Hormones and Behavior 107 (2019) 26–34

Contents lists available at ScienceDirect

Hormones and Behavior

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

Effects of adolescent Bisphenol-A exposure on memory and spine density in ovariectomized female rats: Adolescence vs adulthood

Rachel E. Bowman[a,*], Jennifer Hagedorn[a], Emma Madden[a], Maya Frankfurt[b]

[a] Department of Psychology, Sacred Heart University, Fairfield, CT 06825, United States of America
[b] Department of Science Education, Donald and Barbara Zucker School of Medicine at Hofstra/Northwell, Hempstead, NY 11549, United States of America

ARTICLE INFO

Keywords:
Adolescence
Bisphenol-A
Estradiol
Hippocampus
Prefrontal cortex
Dentate gyrus
Dendritic spine
Memory

ABSTRACT

The endocrine disruptor, Bisphenol-A (BPA), alters many behavioral and neural parameters in rodents. BPA administration to gonadally intact adolescent rats increases anxiety, impairs spatial memory, and decreases dendritic spine density when measured in adulthood. Since BPA's action seems to be mediated through gonadal steroid receptors, the current experiments were done in ovariectomized (OVX) female rats to examine the effects on behavior and spine density of adolescent BPA exposure under controlled hormone conditions. OVX (postnatal day, PND, 21) female Sprague-Dawley rats (n = 66) received subcutaneous injections of BPA (40 μg/kg/bodyweight), 17β-Estradiol (E2, 50 μg/kg/bodyweight), or saline during adolescence (PND 38–49). Following the last injection brains were processed for Golgi impregnation (Exp1), behavioral and spine density in adolescence (Exp2), or in adulthood (Exp3). In Exp1, E2 increased spine density in CA1 pyramidal cells and BPA decreased spine density in granule cells of the dentate gyrus (DG). In Exp2, BPA impaired spatial memory on the object placement (OP) task, E2 increased spine density in CA1, BPA decreased spine density in the DG and the medial prefrontal cortex (mPFC). When measured in adulthood (Exp3), BPA impaired OP and object recognition (OR) performance, E2 increased spine density in CA1, and BPA decreased spine density in CA1, the mPFC and the DG. Results provide novel data on the effects of adolescent BPA in an OVX model and are compared to data in intact animals and within the context of understanding the importance of the profound neuronal alterations occurring during adolescent development.

1. Introduction

Bisphenol-A (BPA), is a synthetic compound commonly found in plastics and other materials which has been demonstrated to have behavioral effects in laboratory animals and humans (for review see Mhaouty-Kodja et al., 2018). It has been suggested that the endocrine disrupter properties of BPA are primarily due to its ability to bind to both estrogen and androgen receptors in the CNS (Ahmed et al., 2018; Mhaouty-Kodja et al., 2018; Nesan et al., 2018). However it must be noted that BPA has also been shown to antagonize thyroid effects at the receptor level (Moriyama et al., 2002) as well as altering other endocrine systems (reviewed by Negri-Cesi, 2015). Many studies have demonstrated that administration of BPA to pre and postnatal rats results in impaired memory (Bowman et al., 2015; Diaz Weinstein et al., 2013; Eilam-Stock et al., 2012; Goncalves et al., 2010; Inagaki et al., 2012; Mhaouty-Kodja et al., 2018; Wang et al., 2014). The importance of synaptic plasticity in mediating the well-established effects of estrogen on memory (Frick et al., 2015; Luine et al., 2018; Luine, 2014; Tuscher et al., 2015) suggests that the mechanism underlying the behavioral effects of BPA also involve synaptic plasticity, which is supported in the literature (Elsworth et al., 2015; Hu et al., 2017; Mhaouty-Kodja et al., 2018; Ogiue-Ikeda et al., 2008). Moreover, several studies demonstrate that BPA can block gonadal steroid induction of dendritic spines in both the hippocampus (CA1) and the medial prefrontal cortex (mPFC) in the rat (Inagaki et al., 2012), and inhibit synaptogenesis in CA1 and the mPFC in both rats (Leranth et al., 2008b; MacLusky et al., 2005) and primates (Leranth et al., 2008a), two regions of the brain critical to memory.

