memory task and a reduction in anxiety levels were observed in young adult mice after BDE-209 exposure [12]. In a neurobehavioral study using zebrafish as the test model, the F0 growth and reproduction of zebrafish were found to be disrupted by a long-term parental exposure to low-dose BDE-209 (0.959–959 μg/L), and accompanied with the behavioral of F1 larvae showed slow locomotion in normal conditions, and hyperactivity under light–dark stimulus [13].

The perception of exogenous information plays a vital role in animal neurobehaviors, of which the visual system is recognized as a potential emerging target of environmental pollutants [14]. The normal visual function is the guarantee of normal neurobehavior for that early neurobehavioral dysfunction of organism was connected with the function change of visual system [15]. Zebrafish model possesses advantages in such studies [16]. Our previous study demonstrated that BDE-47 exposure (50 μg/L) impaired zebrafish vision development and further altered visually guided behaviors in larvae [17]. Chen et al. also showed that acute exposure of zebrafish larvae to DE-71 (commercial mixture, pentaBDE, 31 μg/L) caused biochemical and structural changes in the eye, resulting in optokinetic and phototactic behavioral alterations [18].

Considering the abundant environmental residues and limited toxicological data of BDE-209, the neurobehavioral effects and underlying mechanisms of BDE-209 are essential for the understanding and management of its health and ecological risks. Here using zebrafish larvae model, we demonstrated the neurobehavioral toxicity and visual dysfunction of BDE-209, finding that the neurobehavioral effects induced by low-dose BDE-209 exposure were consistent with the upregulation of four cone opsins in 7 days post fertilization (dpf) larvae. Based on the potential relationship between the two effects, our results would provide the mechanism cue of neurobehavioral toxicity after BDE-209 exposure and call for more attention on the ecotoxicology studies of BDE-209.

Methods and materials

Experimental model and BDE-209 exposure

Healthy embryos, collected from copulatory adult zebrafish (Tubingen strain) soon after light stimulation, were chosen for the following exposure from 3 to 5 h post fertilization (hpf) to 7 dpf. One control group and two BDE-209 exposure groups were employed in the assay. The vehicle of the control group was 10% Hanks' solution with 0.1% DMSO. BDE-209 stock solution of 5.0×10^3 mg/L was prepared by dissolving 5 mg BDE-209 (98.3%, AccuStandard, USA) in 1 mL DMSO completely and then diluted 1000 times to 5 mg/L using sterile water. The nominal concentration of two BDE-209 exposure groups was 5 μg/L and 50 μg/L, respectively with the same vehicle. The nominal concentrations were selected according to the previous study [13]. Every group had 50 embryos in a glass Petri dish with 30 mL exposure solution, which was half renewed every day in the exposure duration. All experiments were performed in triplicates.

BDE-209 analysis

The BDE-209 standard was purchased from AccuStandard. 5 mL exposure solution (1 and 3 dpf), collected from two treatment groups, was subjected to liquid–liquid extraction with 5 mL toluene (HPLC grade, Merck) for three repetitions. Then, the extracts were combined and dried under a gentle stream of nitrogen and reconstituted with 200 μL of toluene for the later analysis. The analysis was performed using a GC–MS with ECNI mode (Agilent, 7890A-5875C) [19]. A concentrated extract (1 μL) was injected onto the column (DB-5HT, 15 m × 0.25 mm,

0.1 μm). The initial temperature was 100 ℃ and then ramping at 30 ℃/min to 320 ℃ followed by isothermal elution at 320 ℃ for 6 min. The SSL injector temperature was 280 ℃ and the MS transfer line temperature was isothermal at 320 ℃. The carrier gas was helium at 1 mL/min.

Neurobehavioral test protocol

Neurobehavioral tests were performed on a ZebraBox platform (ViewPoint, France) according to our previous methods [20, 21], which can realize automatic tracking and high-throughput screening of larvae. Locomotion, path angle, and two-fish social activity of the same batch of exposed larvae were adopted to evaluate the neurobehavioral effects induced by BDE-209 at 5, 6, and 7 dpf, respectively, with light stimulus. The protocol of light stimulus included an initial 10 min of light adaption, followed by three repeated cycles with 10 min of dark period and 10 min of light period.

