Neurotoxicology and Teratology 53 (2016) 1–10

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

journal homepage: www.elsevier.com/locate/neutera

Effects of embryonic exposure to polychlorinated biphenyls (PCBs) on larval zebrafish behavior

Ava K. Lovato [a], Robbert Creton [b], Ruth M. Colwill [a,*]

[a] Department of Cognitive, Linguistic & Psychological Sciences, Brown University, Providence, Rhode Island, United States
[b] Department of Molecular Biology, Cell Biology and Biochemistry, Brown University, Providence, Rhode Island, United States

ARTICLE INFO

Article history:
Received 1 August 2015
Received in revised form 8 November 2015
Accepted 9 November 2015
Available online 10 November 2015

Keywords:
Avoidance behavior
Freezing
Larvae
Polychlorinated biphenyls
Thigmotaxis
Zebrafish

ABSTRACT

Developmental disorders such as anxiety, autism, and attention deficit hyperactivity disorders have been linked to exposure to polychlorinated biphenyls (PCBs), a ubiquitous anthropogenic pollutant. The zebrafish is widely recognized as an excellent model system for assessing the effects of toxicant exposure on behavior and neurodevelopment. In the present study, we examined the effect of sub-chronic embryonic exposure to the PCB mixture, Aroclor (A) 1254 on anxiety-related behaviors in zebrafish larvae at 7 days post-fertilization (dpf). We found that exposure to low concentrations of A1254, from 2 to 26 h post-fertilization (hpf) induced specific behavioral defects in two assays. In one assay with intermittent presentations of a moving visual stimulus, 5 ppm and 10 ppm PCB-exposed larvae displayed decreased avoidance behavior but no significant differences in thigmotaxis or freezing relative to controls. In the other assay with intermittent presentations of a moving visual stimulus and a stationary visual stimulus, 5 ppm and 10 ppm PCB-exposed larvae had elevated baseline levels of thigmotaxis but no significant differences in avoidance behavior relative to controls. The 5 ppm larvae also displayed higher terminal levels of freezing relative to controls. Collectively, our results show that exposure to ecologically valid PCB concentrations during embryonic development can induce functional deficits and alter behavioral responses to a visual threat.

1. Introduction

Polychlorinated biphenyls (PCBs) are lipophilic, chemically stable, chlorinated compounds or congeners that persist in the environment and bioaccumulate in humans and other animals (Herrick et al., 2007; Li et al., 2009; Ling et al., 2008; Safe, 1994; Tiernan et al., 1985). As a result, PCB levels are frequently found at substantially higher concentrations in fish and mammals than in the external environment. Humans are predominantly exposed to PCBs through ingestion of contaminated food, although recent work has identified inhalation of PCB-contaminated dust from deteriorating building products as another exposure risk (Herrick et al., 2007). PCBs remain in the body for prolonged periods of time, providing a means of exposure long after ingestion of the PCB contaminants.

Exposure to PCBs may also occur during the embryological period through maternal consumption of contaminated food. This kind of neurotoxic insult may lead to irreversible alterations of the developing fetal brain. Several large scale epidemiological studies suggest that PCBs can alter endocrine, immune and nervous system function, particularly during embryological and early post-natal growth when rapid changes are taking place (Aoki, 2001; Feeley and Brouwer, 2000; Gladen and Rogan, 1991; Gladen et al., 1988; Huisman et al., 1995a, 1995b; Jacobson et al., 1990; Koopman-Esseboom et al., 1996). Furthermore, embryonic exposure to PCBs can produce detrimental effects on neurocognitive function in children from infancy to adolescence (Darvill et al., 2000; Grandjean and Landrigan, 2006; Jacobson and Jacobson, 1996; Winneke, 2011). Mood disorders such as depression and anxiety have also been documented in humans exposed to PCBs as a result of environmental pollution (Fitzgerald et al., 2008; Plusquellec et al., 2010; Winneke, 2011).

