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Effects of developmental exposure to bisphenol A on spatial navigational learning and memory in rats: A CLARITY-BPA study

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ABSTRACT

Bisphenol A (BPA) is a ubiquitous industrial chemical used in the production of a wide variety of items. Previous studies suggest BPA exposure may result in neuro-disruptive effects; however, data are inconsistent across animal and human studies. As part of the Consortium Linking Academic and Regulatory Insights on BPA Toxicity (CLARITY-BPA), we sought to determine whether female and male rats developmentally exposed to BPA demonstrated later spatial navigational learning and memory deficits. Pregnant NCTR Sprague–Dawley rats were orally dosed from gestational day 6 to parturition, and offspring were directly orally dosed until weaning (postnatal day 21). Treatment groups included a vehicle control, three BPA doses (2.5 μg/kg body weight (bw)/day—[2.5], 25 μg/kg bw/day—[25], and 2500 μg/kg bw/day—[2500]) and a 0.5 μg/kg/day ethinyl estradiol (EE)-reference estrogen dose. At adulthood, 1/sex/litter was tested for seven days in the Barnes maze. The 2500 BPA group sniffed more incorrect holes on day 7 than those in the control, 2.5 BPA, and EE groups. The 2500 BPA females were less likely than control females to locate the escape box in the allotted time (p value = 0.04). Although 2.5 BPA females exhibited a prolonged latency, the effect did not reach significance (p value = 0.06), whereas 2.5 BPA males showed improved latency compared to control males (p value = 0.04), although the significance of this result is uncertain. No differences in serum testosterone concentration were detected in any male or female treatment groups. Current findings suggest developmental exposure of rats to BPA may disrupt aspects of spatial navigational learning and memory.

Introduction

Bisphenol A (BPA) is a mass produced industrial chemical (Environment Canada, 2008; Galloway et al., 2010), with production reported to be approximately 15 billion pounds in 2013 (GrandViewResearch, 2014; Vandenberg et al., 2013a, 2013b). BPA is present in a wide variety of commonly used products and applications, including polycarbonate plastics, metal food can linings, dental sealants, thermal receipt paper, and many other items that are not currently required to be labeled to contain BPA, although the American Medical Association made such a recommendation at its 2011 meeting (American Medical Association, 2011). The widespread prevalence of this chemical has resulted in chronic exposure of humans, including pregnant women, and non-human animals (Bhandari et al., 2015; Braun et al., 2011; Calafat et al., 2005; Vandenberg et al., 2013a, 2013b). The estimated median daily intake for the overall US population is ~34 ng BPA/kg body weight (bw)/day (Lakind and Naiman, 2011).

In 2008, the National Toxicology Program (NTP) determined that there was "*some concern for effects on the brain, behavior, and prostate gland in fetuses, infants, and children at current human exposures to bisphenol A*", but that BPA exposure from food contact materials was below a level that might cause adverse health effects and that it was safe overall, even for infants and young children (NTP, 2008). By

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contrast, the Chapel Hill Report forewarned of negative, and potentially irreversible, outcomes in humans (Vandenberg et al., 2009; vom Saal et al., 2007). Rodent model and human studies show that BPA can be transferred across the placenta (Balakrishnan et al., 2010; Ikezuki et al., 2002; Kawamoto et al., 2007; Nishikawa et al., 2010) and through the milk (Deceuninck et al., 2015; Kurebayashi et al., 2005; Tateoka, 2015; Zimmers et al., 2014), although not all studies agree that this is a substantial route of exposure (Doerge et al., 2011, 2010b). Fetal rodents possess limited ability to metabolize BPA (Doerge et al., 2011, 2010a; Ikezuki et al., 2002; Kawamoto et al., 2007; Nishikawa et al., 2010), and therefore higher internal concentrations of bioactive BPA may result compared to adults. If developing organisms are more susceptible to the potential adverse effects of BPA, perinatal exposure could result in later development of diseases, a hypothesis known as the developmental origin of health and disease.

BPA exposure, especially during the perinatal period, may result in neuroendocrine disruption (reviewed in Leon-Olea et al., 2014) and ultimately compromise the normal organizational and activational effects of endogenous steroid hormones (Arnold and Breedlove, 1985; Morris et al., 2004; Phoenix et al., 1959). Spatial navigational learning and memory is programmed by early exposure to endogenous sex steroid hormones and requires a later surge in adulthood for the normal elaboration of these behaviors, with males tending to exhibit better performance in a variety of species, including humans (Galea et al., 1995; Gaulin et al., 1990, 1992; Jasarevic et al., 2012a, 2012b; Simpson and Kelly, 2012; Williams et al., 1990). As an endocrine disrupting chemical (EDC) that can bind and activate estrogen receptors, as well as other steroidogenic and non-steroidogenic receptors (Lee et al., 2003; Vandenberg et al., 2009; Wetherill et al., 2002; Xu et al., 2005; Zoeller et al., 2005), BPA exposure may negatively impact this cognitive process. Several assessments have been developed that specifically measure spatial navigational learning and memory in rodents, including the Barnes maze, Morris water maze, radial arm maze, and appetite-motivated maze tests (Barnes, 1979; Kuwahara et al., 2014; Morris, 1984; Olton and Samuelson, 1976; Sharma et al., 2010).

