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Effects of embryonic exposure to polychlorinated biphenyls (PCBs) on anxiety-related behaviors in larval zebrafish

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ABSTRACT

The zebrafish (*Danio rerio*) is an excellent model system for assessing the effects of toxicant exposure on behavior and neurodevelopment. In the present study, we examined the effects of sub-chronic embryonic exposure to polychlorinated biphenyls (PCBs), a ubiquitous anthropogenic pollutant, on anxiety-related behaviors. We found that exposure to the PCB mixture, Aroclor (A) 1254, from 2 to 26 h post-fertilization (hpf) induced two statistically significant behavioral defects in larvae at 7 days post-fertilization (dpf). First, during 135 min of free swimming, larvae that had been exposed to 2 ppm, 5 ppm or 10 ppm A1254 exhibited enhanced thigmotaxis (edge preference) relative to control larvae. Second, during the immediately ensuing 15-min visual startle assay, the 5 ppm and 10 ppm PCB-exposed larvae reacted differently to a visual threat, a red 'bouncing' disk, relative to control larvae. These results are consistent with the anxiogenic and attention-disrupting effects of PCB exposure documented in children, monkeys and rodents and merit further investigation.

1. Introduction

Polychlorinated biphenyls (PCBs) are ubiquitous environmental pollutants found in soil, sediment and air as well as in marine and freshwater ecosystems (Alava et al., 2012; Li et al., 2009; Safe, 1994; Tanabe, 1985). Although their production has been banned in the United States for more than three decades and more globally since 2001, PCBs have a very long half-life, are extremely lipophilic and are poorly metabolized leading to their bioaccumulation in animal and human tissues and their biomagnification up the food chain (Jensen, 1987; Porte and Albaigés, 1994; Van Oostdam et al., 2005). High levels of PCBs have been found in the fat deposits of humans and marine mammals with diets consisting primarily of fish and fish-eating mammals (Bernhoft et al., 1997; Dewailly et al., 1993; Hickie et al., 2007; Mos et al., 2010; Ross et al., 2004; Wolkers et al., 2007). Dewailly et al. (1996) found PCB levels in breast milk of 2.9 mg/kg for women in Arctic Quebec, five times higher than for southern Quebec women.Wickizer et al. (1981) reported PCB levels ranging from trace amounts to 5.1 parts-per-million (ppm) in the breast milk of nursing mothers residing in Michigan. Ross et al. (2000) found PCB loads in excess of 250 ppm in male transient orcas feeding on seals.

Maternal consumption of PCB-contaminated fish is a major route of human fetal and neonatal exposure as PCBs are readily transferred to the fetus through the placenta and to the nursing infant in breast milk (Jacobson et al., 1984). The detrimental effects of such exposures have been well documented (Boucher et al., 2009; Darvill et al., 2000; Faroon and Ruiz, 2015; Feeley and Brouwer, 2000; Jacobson et al., 1985; Ribas-Fitó et al., 2001; Rogan et al., 1986; Schantz et al., 2003; Stewart et al., 2000; Verner et al., 2010; Winneke, 2011). Prenatal PCB exposure has been correlated with poor habituation scores, abnormal reflexes, psychomotor deficits, hypotonia, and changes in autonomic function (Huisman et al., 1995a,b; Koopman-Esseboom et al., 1996; Sagiv et al., 2008; Stewart et al., 2000). Cognitive effects have been shown to persist past infancy and into childhood (Grandjean and Landrigan, 2006; Jacobson and Jacobson, 1996; Vreugdenhil et al., 2002) with several studies reporting links between PCB exposure and attention problems, hyperactivity, and impulsivity (Boucher et al., 2012; Jacobson and Jacobson, 1996, 1997; Patandin et al., 1999; Sagiv et al., 2010; Stewart et al., 2000; Verner et al., 2015).

Research in nonhuman animals exposed perinatally to PCBs has produced similar results (Eubig et al., 2010; Tilson et al., 1990). Monkey and rodent studies have demonstrated PCB-induced hyperactivity, perseverative behavior, impaired learning and elevated anxiety-related behavior including increased thigmotaxis

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and social avoidance (Corey et al., 1996; Eriksson and Fredriksson, 1996; Levin et al., 1988; Orito et al., 2007; Rice, 1999; Rice and Hayward, 1997; Schantz et al., 1989, 1991, 1995; Sugawara et al., 2006). Results from some studies (e.g., Rice, 2000) are in agreement with evidence that exposure to PCBs may contribute to attention deficit hyperactivity disorder (ADHD). These findings from human and animal studies underscore the importance of understanding the effect of embryonic exposure to PCBs on neurodevelopment and behavior.