In the locomotion and path angle tests, larvae were transferred into a 48-well microplate from the glass Petri dish at 5 dpf. Each well accommodated one larva with 1 mL exposure solution and each group had 16 larvae. The system can record the swimming distance of larvae as the output of locomotion test. For path angle test, three angle classes, namely straight motion (−10° to 0°, 0° to +10°), average turn (−10° to −90°, +10° to +90°), and responsive turn (−180° to −90°, +90° to +180°), were defined in this test. The system can record the frequency of different classes during the whole test.

The two-fish social activity test was performed with 6-well microplates, where two larvae were placed in each well of the plate. A valid social contact was recorded by the system when the distance of two larvae was less than 0.5 cm. The system can record the frequency of valid contacts and the duration of each contact in the total test period.

Quantitative real-time PCR (qRT-PCR) analysis

At 7 dpf, the expression of five opsin-coding genes (*zfgr1*, *zfblue*, *zfuv*, *zfred*, and *rho*) after BDE-209 exposure was investigated using the qRT-PCR experiment. Trizol (Invitrogen, USA) was used to extract total RNA, and cDNA reverse transcription was performed according to the instructor of High Capacity cDNA Reverse Transcription Kits (ABI, USA). The threshold cycle values for selected genes and housekeeping β-actin were used to calculate the relative RNA amounts. The analysis was performed on a 7500 Real-Time PCR System (Applied Biosystems, USA). Fold changes of genes were defined as the ratio of RNA amounts in the treatments versus the control. More details including the sequences of primers are given in Additional file 1: Table S1.

Statistical analysis

Matlab R2019a (Mathworks, USA) was used to pre-process the raw data of larval behaviors from the Zebrabox system, and formed a processed file for statistical analysis of different behaviors. The $2^{-\Delta\Delta t}$ method was used for the relative quantification analysis of the qRT-PCR data. The software GraphPad Prism 7 was used for statistical analysis and graph drawing. The statistical differences between the treatment groups and the control group were analyzed using one-way ANOVA followed by Dunnett's test and the differences were considered significant when the p value was <0.05. Error bars in the figures represent the standard errors of mean (SEM).

Results and discussion

Concentration in the exposure solution

The measured concentrations of two BDE-209 groups in the exposure solution at 1 dpf and 3 dpf were much lower than the nominal values. At the nominal concentration 5 μg/L group, the measured concentrations at 1 dpf and 3 dpf of BDE-209 were 1.01 ± 0.003 and 1.49 ± 0.11 μg/L, respectively, while at the higher nominal group, the measured concentrations at 1 dpf and 3 dpf were 5.15 ± 2.13 and 19.50 ± 3.33 μg/L, respectively.

Locomotion test

The locomotion changes of 5, 6, and 7 dpf larvae after exposue to BDE-209 are shown in Fig. 1. Similarly with most reports, it could be found that locomotion during dark periods was significantly much more active than that during light periods. The swimming distance of two BDE-209 exposure groups at 5 dpf and 6 dpf had no significant differences with the control group. However, the distance of 7 dpf larvae exposed to 5 μg/L exposure group was longer than the control ($p<0.05$), especially in the first dark and last light periods (Fig. 1c).

Path angle test

For the tests of path angle and social activity, the data only in the dark periods were analyzed because the locomotion during the light periods was much less than the dark periods. The path angles of 5, 6, and 7 dpf zebrafish larvae after BDE-209 exposure were counted and shown in Fig. 2. The angles were divided into three classes as mentioned above. In the straight motion, larvae preferred to turn right rather than left. However, during the average and responsive turns, larvae did not show the distinct preference in the orientation. Consistent with locomotion results, the path angles of 5 dpf larvae in two BDE-209 exposure groups