Animal studies of perinatal PCB exposure have found many of the same effects reported in humans (Tilson et al., 1990). These effects, largely documented in rodents, include spatial learning deficits, hyperactivity, impaired learning and an increase in anxiety-related behaviors including increased thigmotaxis and social avoidance (Corey et al., 1996; Eriksson and Fredriksson, 1996; Rice, 1999; Rice and Hayward, 1997; Schantz et al., 1995; Sugawara et al., 2006). For example, Orito et al. (2007) fed pregnant rats PCB 126 at a dosage of 30 μg/kg in corn oil on gestational day 15. When the offspring were tested at 4–5 weeks of age, they exhibited a significantly greater preference for the edge of an open field than control pups whose mothers were given only the vehicle, corn oil. Further, when placed in an environment with a novel partner, the PCB-exposed pups displayed significantly less time engaged in social interaction than vehicle controls.

The zebrafish has emerged as an excellent model system for large scale studies of vertebrate neurodevelopment and behavior (Ali et al.,

* Corresponding author at: Department of Cognitive, Linguistic & Psychological Sciences, Brown University, Box 1821, Providence, RI 02912, Rhode Island, United States.
E-mail address: ruth_colwill@brown.edu (R.M. Colwill).

http://dx.doi.org/10.1016/j.ntt.2015.11.002
0892-0362/

2011; Bailey et al., 2013; Bruni et al., 2014; Colwill and Creton, 2011a; He et al., 2014; Kalueff et al., 2014). Zebrafish embryos develop externally, are nearly transparent, and are accessible to genetic, chemical and experimental manipulations. Their development is also extremely rapid. Larvae hatch from their chorion at 2–3 days post fertilization (dpf) and acquire a complex repertoire of swimming, hunting, escape, and avoidance behaviors within the first week of development (Colwill and Creton, 2011b; Fero et al., 2011; Spence et al., 2008; Wolman and Granato, 2012). We have recently developed a novel automated imaging system for monitoring developing larvae in multiwell plates and a behavioral assay to assess avoidance behavior in 7 dpf zebrafish larvae to a moving visual stimulus (Colwill and Creton, 2010; Creton, 2009; Pelkowski et al., 2011). Consistent with our view that this stimulus simulates predatory movement and thus constitutes a potential threat, we have found that larvae swim away from the stimulus (avoidance) towards the edge of the well (thigmotaxis) (Pelkowski et al., 2011). We have also confirmed that these behavioral indices of anxiety are appropriately modulated by anxiolytic and anxiogenic pharmaceuticals (Richendrfer et al., 2012).

In the present study, we investigated the effects of exposure to the commercial PCB mixture, Aroclor (A) 1254, during early-stage embryonic development (2 to 26 h post-fertilization, hpf) on visual avoidance behavior in zebrafish larvae at 7 dpf. A1254 is suitable for testing PCB exposure effects in a new model system for several reasons. Exposure to PCB mixtures, not isolated congeners, is common in the environment (Kreiling et al., 2007; Lee et al., 2012); the congener profile of A1254 is similar to that found in human tissues (Angulo et al., 1999; Hansen, 1999; Kodavanti et al., 2011; Yang and Lein, 2010); and A1254 has been used extensively in research studies providing a wealth of information on PCB-exposure effects and mechanisms. To examine visual avoidance behavior, larvae were tested with 5-min periods of no visual stimulus alternating with 5-min periods of either a red 'bouncing' disk (BD assay) or a red 'bouncing' disk and a red stationary disk on the side opposite to the 'bouncing' disk (BD/SD assay) for two hours. We measured avoidance of the moving disk, preference for the edge of the well, and immobility (freezing). Embryonic exposure to 5 and 10 parts-per-million (ppm) but not 2 ppm of A1254 induced specific behavioral defects including reduced avoidance behavior on the BD assay and increased thigmotaxis on the BD/SD assay. These results are consistent with the literature suggesting that PCB-exposure in early development disrupts cognitive processing and increases anxiety-related behaviors.

2. Materials and methods

2.1. Embryo collection

Embryos were collected from a breeding population of adult male and female wild type zebrafish originally obtained from Carolina Biological Supply Co. (Burlington, NC) and maintained at Brown University as a genetically diverse outbred strain. Breeders were housed in 20 gal tanks and maintained on a 14 h light/10 h dark cycle. They were fed a combination of frozen or fresh brine shrimp and flake fish food once or twice per day. Embryos were collected for two hours following light onset in shallow trays placed in the bottom of the tanks.

