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Working memory in bisphenol-A treated middle-aged ovariectomized rats

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ABSTRACT

Over 90% of the U.S. population has detectable bisphenol-A (BPA) in their urine according to recent biomonitoring data. BPA is best known for its estrogenic properties, and most rodent research on the nervous system effects of BPA has focused on determining if chronic exposures during pre- and perinatal development have organizational effects on brain development and behavior. Estrogens also have important impacts on brain and behavior during adulthood, particularly in females during aging, but the impact of BPA on the adult brain is less studied. We have published a series of studies documenting that chronic exposure to various estrogens including 17β-estradiol, ERβ selective SERMs and soy phytoestrogens impairs performance of middle-aged female rats on an operant working memory task. The purpose of this study was to determine if chronic oral exposure to BPA would alter working memory on this same task. Ovariectomized (OVX) middle-aged Long Evans rats were tested on an operant delayed spatial alternation (DSA) task. Rats were treated for 8–10 weeks with either a 0 (vehicle control), 5 or 50 μg/kg bw/day oral bolus of BPA. A subset of the vehicle control rats was implanted with a Silastic implant containing 17β-estradiol (low physiological range) to serve as a positive control. All rats were tested for 25 sessions on the DSA task. BPA treatment did not influence performance accuracy on the DSA task, whereas 17β-estradiol significantly impaired performance, as previously reported. The results of this study suggest that chronic oral exposure to BPA does not alter working memory processes of middle-aged OVX rats assessed by this operant DSA task.

1. Introduction

Bisphenol-A (BPA) is a high production volume chemical used in manufacturing a variety of plastics and plastic-containing products (see Richter et al., 2007), and is found in an assortment of food and beverage containers (see also Erler and Novak, 2010). Human exposure to this chemical is ubiquitous (see Vandenberg et al., 2007), with the majority of exposures occurring orally from the diet (Rudel et al., 2011; von Goetz et al., 2010). Urinary concentrations of BPA in humans are quite variable (Ye et al., 2011), with estimates of daily exposure to adults in the general population ranging from 0.008 to 1.5 μg/kg bw/day (NTP, 2008; World Health Organization, 2010).

BPA is a synthetic estrogen that can bind the nuclear estrogen receptors (ERs), having a higher affinity for ERβ than for ERα (Matthews et al., 2001; Routledge et al., 2000; Takemura et al., 2005). It is considered a weak estrogen agonist as its binding affinity is approximately 10,000 fold lower than that of 17β-estradiol (Kuiper et al., 1998). BPA also possesses both anti-estrogenic and anti-androgenic properties at some doses in particular tissues (Wolstenholme et al., 2011). The current U.S. tolerable daily intake (TDI) set by the EPA is 50 μg/kg/day, but the safety of this oral reference dose has come under scrutiny as recent research suggests the possibility that BPA may have a non-monotonic dose response curve, with some effects in animal models occurring at doses well below the TDI (Vandenberg et al., 2009, 2012).

A large number of animal and human studies have been undertaken to assess the effects of exposure to BPA on a variety of health endpoints. Developmental exposures have been the major focus of research assessing the impact of BPA on learning and memory (see Golub et al., 2010; Palanza et al., 2008), but there is little clarity or consistency in the findings. In rodents, exposures at or below the TDI (≤50 μg/kg/day; see also Sekizawa, 2008) have resulted in a range of effects on memory processes, ranging from deficits, to no effect, to enhancements (Goncalves et al., 2010; Jones and Watson, 2012; Poimenova et al., 2010; Ryan and Vandenbergh, 2006; Viberg et al., 2011; Xu et al., 2011). Studies also suggest that exposure to BPA during development can permanently alter the structure and organization of brain regions important to learning and memory (Matsuda et al., 2010; Xu et al., 2011).