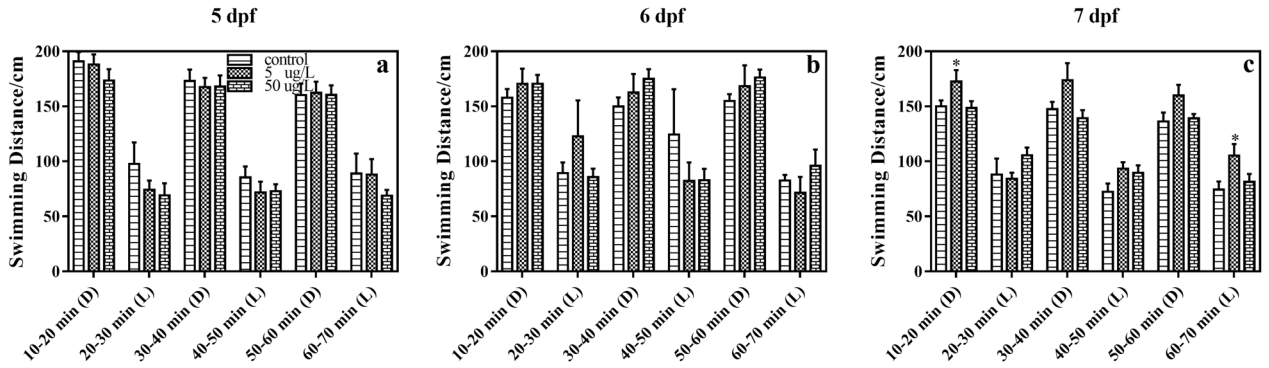

Fig. 1 Locomotion effects of BDE-209 exposure to zebrafish larvae under dark–light stimulus (**a**) 5 dpf; (**b**) 6 dpf; (**c**) 7 dpf. Data were expressed as mean ± SEM ($n = 16$). Significant difference between the control group and the exposure groups was indicated by an asterisk (*$p < 0.05$)