Most studies on BPA have been conducted during the perinatal period. Our studies are designed to assess the potential effects of BPA exposure during the adolescent period when the brain is undergoing rapid neurodevelopmental changes and may be extremely vulnerable (Juraska et al., 2013; Schneider, 2013; Shapiro et al., 2017). To date we have assessed the effects of short-term, low-dose levels of BPA exposure during adolescent development, below the current reference safe daily limit of 50 μg/kg/day set by the United States Environmental Protection

* Corresponding author at: Department of Psychology, Sacred Heart University, 5151 Park Avenue, Fairfield, CT 06825, United States of America.
E-mail address: bowmanr@sacredheart.edu (R.E. Bowman).

https://doi.org/10.1016/j.yhbeh.2018.11.004
Received 26 July 2018; Received in revised form 9 November 2018; Accepted 14 November 2018
Available online 26 November 2018
0018-506X/

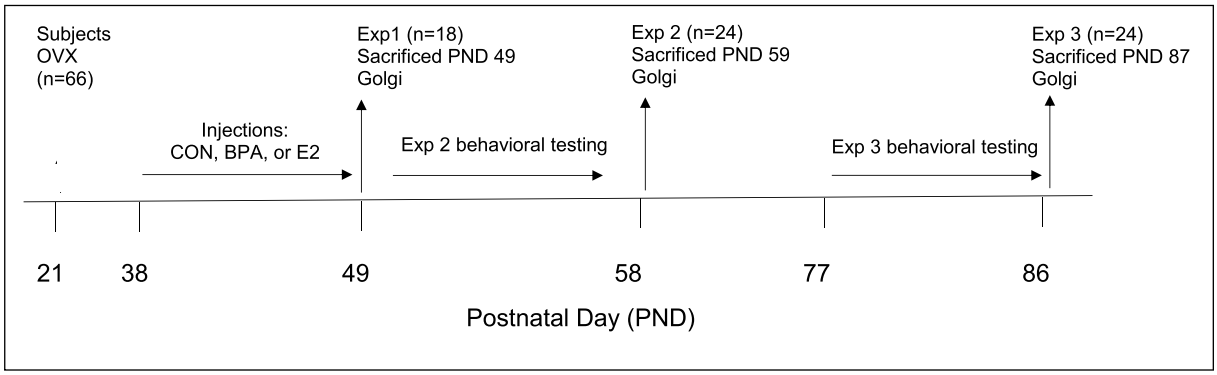

Fig. 1. Overview of experimental research design timeline.

Agency, (United States Environmental Protectional Agency, 1993) on intact adolescent and adult male and female rats. When administered and assessed in adolescence, BPA increased anxiety, impaired spatial memory (Diaz Weinstein et al., 2013) and decreased dendritic spine density in the mPFC and CA1 of both male and female rats (Bowman et al., 2014). Except for some sex differences, the behavioral and morphological changes observed in adolescence were maintained when adolescent BPA exposed subjects were assessed in adulthood (Bowman et al., 2015; Bowman et al., 2014).

Importantly, our previous studies were conducted in gonadally intact rats, which precluded assessing whether previously observed effects were due to BPA or natural fluctuations in gonadal hormones. Additionally, estrogen manipulation alters many aspects of brain structure and function including memory and dendritic spine density in adult and aging rats (Galea et al., 2017; Luine, 2016; Luine and Frankfurt, 2013; Luine, 2014; Scharfman et al., 2007). However, estrogen replacement studies in adolescence in ovariectomized (OVX) rats are limited. Thus, we investigated the effects of adolescent BPA exposure on behavioral measures and dendritic spine density in OVX female rats. The present series of studies was designed to directly compare the adolescent effects of BPA or estradiol on both behavior and dendritic spine density in adolescent and adult OVX female rats. In addition, since it is often hard to determine whether behavior alters spine density or the reverse, rats in the first experiment were sacrificed immediately after their last injection. Specifically, we examined adolescent BPA or estradiol exposure on the following: (1) dendritic spine density in the adolescent brain in the absence of behavior, (2) on behavioral measures and dendritic spine density in adolescence, and (3) behavioral measures and dendritic spine density in adulthood.