In contrast, fewer studies have examined the effects of BPA exposure during adulthood on learning and memory of rodents, and all of the studies to date have been conducted in young OVX rats or gonadally-intact rats. A set of studies found performance on both object placement and recognition memory tasks – thought to be mediated by the hippocampus (Ennaceur et al., 1997) – to be impaired by treatment with BPA. In these tasks, rats were given 3 min to explore 2 objects, and following a delay memory was tested by moving (placement) or replacing (recognition) one of the objects. A single injection of BPA blocked a 17β-estradiol induced improvement in both object placement and

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recognition memory in 3-month old ovariectomized (OVX) female rats (lowest effective dose 4 and 40 μg/kg respectively: Inagaki et al., 2012). Similar effects on both object recognition and placement tasks were seen following a single injection of a 40 μg/kg dose of BPA to gonadally-intact male rats (Eilam-Stock et al., 2012). Further, short term daily oral treatment (14–28 days) with either relatively high (20 mg/kg) or relatively low (2 or 20 μg/kg) doses of BPA impaired memory performance of young adult male rats or mice in the Morris water maze (Jain et al., 2011; Kim et al., 2011).

To date, no studies have assessed the potential of BPA to alter memory processes in aging rodents. This is an important period for estrogen action in the female brain as middle-age represents the time when the transition from cyclical estrogen production to low/null circulating estrogen levels occurs. The question of whether or not estrogen replacement during this period aides or impairs cognitive aging remains unresolved. Furthermore, there are very few studies addressing how exposures to estrogen-active toxicants such as BPA may alter brain and behavior during this time period. Importantly, 17β-estradiol exposures in aging OVX rodents have revealed both brain region and memory system dependent changes in behavior (see Frick, 2009), with estrogens typically enhancing performance on hippocampally-sensitive tasks (Daniel et al., 1997; Daniel and Dohanich, 2001; Davis et al., 2005; Korol and Kolo, 2002; Zurkovsky et al., 2006), but impairing performance on tasks mediated by the prefrontal and striatal systems (Davis et al., 2005; Korol and Kolo, 2002; Neese et al., 2010a; Wang et al., 2008, 2009, 2011; Zurkovsky et al., 2007).

Our research group has established a mnemonic impairing effect of 17β-estradiol on a working memory task in middle-aged (12-month) OVX Long–Evans rats, a rodent model of the perimenopausal woman (Neese et al., 2010a; Wang et al., 2009). Specifically, performance on the operant delayed spatial alternation (DSA) task, which requires a rat to alternate its responses between two retractable levels to receive a food reward, was impaired following chronic treatment (8–10 weeks) with a Silastic implant that delivered a physiological dose (20–30 pg/ml) of 17β-estradiol. The deficits were measured following short intertrial delays (3-, 6- and 9-s) that have been shown to be sensitive to prefrontal cortical disruption (Chudasama and Muir, 1997; Harrison and Mair, 1996; Mair et al., 1998; Sloan et al., 2006; Van Haaren et al., 1985, 1988; Young et al., 1996). This deficit was largely paralleled by treatment with the ERβ agonist diarylpropionitrile (DPN), while treatment with the ERα agonist propyl pyrazole triol (PPT) had little effect (Neese et al., 2010a). In addition, chronic treatment with the soy phytoestrogen genistein, which has a higher binding affinity for ERβ than for ERα, also impaired DSA performance in aging OVX female rats (Neese et al., 2010b, 2012).

The purpose of this study was to determine the potential for BPA to impair performance on this DSA task in middle-aged OVX rats. BPA has been shown to disrupt the performance of young adult OVX rats treated with 17β-estradiol and gonadally-intact adult male rats on hippocampus-sensitive memory tasks, including the Morris water maze, object placement and object recognition tasks (Eilam-Stock et al., 2012; Inagaki et al., 2012; Jain et al., 2011; Kim et al., 2011), but no studies have been conducted in OVX aging female rodents treated with BPA. Given that our prior research has shown that chronic treatment with17β-estradiol or ERβ agonists impairs performance on the DSA task in aging OVX rats, we hypothesized that exposure to BPA would also impair performance in this model. Treatment doses (5 and 50 μg/kg) were selected to address lose-dose effects relevant to human exposures (see Vandenberg et al., 2012).

