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Mice exposed to bisphenol A exhibit depressive-like behavior with neurotransmitter and neuroactive steroid dysfunction

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ABSTRACT

Fetal exposure to endocrine disrupting chemicals (EDCs) has been associated with adverse neurobehavioral outcomes across the lifespan and can persist across multiple generations of offspring. However, the underlying mechanisms driving these changes are not well understood. We investigated the molecular perturbations associated with EDC-induced behavioral changes in first (F1) and second (F2) filial generations, using the model EDC bisphenol A (BPA). C57BL/6J dams were exposed to BPA from preconception until lactation through the diet at doses (10 μg/kg bw/d-lower dose or 10 mg/kg bw/d-upper dose) representative of human exposure levels. As adults, F1 male offspring exhibited increased depressive-like behavior, measured by the forced swim test, while females were unaffected. These behavioral changes were limited to the F1 generation and were not associated with altered maternal care. Transcriptome analysis by RNA-sequencing in F1 control and upper dose BPA-exposed adult male hippocampus revealed neurotransmitter systems as major pathways disrupted by developmental BPA exposure. High performance liquid chromatography demonstrated a male-specific reduction in hippocampal serotonin. Administration of the selective serotonin reuptake inhibitor fluoxetine (20 mg/kg bw) rescued the depressive-like phenotype in males exposed to lower, but not upper, dose BPA, suggesting distinct mechanisms of action for each exposure dose. Finally, high resolution mass spectrometry revealed reduced circulating levels of the neuroactive steroid dehydroepiandrosterone in BPA-exposed males, suggesting another potential mechanism underlying the depressive-like phenotype. Thus, behavioral changes associated with early life BPA exposure may be mediated by sex-specific disruptions in the serotonergic system and/or sex steroid biogenesis in male offspring.

1. Introduction

Mental health disorders are a major contributor to the global burden of disease. Of note, the World Health Organization (WHO) selected depression as the theme for the 2017 World Health Day campaign, bringing worldwide attention to this major global health concern (World Health Organization (WHO), 2017). While genetic risk factors have been identified for mental health disorders (Lohoff, 2010), increased evidence in rodent and human studies has also demonstrated a role for environmental influences on gene regulation and subsequent disease risk (Delgado-Morales, 2017). Additionally, accumulating evidence has linked fetal development with behavioral and mental health outcomes in later life. The developing brain represents a particularly vulnerable window to environmental injury given the incomplete formation of the blood-brain barrier as well as blunted mechanisms for detoxifying exogenous chemicals (Choudhary et al., 2003; de Wildt et al., 1999; Grandjean and Landrigan, 2014).

The developing mammalian brain relies on sex steroid hormones to

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mediate the composition of neural structures and connections that control a broad spectrum of cognitive and behavioral outcomes in adulthood (Palanza et al., 2008). This organizational window in rodents, which occurs just before and after birth, corresponds to the second and third trimesters of pregnancy in humans (McCarthy, 2008). Additionally, a sexually dimorphic increase in estrogen in the developing male brain, through the aromatization of gonadal testosterone, establishes male-specific neural circuitries in late gestation (Palanza et al., 2008). Disruptions in the hormonal milieu during central nervous system (CNS) development, therefore, could impact brain function in a sex-specific manner. Given the well-established role for sex steroid hormones in proper brain development and function, endocrine disrupting chemicals (EDCs) have been proposed as potential neurotoxicants (Grandjean and Landrigan, 2014; McCarthy, 2008).

One EDC of great interest given its ubiquitous presence in the environment is bisphenol A (BPA), a chemical commonly used in the manufacturing of polycarbonate plastics and epoxy resins (Calafat et al., 2008). Given its high production volume, urinary concentrations of BPA are detectable in virtually all sampled individuals in the United States (Calafat et al., 2008). As an EDC, BPA is best studied for its estrogenic activity (Yamasaki et al., 2000), although additional modes of BPA action including disrupted steroidogenesis have also been reported (Hong et al., 2016; Peretz and Flaws, 2013). In humans, maternal exposure to BPA during gestation is associated with altered behaviors in children, including sociability, anxiety, and depression in some, but not all, studies (Mustieles et al., 2015). In rodent models, offspring gestationally and/or lactationally exposed to BPA have also been reported to exhibit comparable behavioral deficits (Palanza et al., 2016). Interestingly, the effects of BPA in both humans and rodents have occasionally been reported to be sex-specific, although the affected sex varies by study (Mustieles et al., 2015; Palanza et al., 2016). Unfortunately, within human epidemiological investigations as well as experiments in rodent models, the heterogeneity in study design limits comparative analyses. Nevertheless, the available literature suggests early life BPA exposure can negatively impact behavior. The mechanisms underlying these changes, however, remain unclear.