2. Materials and methods

2.1. Subjects

Experimentally naïve Sprague Dawley female rats (n = 66) were obtained from Charles River Laboratories (Raleigh, NC, USA). All subjects were bilaterally ovariectomized (OVX) under anesthesia (ketamine, 80 mg/kg bodyweight, and xylazine, 10 mg/kg bodyweight) by the vendor on post-natal day (PND) 21, allowed to recover, and arrived PND28. Subjects were double housed according to treatment condition in a common animal colony room, with temperature at 21.1 °C and maintained on a 12/12-h light/dark cycle (lights on 7:00 am), weighed weekly, had free access to rat chow (Harlan 2018 Teklad Global) and water (Glass water bottles, Ancare Corporation, Bellmore, NY). 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.

2.2. Injections

Subjects were randomly assigned to a control (vehicle control), BPA, or 17β-Estradiol (E2) treatment group. BPA (≥99% purity grade) and E2 (≥98% purity grade) were obtained from Sigma-Aldrich Corp (St. Louis, MO). Each rat received daily subcutaneous (SC) injections of BPA (40 μg/kg/bodyweight), 17β-Estradiol (E2, 50 μg/kg/bodyweight), or saline during adolescence (PND 38–49). The BPA and E2 were initially dissolved in ethanol and diluted with saline (BPA) or corn oil (E2) for the stock solutions (final ethanol concentration < 0.006%, Inagaki et al., 2012). In the present study SC injections of BPA were used because it has been shown that circulating BPA levels in neonatal mice are the same following oral or SC exposure (Taylor et al., 2008) and no differences were observed between SC and oral administration of BPA on the reduction in spine synapses in hippocampus and prefrontal cortex in adult rats (Hajszan and Leranth, 2010). Furthermore, the dosing paradigm used in the current study is the same as all of our previous BPA-related work (Bowman et al., 2015; Bowman et al., 2014; Diaz Weinstein et al., 2013). The internal dose equivalent associated with our dosing paradigm is approximately three ng/ml based on the Taylor et al. (2008) study.

2.3. Experimental timeline

Fig. 1 illustrates the overall research design timeline for the three experiments. In Experiment 1, a cohort of animals (n = 18) was sacrificed immediately following injections on PND 49. As described below, brains were processed for Golgi impregnation. In Experiment 2 (n = 24), injections were immediately followed by behavior testing during adolescence on PND 49–58. Subjects were sacrificed PND 59 and brains were processed for Golgi impregnation. In Experiment 3, subjects (n = 24) were aged until young adulthood and received behavioral testing PND 77–86. Subjects were sacrificed PND 87 and brains were processed for Golgi impregnation.

2.4. Behavioral measures

Behavioral measures were conducted in designated behavioral testing rooms (21.1 °C, 43 lm/m^2) and all behavioral testing occurred between 9:00 and 14:00 h. Animals received one behavioral test/day for ten days. The elevated plus maze (EPM) testing was conducted first (behavioral testing day 1), followed by open field (behavioral testing day 2), and object placement (OP, behavioral testing days 3–6) and object recognition (OR, behavioral testing day 7–10) trials. To minimize olfactory cues, all behavioral equipment was wiped down with disinfectant spray and allowed to dry between each individual trial/animal.