All protocols involving animals were approved by the Institutional Animal Care and Use Committee of Brown University (Providence, RI) prior to the initiation of experimentation.

2.2. PCB exposure

A stock solution of 50 mg/ml of Aroclor (A) 1254 (Ultra Scientific, Kingston, RI) in dimethyl sulfoxide (DMSO) was diluted to final concentrations of 2, 5, and 10 ppm in egg water (60 mg/l Instant Ocean in deionized water and 0.25 mg/l methylene blue). A1254 is a commercial PCB mixture consisting mainly of non-coplanar, ortho-substituted congeners. Embryos at the 4 cell stage with intact chorions were statically exposed for 24 h at a density of approximately 25 embryos per 50 mL petri dish (Corning no. 430591) and incubated at 28.5 °C. The PCB concentrations selected for study have been shown to be environmentally relevant (Li et al., 2009; Wickizer et al., 1981). Egg water (EW) and DMSO at a final concentration of 0.1% in EW were used as the two controls.

Following treatment exposures, all embryos were transferred to microfuge tubes and triple-rinsed in EW. They were then placed in 1 L plastic breeding tanks (Aquatic Habitats) containing approximately 500 mL of EW and incubated at 28.5 °C until they reached 7 dpf. During this period, any dead larvae and other particles were removed and EW in each tank was replaced to maintain water quality. The developing larvae were examined for pericardial and yolk sac edemas, curved body axis and sidewise position. Any larvae exhibiting these visible malformations were not used in the behavioral assays. We observed no PCB-related delays in swim bladder inflation or increases in mortality. Food supplements were not provided because developing zebrafish larvae absorb nutrition from their yolk sac through 7 dpf (Jardine and Litvak, 2003).

2.3. Image collection

Details of the imaging system have been described previously (Pelkowski et al., 2011). Flat-bottom 6-well plates (Corning Costar no. 3506) were prepared for behavioral testing by filling each well with 5 ml of agarose (0.5% *w/v* in deionized water). Agarose was allowed to set and then a center portion was punched out to create a 27 mm diameter × 5 mm deep swimming area surrounded by an agarose ring. Four 6-well plates with one larva per well were placed on the LCD screen (1366 × 768 pixel resolution and a brightness of 220 cd/m^2) of an inverted laptop on the bottom shelf of a tall cabinet. A plastic diffuser (Pendaflex 52345) was placed between the multiwell plates and the screen to avoid moiré patterns. Larvae were imaged from above by a camera (Canon EOS Rebel T1i digital camera with a Canon EF-S 55-250 mm f/4.0–5.6 IS Zoom Lens) using Canon's remote capture software. Acquired images were compressed as 0.6 MB JPEGs and stored on a standard desktop computer (Dell OptiPlex).

2.4. Behavioral testing

Larvae were tested at 7 dpf with alternating 5 min periods of a white background with no visual stimuli (Fig. 1A) and 5 min periods of either a red 'bouncing' disk (BD assay, Fig. 1B) or a red 'bouncing' disk and a red stationary disk (BD/SD assay, Fig. 1C). Images were captured every 6 s for 125 min. The visual stimuli were created in Microsoft PowerPoint. For the BD and BD/SD assays, a red disk (RGB values were 255, 0, 0) with a 1.35 cm diameter was programmed to travel back and forth in a straight line across the upper half of each well at a rate of 1.65 cm/s. For the BD/SD assay, a red stationary disk also appeared in the lower half of each well throughout the time that the 'bouncing' disk was present in the upper half. Over three replications, a total of thirty larvae per group were tested using the BD assay; forty-eight larvae per group were tested using the BD/SD assay. Data from one of the 48 EW controls were discarded from analysis because the larva did not move during behavioral testing.

2.5. Image analysis and quantification of behavior

Images were imported into ImageJ (http://rsb.info.nih.gov/ij/index.html) and an automatic macro was used to split color channels to remove the red disk(s), subtract the background, apply a threshold, and identify larvae on the basis of particle size. The X,Y coordinates of each larva's centroid obtained from Image J were imported into a customized Microsoft Excel template and compared to the X,Y coordinates of the midpoint of the larva's well to determine if the larva was (1) on the