The hippocampus has long been associated with emotional regulation (Bannerman et al., 2014) and is susceptible to BPA. Morphologically, developmental exposure to BPA is associated with impaired synaptogenesis and spinogenesis, two estrogen-mediated processes, that can persist into adulthood (Kimura et al., 2016; Tiwari et al., 2015; Wang et al., 2014; Xu et al., 2014). Molecular assessments of hippocampal gene expression changes following developmental BPA exposure have, until recently, utilized candidate approaches (Chen et al., 2015; Kumar and Thakur, 2017; Kundakovic et al., 2015; Wang et al., 2014; Xu et al., 2014). Given the pleiotropic effects associated with BPA exposure, unbiased approaches have gained favor in the identification of major pathways responsible for exposure-associated behavioral outcomes (Arambula et al., 2016).

We previously reported whole brain-specific expression changes of clinically relevant imprinted genes at midgestation in offspring exposed to BPA in utero (Susiarjo et al., 2013). The postnatal molecular and behavioral consequences of exposure in our model, however, remain to be determined. Moreover, alterations in behavior have been reported to persist across multiple generations of offspring directly exposed to BPA as well as generations that have received no direct exposure, known as multi- and transgenerational effects, respectively (Wolstenholme et al., 2012, 2013). Other than social deficits, however, multi- and transgenerational behavioral phenotypes associated with developmental BPA exposure have not been reported.

The aim of the present study was to assess multigenerational adult behavioral consequences associated with developmental BPA exposure and to identify underlying molecular dysregulation contributing to the behavioral changes. We identified male-specific disruptions in affective behavior that were associated with reduced levels of the hippocampal serotonin and systemic dehydroepiandrosterone, a neuroactive steroid in the sex steroidogenesis pathway. The depressive-like phenotype in F1 generational males did not persist into the F2 generation.

2. Materials and methods

2.1. Animals and treatments

All animal studies were approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania. The animals used in this study were treated humanely and with regard for alleviation of suffering. Mice were maintained on a 12:12-h light/dark cycle, with lights on at 07:00 (based on a 24-hour time system) and temperature maintained at 25 °C ± 2 °C. Drinking water was provided in polypropylene bottles. To verify the robustness of phenotypes, a total of five cohorts from two different animal facilities were examined over a two-year period. When possible, experiments were performed on animals and tissues from multiple cohorts of exposures and has been denoted in the figure legends. A comprehensive summary of assays performed on adult animals of each sex and generation can be found in Supplemental Table 1. All sections of this report comply with ARRIVE guidelines (Kilkenny et al., 2010); a completed checklist is included (Supplemental Checklist 1).

BPA (CAS 80-05-7; Sigma ≥99% purity)-supplemented feed was purchased from *Envigo* (Madison, WI). The control diet was a modified, low phytoestrogen, AIN 93G diet (TD 95092 with 7% corn oil substituted for 7% soybean oil to minimize exposure to other estrogen-like compounds that could confound BPA-related effects), and BPA diets included 50 μg BPA/kg diet (lower dose BPA; TD 110337) and 50 mg BPA/kg diet (upper dose BPA; TD 06156) supplemented into the control diet to approximate exposures of 10 μg and 10 mg BPA per kg bw/d (Dolinoy et al., 2007). The levels of exposure fall below established reference doses for BPA that are considered safe for humans: the tolerable daily intake (TDI; 50 μg/kg bw/d), defined by the European Food Safety Authority (Bolt and Stewart, 2011), and the lowest observed adverse effect level (LOAEL; 50 mg/kg bw/d), defined by the United States Environmental Protection Agency (Integrated Risk Information System (IRIS), 1988). Moreover, we previously assessed circulating levels of BPA in F0 maternal serum (Susiarjo et al., 2013) and found levels to be comparable to human exposures (Schönfelder et al., 2002; Vandenberg et al., 2007).

Six-week-old virgin C57BL/6J female mice (designated F0 generation, purchased from The Jackson Laboratory) were randomly assigned to control or BPA-containing diets beginning two weeks prior to mating. At least six F0 females per treatment group were exposed per cohort. Dietary exposure, a common route of human exposure, minimizes stress and provides a more chronic exposure throughout the duration of the experiment. Females were mated to unexposed C57BL/6J male mice during each cohort of exposure. Upon detection of a vaginal plug, females were separated from males. No females were mated for more than two weeks. Exposures continued throughout mating, gestation, and lactation. We previously reported no differences in maternal food consumption between control and BPA-exposed dams (Susiarjo et al., 2015). The start of this exposure window coincides with the acquisition of epigenetic marks in late oocyte maturation that may be susceptible to environmentally-induced disruption and subsequently influence offspring health (Smallwood et al., 2011). Postnatal exposure to BPA was included in our study because the differentiation and development of the rodent brain corresponding to the second and third trimesters of human fetal brain development extend into the early postnatal period (Palanza et al., 2016; Williams et al., 2013).

All F1 generation offspring were weaned on postnatal day (PND) 21 and maintained on control diets throughout the remainder of the studies. No differences in litter size or sex ratio were observed, and no culling of litters was performed. To generate F2 offspring, a subset of six-week-old F1 females (one to two females per litter) were randomly selected to be mated to unexposed males. Animals used for adult