2. Methods

2.1. Animals and exposure

One hundred fifty-seven 10–12 month old female Long–Evans retired breeder rats were obtained from Harlan (Indianapolis, IN) in three separate cohorts and maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). Rats were housed in a temperature and humidity controlled room (22 °C, 40–55% humidity) on a 12-hour reverse light–dark cycle (lights off at 8:30 am). All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Illinois at Urbana-Champaign and were in accordance with the guidelines of the *Public Health Service Policy on Humane Care and Use of Laboratory Animals* (National Institutes of Health, 2002) and the *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* (National Research Council, 2003).

Rats were pair-housed by treatment group in polysulfone cages (45×24×20 cm) with woodchip bedding. All rats were subject to isoflurane gas anesthesia prior to OVX. A subset of rats was implanted with a Silastic capsule that contained 17β-estradiol at the time of OVX, and served as the positive control group (see Neese et al., 2010a; Wang et al., 2009). The Silastic capsule was 1 cm in length (1.5 mm i.d., 1.96 mm o.d.) and was plugged with silicone and dried overnight before packing with a 10% 17β-estradiol/cholesterol mixture (Sigma, St. Louis, MO), after which the other end was plugged with silicone. Capsules were soaked in sterile saline at 37 °C overnight before insertion during surgery. Previous research in this lab has shown that these 17β-estradiol implants produce stable serum estradiol concentrations of about 20–30 pg/ml for at least 10 weeks (Neese et al., 2010a).

Standard rodent diets contain soy phytoestrogens which can influence performance of the DSA task (see Neese et al., 2010b, 2012). There is also a wide variability of soy content seen across lots of standard rodent chow (Brown and Setchell, 2001; Thigpen et al., 2004, 2007). To avoid this confound, the rats in this study were maintained on a low-soy diet (Harlan diet 2016, Madison, WI). BPA-free water (Ultra-Reverse Osmosis System, freedrinkingwater.com; confirmed BPA free via HPLC with CoulArray detection, personal communication, Fred Vom Saal) was available ad libitum in glass water bottles. Beginning one week after OVX surgery, rats were weighed daily and food was restricted to maintain them at 85% of their free-feeding body weights. Operant training began two weeks following OVX and occurred once daily, six days/week during the dark phase of the light cycle. Rats were fed 1 h after the daily test session was completed.

2.2. BPA treatment

BPA was purchased from Sigma-Aldrich (#239658, St. Louis, MO) and dissolved in a 2% ethanol solution prior to mixing in tocopherol stripped corn oil (MP Biomedicals, #901415, Solon, OH). BPA at 50, 5, or 0 (vehicle control) μg/kg/bw was delivered via an oral bolus once per day with a polysulfone pipette tip (#9400263, Thermo Scientific). All rats received a single treatment each day prior to training/testing, and a single treatment on Sunday (non-operant testing day). BPA is quickly metabolized following oral bolus exposure and this glucuronidated form is not estrogenic (Matthews et al., 2001). In rodents, peak serum concentrations of unconjugated BPA occur within 30–60 min (see Doerge et al., 2010, 2011; Taylor et al., 2011). The operant DSA training and testing tasks typically take 45–60 min to complete, therefore rats were treated with BPA approximately 10–30 min prior to operant training or testing each day. Rats treated with 17β-estradiol (positive control) were also given a single oral dose of the 0 μg/kg treatment (vehicle control) prior to training or testing each day. Daily BPA treatments lasted for 8–10 weeks depending upon the number of sessions needed for individual rats to achieve criterion for response shaping, lever press training, and cued alternation training (Fig. 1 and see below). Few rats needed more than 9 weeks to complete training and testing, and the length of treatment did not differ across BPA exposure groups or cohorts.