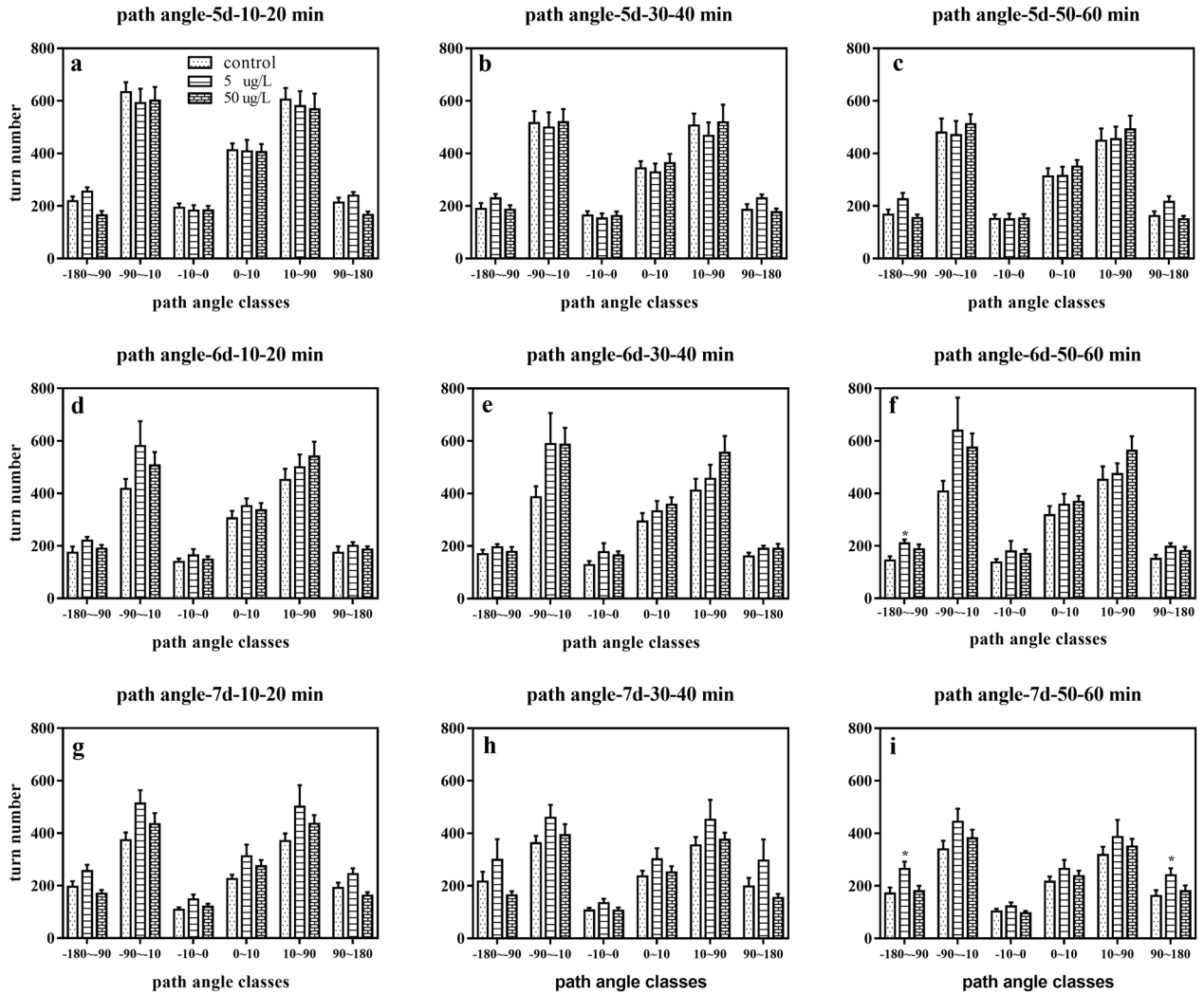

Fig. 2 Path angle effects of BDE-209 exposure to zebrafish larvae during dark period (**a**–**c**) 5 dpf; (**d**–**f**) 6 dpf; (**g**–**i**) 7 dpf. Data was expressed as mean ± SEM ($n = 16$). Significant difference between the control group and the exposure groups was indicated by an asterisk (*$p < 0.05$)

had no significant differences with the control group. The numbers of responsive turns of 6 and 7 dpf larvae in 5 μg/L exposure group were significantly more than the control group at the last dark period (Fig. 2f, i). Straight motion and average turns at 7 dpf also showed the same tendency but had no significant differences.

Two-fish social activity test

Two-fish social activity in the dark periods of 5, 6, and 7 dpf larvae after BDE-209 exposure is shown in Fig. 3. The duration per contact between two larvae was adopted as the indicator of analysis. At 5 dpf, social activity of the first dark period was lower than the subsequent two dark periods; however, this tendency was not observed at 6 and 7 dpf. The effects on social activity of 6 dpf larvae increased with the exposure concentration increasing at the last two dark periods, in which the activity of 50 μg/L exposure group was significantly higher than the control group.

Gene expression test

The functions of visual opsins, which lie in the photoreceptor layer of retina, are associated with photosensitivity and circadian rhythm of animals. The gene expression of four cone opsins and rhodopsin (*rho*) was investigated in the present study. The four cone opsins are in charge of green, red, blue, and ultraviolet light perception (*zfgr1*, *zfred*, *zfblue*, and *zfuv*). The influence of BDE-209 exposure on the expressions of larval opsin genes is shown in Fig. 4. The results showed a nonlinear concentration-dependent effect of BDE-209 exposure in the expression of four cone opsins, in which 5 μg/L BDE-209 induced the upregulation and 50 μg/L BDE-209 induced the downregulation for all genes. However, the gene expression of rod opsin (*rho*) was not changed in both exposure groups.

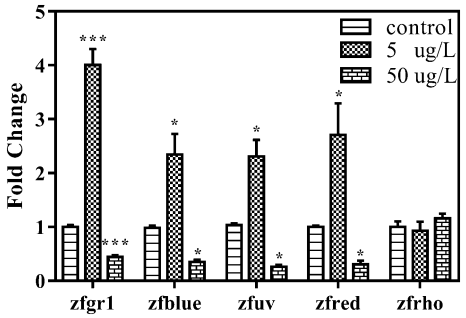

Fig. 4 Gene expression effects of BDE-209 exposure to zebrafish larvae at 7 dpf. Data were expressed as mean ± SEM ($n = 3$). Significant difference between the control group and the exposure groups was indicated by an asterisk (*$p < 0.05$) or three asterisks (***$p < 0.001$)

Discussion

The potentials of zebrafish model in studying neurobehavioral effects of environmental pollutants have been uncovering [22, 23]. Here, the classical intermittent light stimulus to larvae was adopted to study the potential relationship between the neurobehavioral toxicity and visual dysfunction induced by BDE-209, in which larvae performed a pattern of hyperactivity during the dark period followed by quiescent state during the light period [24]. From the analysis data, we can see that the determined concentrations of the low dose group were 20.2% and 30.0% of the nominal 5 μg/L group at 1 dpf and 3 dpf, respectively. For the high dose group, the measured concentrations were 10.3% and 39.0% of the nominal 50 μg/L group at 1 dpf and 3 dpf, respectively. The results were consistent with a previous study, which showed at the nominal concentrations of 0.2 and 0.6 mg/L groups, the measured concentrations of BDE-209 were 27.0% and 13.8% nominal concentrations, respectively [25].