The zebrafish (*Danio rerio*) is an excellent model system for studying toxicological effects on behavior (Bailey et al., 2013; de Esch et al., 2012; He et al., 2014; Liu et al., 2012; Padilla et al., 2012; Reif et al., 2015; Richendrfer et al., 2014; Sipes et al., 2011; Sloman and McNeil, 2012; Truong et al., 2014). Eggs are fertilized externally and in large numbers, providing an opportunity for immediate large-scale embryonic exposure to toxicants in multiple concentrations and mixtures. Early development is rapid, synchronous and well-characterized (Kimmel et al., 1995). Larvae hatch from the chorion at 2–3 days post fertilization (dpf) and are free swimming following swim bladder inflation at 4–5 dpf. Within a week of fertilization, zebrafish larvae exhibit a range of locomotor, foraging, predatory and defensive behaviors (Budick and O'Malley, 2000; Colwill and Creton, 2011b; Spence et al., 2008; Wolman and Granato, 2012). Moreover, larvae at this stage are still small enough to be imaged in multiwell plates. The development of high-throughput automated imaging systems has facilitated rapid screening of the behavior of large numbers of subjects to capitalize on these attributes of the developing zebrafish (Berghmans et al., 2007; Bruni et al., 2014; Delvecchio et al., 2011; Gerlai, 2010; Kokel et al., 2010; Lessman, 2011; Mathias et al., 2012; Muto et al., 2005; Raldúa and Piña, 2014; Rihel and Schier, 2012; Rihel et al., 2010).

Although the developing zebrafish has been used extensively to examine the effects of pesticides and pharmaceuticals on neurodevelopment and behavior, relatively few studies have taken advantage of this model system to examine PCB exposure effects. Chronic exposure to the commercial PCB mixture Aroclor (A) 1254 and to individual congeners may induce significant developmental defects including cardiac, craniofacial, skeletal and retinal abnormalities in zebrafish embryos and larvae (Billsson et al., 1998; Grimes et al., 2008; Ju et al., 2012; Li et al., 2014; Olsson et al., 1999; Orn et al., 1998; Şişman et al., 2007; Wang et al., 2012). Continuous exposure to A1254 from immediately after fertilization until testing at 7 dpf has also been found to disrupt performance on an optomotor assay consistent with impaired visual acuity (Zhang et al., 2015). Finally, offspring of adult zebrafish that had been exposed to a PCB mixture of 13 congeners for 8 months were found to be hyperactive at 5 dpf (Péan et al., 2013).

Recently, our laboratory reported that exposure to 5 ppm and 10 ppm of A1254 during early-stage embryonic development (2–26 h post-fertilization, hpf) altered anxiety-related behaviors in 7 dpf zebrafish larvae on a visual avoidance assay (Lovato et al., 2016). Briefly, in one experiment, the PCB-exposed larvae were less likely than the control larvae to avoid a red 'bouncing' disk, a stimulus that is perceived as a visual threat (Pelkowski et al., 2011), that was presented intermittently for two h. In a second experiment, the PCB-exposed larvae displayed a stronger edge preference (thigmotaxis), a commonly used index of anxiety (Ahmad and Richardson, 2013; Champagne et al., 2010; Colwill and Creton, 2011a; Maximino et al., 2010; Schnörr et al., 2011). In the present study, we modified our visual avoidance assay to examine the effects of early embryonic PCB exposure on free swimming (phase 1) and on visual startle behavior (phase 2) in 7 dpf zebrafish larvae. Our goals were to explore further the effects of PCB exposure on anxiety-related behaviors and to devise a visual startle assay suitable for video recording and more precise measurement of PCB exposure effects on avoidance behavior.

2. Materials and methods

2.1. Embryo collection

Details of our breeding population of adult male and female wild type zebrafish, their housing and maintenance schedule, and our protocol for embryo collection were the same as those described in Lovato et al. (2016). Briefly, mixed sex adults were housed in 20 gallon 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 ninety minutes 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

Details of our PCB exposure and embryo rearing protocols were identical to those described in Lovato et al. (2016). Briefly, individual stock solutions of 2, 5 and 10 mg/ml of Aroclor (A) 1254 (Ultra Scientific, Kingston, RI) in dimethyl sulfoxide (DMSO) were diluted (matching the 0.1% DMSO control) 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). 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. Egg water (EW) and DMSO in EW at a final concentration of 0.1% were used as the two controls. A1254 is a commercial PCB mixture consisting mainly of non-coplanar, ortho-substituted congeners. Its congener profile is similar to the congeners found in human tissues (Angulo et al., 1999; Hansen, 1999; Kodavanti et al., 2011; Yang and Lein, 2010) indicating its suitability for testing PCB exposure effects in a model system.

Following treatment exposures, all embryos were transferred to microfuge tubes and triple-rinsed in egg water. They were then placed in 1 L plastic breeding tanks (Aquatic Habitats) containing approximately 500 mL of egg water and incubated at 28.5 °C until they reached 7 dpf. During this period, any dead larvae and particulates were removed and egg water in each tank was replaced to maintain water quality. The developing larvae were examined for malformations such as pericardial and yolk sac edemas, curved body axis and sidewise position. Larvae exhibiting such defects were not used for behavior assessment. 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 and preparation of the 6-well plates with agarose rings (Corning Costar no. 3506) have been described previously (Pelkowski et al., 2011; Lovato et al., 2016). 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 an 18 megapixel Canon EOS Rebel T2i digital camera with an EF-S 55-250 mm f/4.0–5.6 IS zoom lens with image quality = 5184 × 3456 pixels using Canon's remote capture software. Acquired images were compressed as 0.6MB JPEGs and stored on a standard desktop computer (Dell OptiPlex).