2.5. Elevated plus maze

Anxiety was measured using the EPM, which is 50.8 cm high and consists of two open arms, 50.8 × 12.7 cm, and two enclosed arms, 50.8 × 12.7 × 40.6 cm, with an open top. The two open arms and the two closed arms are arranged opposite one another around a 10.2 cm square center. Each rat received one trial on the EPM lasting 5 min during which the number of entries and duration of time spent in open and closed arms was recorded. An entry occurred when the animal placed both forelimbs and more than half of their trunk into the arm. The number of visits to and duration of time spent in open arms is used as an anxiety index, with fewer open arm visits and/or decreased time spent on open arms being indicative of increased anxious behavior.

2.6. Open field

All animals were tested on the OF for measures of locomotor activity (peripheral grid visits) and anxiety-related behaviors (central grid visits) (Tovote et al., 2015). Rats were placed one at a time in the OF from a common starting area of a 117 × 70 × 45 cm wooden open top box, with the floor divided into 23 cm square grids (5 × 3). Activity in the field was scored for 6 min. Behaviors recorded included the number of peripheral grid visits (movements across squares and a measure of general locomotor activity), central grid visits (an anxiety-related behavior), and total grid visits. Grid visits were scored when the subjects' full body, excluding tail, entered a grid.

2.7. Object placement and object recognition trials

Spatial memory was assessed using the OP task. All trials were conducted in an enclosed arena measuring 70 × 70 × 40 cm. The arena was wood with an open top. Three walls were painted grey and one wall was painted black and white striped which provided an intra-maze spatial cue. Trials consisted of a sample trial (T1) and a retention trial (T2), separated by an inter-trial delay. In T1, two identical objects were placed at one end of the open field and amount of time spent exploring the two objects was recorded for 3 min. For T2, one object was moved to a novel location within the field. In T2, the time spent exploring the object at the old location and the new location was recorded for 3 min. Thus, the percentage of time spent with the object in the new location during the total exploration time during T2 is used as an index of OP performance, (time with new location) / (time with old location + time with new location) × 100. This measure is referred to as Ratio. All animals received a 1 min delay acclimation trail (data not shown) and then were tested for OP memory with 10 min, 30 min, and 1 h inter-trial delays.

Non-spatial, visual memory was assessed using the OR task. The testing field was identical to that used for the OP trials with the exception that all four walls were painted grey. The OR task is identical to the OP procedure, except that during T2, the retention trial, one of the original objects is replaced with a new distinct object. Thus, the percentage of time spent with the novel object during the total exploration time during T2 is used as an index of OR performance. Subjects received a 1 min delay acclimation trail (data not shown) and were then tested for object recognition memory with 10 min, 30 min, and 1 h inter-trial delays.

During OP and OR trials, object exploration was defined as subjects sniffing, whisking, or visually examining the object from no > 2 cm away. The objects used for trials included identical pairs of bottles, cans, ceramic and glass figures. The new location/object was counterbalanced across treatment.

2.8. Golgi impregnation

Dendritic spine density was measured in brain regions known to be involved in spatial memory (the hippocampus) and visual working memory (the mPFC). At sacrifice, brains were removed from subjects, cut into an anterior block (anterior to the optic chiasm) and posterior block (between the optic chiasm and the brainstem), and placed in solutions provided in the Rapid Golgi Stain Kit (FD NeuroTechnologies, Ellicott City, MD). Golgi impregnation was performed as previously described (Frankfurt et al., 2011; Inagaki et al., 2012). Secondary basal dendrites and tertiary apical dendrites were analyzed blindly from pyramidal cells from the CA1 region of the dorsal hippocampus and layer II/III of the prelimbic portion of the mPFC and granule cells of the suprapyramidal blade of the dentate gyrus (DG). Six cells per region/brain were included in the analysis and approximately 6 brains were quantified per group. Neurons in all areas were chosen for analyses as follows: (1) cell bodies and dendrites were well impregnated; (2) dendrites were clearly distinguishable from adjacent cells and continuous. Spines were counted under oil (100×) using a hand counter and dendritic length measured using the Olympus CellSens 1.16 software and an Olympus BX-41 microscope. Spine density was calculated by dividing spine number by the length of the dendrite and data expressed as number of spines/10 μm dendrite.

