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Two-hit exposure to polychlorinated biphenyls at gestational and juvenile life stages: 2. Sex-specific neuromolecular effects in the brain

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ABSTRACT

Exposures to polychlorinated biphenyls (PCBs) during early development have long-lasting, sexually dimorphic consequences on adult brain and behavior. However, few studies have investigated their effects during juvenile development, a time when increases in pubertal hormones influence brain maturation. Here, male and female Sprague Dawley rats were exposed to PCBs (Aroclor 1221, 1 mg/kg/day) or vehicle prenatally, during juvenile development, or both, and their effects on serum hormone concentrations, gene expression, and DNA methylation were assessed in adulthood. Gene expression in male but not female brains was affected by 2-hits of PCBs, a result that paralleled behavioral effects of PCBs. Furthermore, the second hit often changed the effects of a first hit in complex ways. Thus, PCB exposures during critical fetal and juvenile developmental periods result in unique neuromolecular phenotypes, with males most vulnerable to the treatments.

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1. Introduction

Polychlorinated biphenyls (PCBs) are some of the most widespread environmental endocrine-disrupting chemicals (EDCs), as they persist in the food chain and are detectable in tissues of virtually all humans (Agency for Toxic Substances and Disease Registry, 2000). While banned in the 1970s, recent epidemiological data show that PCB body burdens continue to be associated with impaired reproductive and neurobiological health in humans (Boucher et al., 2009; Buck Louis et al., 2013; Engel and Wolff, 2013). In addition, rodent studies demonstrate that PCBs exert subtle but chronic effects on a range of social and anxiety related behaviors (Elnar et al., 2012; Jolous-Jamshidi et al., 2010; Reilly et al., 2015; Tian et al., 2011). Many of these behaviors are sexually dimorphic and organized by neonatal exposure to steroid hormones (Adler et al., 1999; Auger and Olesen, 2009; Bitran, 1993; Henley et al., 2011; Mora et al., 1996). As some PCB congeners, including those in the current study (Aroclor 1221, A1221) are weakly estrogenic (Jansen et al., 1993), the majority of behavioral studies focus on gestational or neonatal exposure, a life stage when brain sexual differentiation occurs, and when hormonal perturbations were predicted to have the most profound effects (McCarthy et al., 2009).

Juvenile development is also a time of continued sensitivity to organizational effects of gonadal hormones, as well as activation of neural pathways that were organized earlier in life (Schulz et al., 2009; Sisk and Foster, 2004). We recently demonstrated that exposing rats to PCBs during juvenile development, with or without prior prenatal exposure, affected several types of behavior in a sex- and age-specific manner (Bell et al., 2016). Two hits of PCBs, the first in late gestation and the second in juvenile development, resulted in abnormal levels of play and anxiety-like behavior in adolescent females, and caused disruptions of opposite-sex partner preference in adult males. In some cases, juvenile exposure modified or unmasked the effects of a previous prenatal exposure, especially in the male rats.

The goals of this study were to determine how two hits of PCB exposure, given during prenatal or juvenile development, or both, interact to change expression of genes in the adult brain as potential molecular substrates related to the observed behavioral changes. Neural regions were selected based on their roles in sexually dimorphic sociosexual and anxiety-like behaviors and in mediating the rewarding qualities of these social interactions

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(Burgdorf et al., 2007; Davis et al., 2010; Gordon et al., 2002; Harding and McGinnis, 2005; Newman et al., 1997; Pfaff and Sakuma, 1979). Genes that were studied within these regions included those involved in dopaminergic and endogenous opioid signaling, the vasopressin and oxytocin systems, and steroid hormone receptors that regulate social and anxiety-like behaviors (Bale et al., 2001; Bielsky et al., 2004; Buck et al., 2014; Bychowski et al., 2013; Egashira et al., 2007; Ferguson et al., 2000; Harding and McGinnis, 2004; Lim and Young, 2006; Matochik et al., 1992; Trezza et al., 2010; Veenema et al., 2013). We hypothesized that changes in gene expression would be correlated with changes in behavior, that a second PCB hit would change the developmental trajectory of gene expression in the brain in a manner not predicted by either hit alone, and that the sexes would differ in their sensitivity to PCB effects.

2. Methods

2.1. Animals and husbandry

All animal protocols were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and approved by The University of Texas at Austin's Institutional Animal Care and Use Committee. Sprague–Dawley rats were purchased from Harlan Laboratories (Houston, Texas) and were housed in a humidity- and temperature-controlled room with a 12:12 reversed light cycle (lights out at noon) at 21–23 °C. 2–3 animals were group-housed in polycarbonate cages (43 × 21 × 25 cm) with aspen bedding (PJ Murphy Forest Products, Sani-Chip), a PVC tube for enrichment, and weekly cage changes. Rats were fed low phytoestrogen Harlan-Teklad 2019 Global Diet (Harlan-Teklad, Indianapolis, Indiana) *ad libitum* for the duration of the experiment. Upon arrival, rats were handled daily to acclimate them to their new housing conditions, and mating began at least two weeks later.