Conflicting results, however, have been obtained using such assessments in laboratory rodents exposed to BPA. Some reports have suggested that BPA exposure impairs spatial learning and memory (Diaz Weinstein et al., 2013; Eilam-Stock et al., 2012; Goncalves et al., 2010; Jasarevic et al., 2011, 2013; Kim et al., 2011; Kuwahara et al., 2013; Viberg et al., 2011; Xu et al., 2013); while others suggest minimal or no BPA effects on spatial navigation (Ferguson et al., 2012; Kuwahara et al., 2014; Neese et al., 2013; Ryan and Vandenbergh, 2006; Sadowski et al., 2014a; Williams et al., 2013). Differences in species, age at and/or route of exposure, BPA dose, and testing methods may account for those varying results. Here, National Center for Toxicological Research (NCTR) Sprague–Dawley rats were exposed daily during the developmental period to BPA (2.5 μg/kg bw, 25 μg/kg bw, or 2500 μg/kg bw) and spatial navigation was assessed at adulthood. Concurrent negative (vehicle) and reference estrogen (ethinyl estradiol, EE) controls were also included. This work was performed as part of the larger National Institute of Environmental Health Sciences (NIEHS)/National Toxicology Program (NTP)/Food and Drug Administration (FDA) Consortium Linking Academic and Regulatory Insights on BPA Toxicity (CLARITY-BPA) program, in which other research groups are examining additional behavioral and phenotypic endpoints (Birnbaum et al., 2012; Schug et al., 2013).

Materials and methods

Animal husbandry and dosing

Comprehensive details on animal husbandry, treatment, and dosing procedures have been published (Heindel et al., 2015). Therefore, only brief details are described here. All animal use and procedures were approved in advance by the NCTR Institutional Animal Care and Use Committee and were conducted in an Association for Assessment and Accreditation of Laboratory Animal Care (AALAC)-accredited facility. Experiments were performed in accordance with the "Guide for the Care and Use of Laboratory Animals" (NRC, 2011). The animal rooms for breeding, gestation, and pre- weaning housing were maintained at 23 ± 3 °C with a humidity level of 50 ± 20%, and a 12 h:12 h light/dark cycle with lights on at 6:00. After weaning on postnatal day (PND) 21, the light cycle was changed to a reverse cycle (light on at 11:00; off at 23:00). A low phytoestrogen diet (5K96 verified casein diet 10 IF, round pellets, γ-irradiated, Test Diets, Purina Mills, Richmond, IN) and Millipore-filtered water in glass water bottles with silicone stoppers (#7721 clear, The Plasticoid Co., Elkton, MD) were provided ad libitum. Housing cages were polysulfone with microisolator tops (Ancare Corp., Bellmore, NY) and contained hardwood chip bedding. Drinking water, cage, and bedding extracts were tested for BPA and none had levels detectable above the level of the average analytical blanks. Diet was tested for BPA, genistein, daidzein, zearalenone, and coumesterol, and only lots with <5 ppb BPA, <1 ppm genistein and daidzein, and <0.5 ppm zearalenone and coumesterol were used. Breeder male and female Sprague–Dawley (SD) rats (i.e., F_0) from the NCTR breeding colony were placed in the above conditions (e.g., low phytoestrogen diet, glass water bottles, polysulfone cages) at weaning on PND 21. SD rats were chosen for several reasons. They are one of the most common rodent strains in toxicological research, and the studies described herein are part of the larger CLARITY-BPA studies where all investigators are using this animal model (Birnbaum et al., 2012; Heindel et al., 2015; Schug et al., 2013). Additionally, SD rats are widely used in various Barnes maze experiments (Barrett et al., 2009; Locklear and Kritzer, 2014; Morel et al., 2015), including those performed at the NCTR (Ferguson et al., 2012), and we sought to compare the current findings to those prior studies. Another study suggested that adult SD perform better than Dark Agouti rats in this behavioral test (Barrett et al., 2009). Finally, SD rats are easy to handle and are more resistant to injuries that might result from the gavage method that was used to dose the animals (described below) (Germann and Ockert, 1994; Germann et al., 1998; Germann et al., 1995).

Approximately 2 weeks prior to mating, females were assigned to one of five treatment groups (vehicle control, 2.5 BPA, 25 BPA, 2500 BPA, or EE) based on body weight ranking to produce approximately equal mean body weights in each group. Males were randomly mated with females with the stipulation that no sibling or first cousin pairing was permitted. Breeding occurred in five "loads" or "cohorts" each spaced four weeks apart. Offspring from the last two breedings (i.e., loads 4 and 5) were used for the behavioral studies described here and in Rebuli et al. (2015).

Dams were considered pregnant when a sperm plug or a sperm-positive vaginal cytology was observed [mating day = gestational day (GD) 0)]. Beginning on GD 6, dams were gavaged daily with 0.3% carboxymethylcellulose (CMC or vehicle), 2.5 μg BPA/kg bw/day, 25 μg BPA/kg bw/day, 2500 μg BPA/kg bw/day, or 0.5 μg EE/kg bw/day. These BPA doses were selected to provide low, middle, and upper levels of exposure and are below the no-observed-adverse-effect level (NOAEL) of 5 mg/kg bw/day as detailed in Tyl et al. (2002, 2008). These doses are also within the dose range used in prior studies conducted at the NCTR with the same animal model (Delclos et al., 2014; Ferguson et al., 2014). The highest EE dose available in the CLARITY-BPA study was selected to better compare to a 10-fold greater dose (5 μg EE/kg bw/day) employed in a prior study (Ferguson et al., 2012). The materials used for dose formulation were BPA (CAS # 80-05-7, TCI America, Portland, OR; catalogue # B0494, Lot # 111909/AOHOK [air-milled], ≥99.9 purity), EE (CAS # 57-63-6, Sigma-Aldrich, St. Louis, MO; catalogue # E4876, Lot # 071M1492V, >99% purity), and CMC (Sigma-Aldrich; catalogue # C5013, Lot # 041M0105V). Dams were gavaged daily at a volume of 5 ml/kg bw using a modified Hamilton Microlab® ML511C programmable 115 V pump (Hamilton Co., Reno, NV; Lewis et al., 2010). No treatment occurred on the day of parturition,