2.9. Data analysis

Data were analyzed using NCSS software (Kaysville, UT, USA). One-way (treatment), between-subject ANOVAs were used to test for group differences. Type I error rate was set at 0.05. Effect sizes were calculated using Eta-squared and Tukey-Kramer Multiple-Comparison Tests were used for post-hoc analysis, where appropriate.

3. Results

3.1. Experiment 1

In experiment 1, subjects were sacrificed immediately following the last injection (PND49) in adolescence.

3.1.1. Golgi analysis

As shown in Fig. 2, adolescent hormonal manipulations altered dendritic spine density in a region-specific manner. In the hippocampus on pyramidal cells in CA1, spine density was significantly increased by E2 treatment compared to CON and BPA groups, on both basal, $F(2,16) = 7.49$, $p < 0.006$ $\eta^2 = 0.52$, and apical dendrites, $F(2,16) = 6.89$, $p < 0.008$ $\eta^2 = 0.50$. In the DG, BPA decreased dendritic spine density on granule cells when compared to CON and E2 groups, $F(2,16) = 9.43$, $p < 0.003$ $\eta^2 = 0.57$. No significant changes were observed in the mPFC, $p > 0.05$.

3.2. Experiment 2

In experiment 2, adolescent exposure to E2, BPA, or CON was followed by behavioral testing PND 49–58. Subjects were sacrificed in adolescence, PND 59.

3.2.1. Behavioral measures

3.2.1.1. Elevated plus maze. As shown in Table 1, there were no differences between the groups for the number of visits to open, $F(2,22) = 0.27$, $p = 0.77$ $\eta^2 = 0.03$, or closed arms, $F(2,22) = 2.91$,$p = 0.08$ $\eta^2 = 0.23$. There were also no significant differences in the amount of time spent on open, $F(2,22) = 1.11$, $p = 0.35$ $\eta^2 = 0.09$, or closed, $F(2,22) = 0.71$, $p = 0.50$ $\eta^2 = 0.07$, arms.

3.2.1.2. Open field. Also shown in Table 1, is the OF data. There were no significant effects of adolescent hormone exposure on the number of peripheral, $F(2,23) = 2.22$, $p = 0.13$ $\eta^2 = 0.17$, central, $F(2,23) = 1.31$, $p = 0.29$ $\eta^2 = 0.11$, or total, $F(2,23) = 2.16$, $p = 0.14$ $\eta^2 = 0.17$, grid crossings.