Females (3–4 months old, virgin) were mated with sexually experienced untreated male rats (~6 month old); for balance, each stud male sired two litters, one that was subsequently treated with the vehicle and the other with PCBs. The morning after successful mating (sperm-positive vaginal smear), termed embryonic day (E) 1, dams were singly housed. Dams were provided with nesting materials several days prior to expected parturition on E23. On the day after birth [postnatal day (P) 1], litters were culled to equal sex ratios, with final litter size ranging from 6 to 8 pups. Weaned pups were housed with same sex littermates (2–3 per cage), and were weighed and handled for at least 5 min weekly. Animals were tested for social and anxiety behaviors in adolescence and adulthood, with results published in a sister paper (Bell et al., 2016). Because of the large number of animals necessary for both studies, the animals were raised in 3 cohorts over 1.5 years, with treatments equally distributed across each cohort.

2.2. Treatments

Aroclor 1221 (A1221, AccuStandard, New Haven, CT, Cat No: C-221N-50MG, Lot: 23683) is a mix of ~45 lightly chlorinated PCB congeners with known estrogenic (Layton et al., 2002; Shekhar et al., 1997), anti-aromatase (Woodhouse and Cooke, 2004), and anti-androgen (Schrader and Cooke, 2003) actions, but without effect on aryl hydrocarbon receptor (Poland and Glover, 1977). It was dissolved in a 4% dimethylsulfoxide vehicle (Veh, Cat No D4540; Sigma, St Louis, Missouri in sesame oil) for intraperitoneal injection at 1 mg/kg dam body weight. Dams were randomly assigned to either Veh (n = 6) or A1221 (n = 6), and each litter contributed no more than two animals per group. On E16, E18, and E20, during the period of sexual differentiation of the rat brain (Breedlove, 1992; Ramaley, 1979; Rhees et al., 1990; Tobet and Fox, 1989; Wagner et al., 1998), dams were weighed and injected with 0.1 ml of Veh or A1221 solution using a 1 ml syringe with a 25 gauge needle, 3 h prior to lights out. This mixture and dosage is not toxic to dams, does not cause fetal loss, and was selected so that outcomes of the current study could be compared with findings from several other previous studies using a very similar exposure regime (Dickerson et al., 2011a; Reilly et al., 2015; Steinberg et al., 2008; Walker et al., 2014). Although we did not measure body burden, we estimated that each pup is exposed to approximately 2 μg/kg A1221 based on (Takagi et al., 1976). This is within the range of human exposure according to levels found in maternal serum, cord blood and milk fat (Agency for Toxic Substances and Disease Registry, 2000; Karmaus et al., 2002; Lackmann, 2002; Law et al., 2005; Longnecker et al., 2005; Matthews and Anderson, 1975; Patterson et al., 2009; Schantz, 1996).

Rats were given an additional set of juvenile injections, either Veh or A1221 (1 mg/kg), again at 0.1 mL volume, ip, on P24, 26, and 28, when puberty is beginning, estrogen-positive feedback is being established (Andrews et al., 1981) and the brain is highly sensitive to organizational and activational effects of gonadal steroids (Döhler and Wuttke, 1975; Saksena and Lau, 1979; Schulz et al., 2009; Smyth and Wilkinson, 1994; Vetter-O'Hagen and Spear, 2011). Littermates within a cage were given the same treatment to prevent cross-contamination. With both gestational and juvenile exposures, there were four experimental groups in a 2 × 2 balanced design (first hit prenatal, second hit juvenile): Veh–Veh, A1221-Veh, Veh-A1221, and A1221-A1221. Final Ns per group were between 9 and 12 for all measures, from 6 litters per treatment. The experimenters were blind to treatment throughout the duration of the experiment.

2.3. Tissue collection

Rats were euthanized in adulthood (between P93–P108) by rapid decapitation 1–3 h before lights out, on proestrus in females and 3–9 days after the last behavioral test (reported in the companion study; Bell et al., 2016) in both sexes. Brains were immediately removed and placed in ice for 5 min prior to placing in an ice-cold stainless steel brain matrix. After cutting the optic chiasm, a razor blade was inserted through the center of this landmark, and three 2-mm (rostral) and two 1-mm (caudal) coronal sections were taken. Sections were placed on an ice-cold microscope slide, and snap frozen on dry ice. One to 11 months later, frozen sections were placed on a freezing stage, allowed to equilibrate to −18 °C, and micropunches (0.98 mm diameter) were taken from each region of interest according to Paxinos and Watson, 2009 (Paxinos and Watson, 2009). Photographs were captured of sections before and after punching to ensure consistency across the cohorts (Fig. 1). Samples were placed in a cold Eppendorf tube and stored at −80 °C for 2–9 months until nucleic acid isolations. Trunk blood samples were collected and allowed to clot for 30 min before centrifugation (1500 × g for 5 min). Sera were collected and stored at −80 °C until use, 1–2 years later.

2.4. Serum hormone quantification

Total serum testosterone (T) was determined in male animals via radioimmunoassay (ImmuChem Double Antibody ^{125}I RIA kit, Cat No 07-189105, Lot# RTK1420, MP Biomedicals, Costa Mesa,CA), according to manufacturer directions. All samples were run in a single assay, and duplicate volumes of 50 μl serum were used. The assay limit of detection was 0.03 ng/mL, and the intraassay C.V. was 1.41%. This assay is not sensitive enough to run T in females. Total serum estradiol (E_2) was determined in male and female rats via

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