Hormones and Behavior 69 (2015) 89–97

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Regular article

Bisphenol-A exposure during adolescence leads to enduring alterations in cognition and dendritic spine density in adult male and female rats

Rachel E. Bowman [a,*], Victoria Luine [b], Samantha Diaz Weinstein [a], Hameda Khandaker [b], Sarah DeWolf [a], Maya Frankfurt [c]

[a] Department of Psychology, Sacred Heart University, Fairfield, CT 06825, USA
[b] Department of Psychology, Hunter College, CUNY, New York, NY 10065, USA
[c] Department of Science Education, Hofstra North Shore-LIJ School of Medicine, Hempstead, NY 11549, USA

ARTICLE INFO

Article history:
Received 3 December 2014
Revised 16 December 2014
Accepted 20 December 2014
Available online 30 December 2014

Keywords:
Bisphenol-A
Adolescence
Anxiety
Memory
Exploration
Spine density

ABSTRACT

We have previously demonstrated that adolescent exposure of rats to bisphenol-A (BPA), an environmental endocrine disrupter, increases anxiety, impairs spatial memory, and decreases dendritic spine density in the CA1 region of the hippocampus (CA1) and medial prefrontal cortex (mPFC) when measured in adolescents in both sexes. The present study examined whether the behavioral and morphological alterations following BPA exposure during adolescent development are maintained into adulthood. Male and female, adolescent rats received BPA, 40 μg/kg/bodyweight, or control treatments for one week. In adulthood, subjects were tested for anxiety and locomotor activity, spatial memory, non-spatial visual memory, and sucrose preference. Additionally, stress-induced serum corticosterone levels and dendritic spine density in the mPFC and CA1 were measured. BPA-treated males, but not females, had decreased arm visits on the elevated plus maze, but there was no effect on anxiety. Non-spatial memory, object recognition, was also decreased in BPA treated males, but not in females. BPA exposure did not alter spatial memory, object placement, but decreased exploration during the tasks in both sexes. No significant group differences in sucrose preference or serum corticosterone levels in response to a stress challenge were found. However, BPA exposure, regardless of sex, significantly decreased spine density of both apical and basal dendrites on pyramidal cells in CA1 but had no effect in the mPFC. Current data are discussed in relation to BPA dependent changes, which were present during adolescence and did, or did not, endure into adulthood. Overall, adolescent BPA exposure, below the current reference safe daily limit set by the U.S.E.P.A., leads to alterations in some behaviors and neuronal morphology that endure into adulthood.

Introduction

Bisphenol-A (BPA), is a known endocrine disruptor, documented to have estrogenic, anti-estrogenic, androgenic, and anti-androgenic effects (Negishi et al., 2003; Sohoni and Sumpter, 1998) on various hormone-induced physiological and behavioral phenomena. Detectable levels of BPA have been reported in body fluids of humans and animals (Biedermann et al., 2010; Geens et al., 2011; Rubin, 2011) and, thus, BPA exposure has potential health hazards (Rubin, 2011; Rubin and Soto, 2009; Talsness et al., 2009; vom Saal and Hughes, 2005).

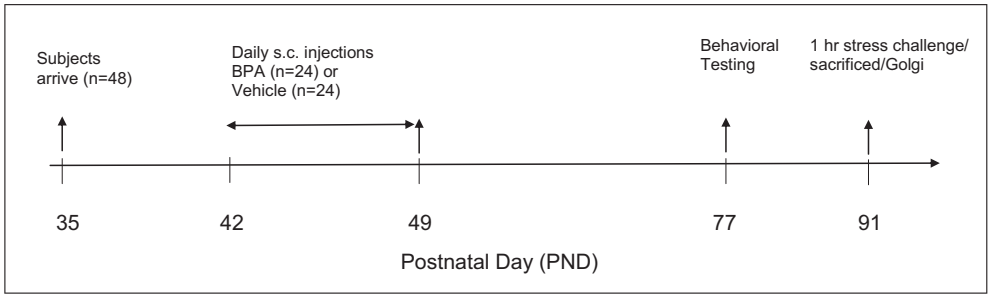
Exposure to BPA during the perinatal period has been shown to reverse or abolish sexual dimorphisms in several brain regions in the rodent (Cao et al., 2014; Kubo et al., 2003; Patisaul et al., 2006; Rubin et al., 2006) and rhesus monkeys (Elsworth et al., 2013). Additionally, perinatal BPA exposure has been shown to reverse sex differences in sweet taste preference, a marker of anhedonia (Katz, 1981), by increasing sucrose preference in adult males and decreasing it in adult females (Xu et al., 2011). In adolescent rats, perinatal BPA exposure increases hyperactivity in males (Ishido et al., 2004; Kiguchi et al., 2008) and eliminates sex differences in both open-field behavior (Fujimoto et al., 2006; Kubo et al., 2003) and the forced swimming test (Fujimoto et al., 2006).

Aggression and anxiety in adult rats are increased following perinatal exposure to BPA (Patisaul and Bateman, 2008; Patisaul et al., 2012), and exploratory behaviors in both adolescent (Fujimoto et al., 2006) and adult rodents are decreased (Farabollini et al., 1999; Goncalves et al., 2010). Importantly, perinatal BPA exposure impairs spatial memory of both male and female adolescent rats (Poimenova et al., 2010). In addition, BPA administration in adulthood alters both object recognition (OR), spatial memory (object placement, OP) and dendritic spine density in male and female rats (Goncalves et al., 2010; Eilam-Stock et al., 2012; Inagaki et al., 2012). Thus BPA has been demonstrated to have both behavioral and morphologic effects through development and in adulthood.