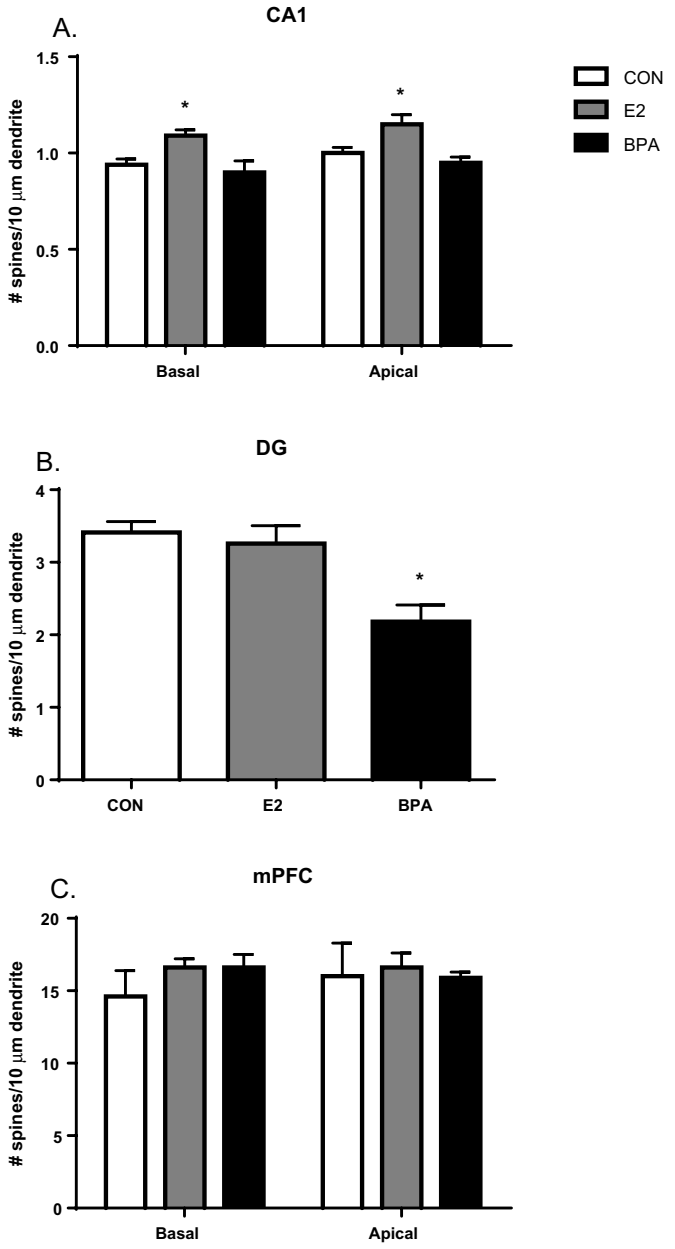

Fig. 2. Effects of adolescent hormone exposure on dendritic spine density in adolescence, in the absence of behavioral testing. Entries are the average # spines/10 μm ± SEM. All significant effects are $p < 0.05$ and group differences are denoted by *. (Panel A) Adolescent E2 exposure led to increased spine density on both basal and apical dendrites in CA1. (Panel B) BPA decreased dendritic spines on granule cells. There were no significant effects of adolescent hormone exposure in mPFC (Panel C).

3.2.1.3. Object placement. There were no differences in exploration times during the sample (T1) or retention (T2) trials between the groups for any of the OP inter-trial delays (data not shown, $p > 0.05$). Fig. 3A shows percentage of time spent with the object in the new location (ratio) across inter-delay trials. While there were no effects of adolescent hormone exposure on spatial memory at the 10 min inter-trial delay, BPA exposure significantly impaired spatial memory compared to the CON and E2 groups at the 30 min, $F(2,22) = 6.45$, $p = 0.007$ $\eta^2 = 0.39$, and 1 h, $F(2,22) = 9.13$, $p = 0.002$ $\eta^2 = 0.48$ delays.

3.2.1.4. Object recognition. There were no differences in exploration times during the sample (T1) across any of the OR inter-trial delays (data not shown, $p > 0.05$); however, as shown in 3B, a significant treatment effect was observed during the retention (T2) trials of the 10 min, $F(2,21) = 4.23$, $p < 0.03$ $\eta^2 = 0.31$, and 1 h, $F(2,20) = 4.47$, $p = 0.03$ $\eta^2 = 0.33$, inter-trial delays. Post-hoc testing revealed that E2 decreased T2 exploration times compared to CON (10 min delay) and both CON and BPA (1 h delay). Fig. 3B shows percentage of time spent with the new object (ratio) across the inter-delay trials. All groups had intact non-spatial working memory (i.e., ratios above chance performance) and there were no significant group differences, $p > 0.05$, regardless of adolescent hormone exposure. Thus, E2 treatment altered T2 exploration but did not alter performance of the OR task.