The sudden dark stimulus is a signal to zebrafish larvae, which may motivate the larval instinct of shelter seeking.

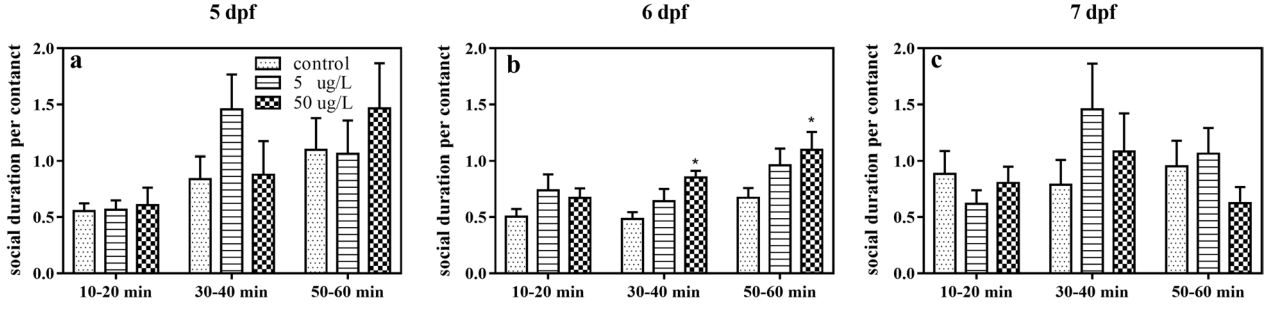

Fig. 3 The effects of BDE-209 exposure on social activity of zebrafish larvae during dark period. **a** 5 dpf; **b** 6 dpf; **c** 7 dpf. Data were expressed as mean ± SEM ($n = 6$). Significant difference between the control group and the exposure groups was indicated by an asterisk (*$p < 0.05$)

The driving force behind such response is dependent on the health of the visual system. The results in locomotion showed that 5 μg/L BDE-209 exposure caused hyperactivity of larvae compared with the control at 7 dpf. Path angle means the turning direction in the moving direction of larvae [26]. The results in path angle test also revealed 5 μg/L BDE-209 exposure caused more responsive turns at 7 dpf. Consistent with these neurobehavioral results, 5 μg/L of BDE-209 exposure was found to induce the upregulation of gene expression of four cone opsins at 7 dpf. Therefore, it was possible that low concentration of BDE-209 exposure induced the hyperactivity and more responsive turns by disrupting the light perception of larvae. Though there were limited evidence to confirm the light perception effects of BDE-209, our previous study demonstrated that BDE-47 exposure significantly inhibited the expressions of *zfrho*, *zfblue,* and *zfgr* and damaged the retina [17], while another study showed DE-71 exposure significantly upregulated the expressions of *zfrho* and *zfgr1*, but had no effects on the expressions of *zfred*, *zfblue*, and *zfuv* [18]. It can be seen that different PBDE congeners obviously led to the different effects on the different opsin genes. However, a recent study found that just BDE-47 could damage the structure of eyes but not BDE-209 [27]. To sum up, the visual system is still a potential target organ of PBDEs congeners, which is worthy of more attention in future studies.

Another interesting result is that only the exposure of low-concentration BDE-209 induced the changes in locomotion and path angle tests at 7 dpf other than the higher concentration exposure. However, it was reported that BDE-209 at low doses was not an acute lethal toxicant and may just pose a low risk to marine rotifers [25]. Besides the potential influences of different tested species, the difference was possibly due to the fact that BDE-209 is easy to be bioaccumulated in sediment and organisms, which could cause a tenfold accumulation in zebrafish larvae in unspiked sediment after 8 days [28]. Some mechanism studies indicated that the toxicity of BDE-209 was through the debromination effects to more toxic lower PBDEs' congeners [29]. By far most studies used to contribute neurobehavioral changes induced by pollutants to the primary effects in thyroid function [30, 31]. The hypothalamic–pituitary–thyroid axis can be evaluated to determine thyroid endocrine disruption by BDE-209 in developing zebrafish larvae [32]. However, one study found that BDE-209 impacted the expression of neurological pathways and altered behavior of zebrafish larvae, but had no explicit effects on thyroid function, motoneuron, and neuromast development [28]. Therefore, the consistent effects of neurobehavior and gene expression in our results may offer a new insight into the mechanism of neurobehavioral effects of BDE-209. The supporting evidence is from a study using apolipoprotein E (apoE2) transgenic mice model, in which high dose of BDE-209 exposure retarded the eye opening of mice without affecting other developmental features [33].