* Corresponding author at: Department of Psychology, Sacred Heart University, 5151 Park Avenue, Fairfield, CT 06825, USA. Fax: +1 203 371 7998.
E-mail address: bowmanr@sacredheart.edu (R.E. Bowman).

http://dx.doi.org/10.1016/j.yhbeh.2014.12.007
0018-506X/

Table 1
Methodological timeline.



BPA has also been shown to alter the hypothalamic–pituitary–adrenal axis. Low dose exposure to BPA during the perinatal period increased corticosterone levels under both basal and stress conditions in adolescence (Panagiotidou et al., 2014; Poimenova et al., 2010), altered concentrations of hippocampal glucocorticoid and mineralocorticoid receptors and induced sex differences in plasma corticosterone levels at an earlier developmental age (pre-pubertal) than previously reported (Malendowicz and Mlynarczyk, 1982; Panagiotidou et al., 2014; Poimenova et al., 2010).

Only more recently have investigators turned their attention to the possible effects of BPA during the period of adolescence, which is characterized by hormonal changes, structural alterations in the brain and further programming of some sexually dimorphic behaviors (Juraska et al, 2013). Environmental stressors during this period have also been shown to affect adult behaviors and alter neural plasticity (Holder and Blaustein, 2014). We have previously demonstrated that short term, low-dose BPA adolescent exposure (below the current reference safe daily limit of 50 μg/kg day set by the United States Environmental Protection Agency, (U.S.E.P.A., 1993) increased anxiety on the elevated plus maze (EPM) and open field and impaired spatial memory on the OP task (Diaz Wienstein et al., 2013). In addition, we found that adolescent BPA exposure increased sucrose preference, and all of these BPA changes occurred independent of sex when tested during adolescence (Diaz Wienstein et al., 2013). Golgi impregnation studies demonstrated that BPA exposure during adolescence resulted in a decreased dendritic spine density on pyramidal cells in both the mPFC region and the CA1 region of the hippocampus during adolescence, an effect that persisted into adulthood (Bowman et al., 2014).

The current study was designed to answer several questions stemming from our previous two studies (Diaz Wienstein et al., 2013; Bowman et al., 2014) — specifically whether the behavioral and morphological changes observed in adolescence following adolescent BPA are maintained when treated subjects are evaluated at adulthood. We investigated whether BPA exposure during adolescence (postnatal days [PND] 42–49) alters anxiety, cognitive functioning, and sucrose preference in male and female rats tested in adulthood (11 weeks of age). Furthermore, whether adolescent BPA exposure alters serum corticosterone levels in response to a stress challenge was examined. Lastly, the current study determined whether possible differences in adult anxiety and memory following adolescent BPA exposure are accompanied by alterations in spine density in the mPFC and CA1 regions in adult male and female, BPA treated subjects.

Experimental procedures

Subjects

Thirty two experimentally naïve 5 week old Sprague Dawley rats (n = 16 males, n = 16 females) were obtained from Charles River Laboratories (Maryland, USA) and maintained on a 12/12-hr light/dark cycle (lights on 7:00 am). All experimental procedures were approved by the Sacred Heart University Institutional Animal Care and Use Committee and in accordance with the NIH Guide for the Care and Use of Animals. Subjects were double housed according to sex and treatment conditions in a common animal colony room, temperature regulated at 21.1 °C, and had free access to rat chow (Harlan 2018 Teklad Global) and water (Glass water bottles, Ancare Corporation, Bellmore, NY). All animals were weighed regularly. Table 1 illustrates the overall research design timeline.

Injections

Following a one-week acclimation period during which animals were allowed to adjust to the new housing conditions, male and female adolescent subjects (Andreollo et al., 2012; Kwekel et al., 2010; Quinn, 2005), now aged 6 weeks, were randomly assigned to either a control (vehicle only) or experimental group (BPA exposed). BPA (>99% purity grade) was obtained from Sigma-Aldrich Corp (St. Louis, MO). Each rat received a daily subcutaneous injection, 40 μg/kg bodyweight, at the nape of the neck for one week. The BPA was initially dissolved in ethanol for stock solutions and diluted with saline for the injection. While an important consideration when interpreting the current data is the subcutaneous method of BPA administration used compared to an oral route of exposure, the dosing paradigm used in the current study is consistent with previous, related work (Bowman et al., 2014; Diaz Wienstein et al., 2013; for review, Hajszan and Leranth, 2010).

Behavioral measures

Following injections, rats were allowed to mature to 11 weeks of age and then underwent a series of behavioral assessments. Open field (OF) testing was conducted first, followed by EPM, OP, OR, and finally sucrose preference testing. Behavioral measures were performed in designated behavioral testing rooms (21.1 °C, 43 lm/square meter) and all behavioral testing occurred between 9:00 and 14:00 h. Behavioral measures were obtained in real time by trained laboratory researchers who were blind to treatment assignment consistent with past studies (Bowman and Kelly, 2012; Bowman et al., 2001, 2002; Diaz Wienstein et al., 2013; Eilam-Stock et al., 2012). In addition, a paired sample *t*-test was conducted on open field measures scored in real-time versus from video for a group of animals (not otherwise used in the study, n = 5) and there are no differences between the two (see Table 2). Furthermore, inter-rater reliability for real-time open field measurements was assessed using a bivariate correlation. Positive significant correlations were obtained for outer crossings ($r(4) = .999$, $p = .000$), inner crossings ($r(4) = .991$, $p = .001$), total crossings ($r(4) = .998$, $p = .000$), wall climbs ($r(4) = .984$, $p = 000$), rears ($r(4) = .962$, $p = .009$), and grooms ($r(4) = .932$, $p = .021$). Behavioral testing occurred during PND 77–90.