Zebrafish is a highly social species, and has the shoaling behavior promoting individuals to form tight groups [34]. Two-fish social activity offered the information of contact distance and duration between two larvae, which could be regarded as the basis of the shoaling behavior. The two-fish test actually requires the larva to sense the other larva in similar age and size, and this process is mainly dependent on vision [35]. Our results showed 50 μg/L BDE-209 exposure induced more active social activity at 6 dpf, indicating high-concentration BDE-209 group may affect the social activity. One study showed the relationship between social activity and thigmotaxis, a valid index of anxiety, in which the isolated zebrafish exhibited the reduced thigmotaxis and appeared to have obvious anxiolytic effects [36]. In our study, the two-fish social activity could also offer the information of anxiety to some degree, which remains to be improved in the future study.

Recently, neurobehavioral test of environmental pollutants was suggested to be integrated in Adverse Outcome Pathways (AOPs) [37]. There are at least two difficulties in the process. One is that how to interpret subtle changes in behavior in terms of population demographics. The second is that neurobehavioral effects induced by pollutants are difficult for cross-species extrapolations [37, 38]. Zebrafish larvae may become one potential model animal to come true embedding behavior into an AOP [39, 40]. In our study, the associations between measurable disruptions of gene expression and neurobehavioral changes of BDE-209 can be a key event, though it is far away from a whole AOP frame. There were also many examples offering the possibilities of involving the neurobehavioral studies into an AOP of PBDEs, such as locomotion behavioral changes of zebrafish induced by BDE-47 through inhibiting dopaminergic function [41], feeding activity of Daphnia magna induced by BDE-47 and its two derivatives [42], oxidative and nitrosative stress in the neurotoxicity of BDE-153 [43]. Meantime, some studies focused on the molecular mechanism of PBDEs toxicity [44] and long-term assessment associated with multimedia exposure [45], which are also important key events of an AOP. In addition, developing high-throughput behavior analysis methods may help interpret behavior changes in terms of population demographics, which is also one aim of our future study.

Conclusion

In general, zebrafish larvae model was used herein to investigate the potential relationship between neurobehavioral toxicity and visual dysfunction after exposure to BDE-209. Overall, BDE-209 exposure could not induce the changes of locomotion and path angle in 5 dpf larvae, but 5 μg/L BDE-209 exposure caused locomotor hyperactivity and more responsive turns at 7 dpf. Meantime, 5 and 50 μg/L exposure caused the upregulation and downregulation of four cone opsin genes, respectively. In addition, the social activity of 50 μg/L exposure group was significantly higher than the control group at 6 dpf. Our results showed that the neurobehavioral effects of 5 μg/L BDE-209 exposure were consistent with the upregulation of four cone opsins at 7 dpf, providing a new mechanism cue of neurobehavioral toxicity after exposure to BDE-209 and calling for more attention on the future ecotoxicology studies of BDE-209.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12302-020-00308-7.

Additional file 1: Table S1. Primer sequences used in qRT-PCR assay.

Abbreviations

c-DecaBDE: Commercial Decabromodiphenyl ether; POPs: Persistent organic pollutants; c-PentaBDE: Commercial Pentabromodiphenyl ether; c-OctaBDE: Commercial Octabromodiphenyl ether; PBDEs: Poly brominated diphenyl ethers; hpf: Hours post fertilization; dpf: Days post fertilization; qRT-PCR: Quantitative Real-time PCR; AOPs: Adverse Outcome Pathways.

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Authors' contributions

BZ and TX were involved in the experiments and manuscript writing. BZ and WS were involved in the preprocessing of the samples and the data analysis. BZ, DY, and TX designed the study. TX and DY contributed to correction of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study is in accordance with guidelines approved by the Animal Ethics Committee of Tongji University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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