3.2.2. Golgi analysis

Fig. 4 is a photomicrograph illustrating Golgi impregnated dendrites on granule cells in BPA and CON treated subjects and Fig. 5 shows the region-specific effects of adolescent hormone treatment exposure on dendritic spine density following adolescent behavior testing. The same pattern in dendritic spine density observed in experiment 1 was observed in experiment 2 (Fig. 5). E2 treatment significantly increased spine density in CA1 compared to CON and BPA groups on both basal, $F(2,23) = 5.36$, $p < 0.02$ $\eta^2 = 0.34$, and apical, $F(2,23) = 16.57$, $p < 0.0001$ $\eta^2 = 0.61$, dendrites. In the DG, BPA decreased dendritic spine density compared to CON, but not E2 groups, $F(2,16) = 4.4$, $p < 0.03$ $\eta^2 = 0.39$. BPA exposure decreased spine density in the mPFC on both basal, $F(2,21) = 33.87$, $p < 0.0001$ $\eta^2 = 0.78$, and apical, $F(2,21) = 80.56$, $p < 0.0001$ $\eta^2 = 0.90$, compared to both CON and E2 groups.

3.3. Experiment 3

In experiment 3, adolescent exposure to E2, BPA, or CON was followed by behavioral testing in young adulthood PND 77-86 and subjects were sacrificed PND 87.

3.3.1. Elevated plus maze

As shown in Table 1, adolescent hormone treatment exposure did not impact the number of visits to open $F(2,22) = 3.12$, $p = 0.07$ $\eta^2 = 0.23$, or closed arms, $F(2,22) = 0.79$,$p = 0.47$ $\eta^2 = 0.07$, or the amount of time spent on open arms, $F(2,22) = 1.7$, $p = 0.21$ $\eta^2 = 0.15$ and closed arms, $F(2,22) = 3.32$, $p = 0.11$ $\eta^2 = 0.20$.

3.3.2. Open field

Also shown in Table 1, is the OF data. There were no significant effects of adolescent hormone exposure on the number of peripheral, $F(2,23) = 1.87$, $p = 0.18$ $\eta^2 = 0.15$, central, $F(2,23) = 0.38$, $p = 0.68$ $\eta^2 = 0.04$, or total, $F(2,23) = 1.76$, $p = 0.20$ $\eta^2 = 0.14$, grid crossings when measured in adulthood.

3.3.3. Object placement

There were no differences in exploration times during the sample (T1) or retention (T2) trials between the groups for any of the OP inter-trial delays (data not shown, $p > 0.05$). Fig. 6A shows percentage of time spent with the object in the new location (ratio) across inter-delay trials. BPA exposure significantly impaired spatial memory compared to the CON and E2 groups at the 10 min, $F(2,23) = 8.35$, $p = 0.002$ $\eta^2 = 0.44$, but not the 30 min ($p = 0.068$) or 1 h ($p = 0.065$) delays.

3.3.4. Object recognition

There were no differences in exploration times during the sample (T1) or retention (T2) trials between the groups for any of the OR inter-trial delays (data not shown, $p > 0.05$). Fig. 6B shows percentage of time spent with the new object (ratio) across inter-delay trials. There were no differences between the treatment groups at the 10 min inter-trial delay; however, adolescent BPA exposure lowered non-spatial working memory performance at both the 30 min, $F(2,23) = 5.05$,

Table 1
Adolescent hormone exposure does not alter EPM and OF behaviors in adolescence (exp 2) and young adulthood (exp 3).

	Elevated plus maze				Open field		
	Number of visits		Time spent (secs)		Number of grid crossings		
	Open arms	Closed arms	Open arms	Closed arms	Peripheral	Central	Total
Experiment 2:							
CON	3.89 ± 0.9	11.0 ± 0.7	36.8 ± 8.8	200.0 ± 8.8	104.5 ± 11.3	5.9 ± 1.2	110.4 ± 12.5
E2	3.63 ± 0.8	7.25 ± 1.3	60.8 ± 14.3	186.9 ± 10.0	63.4 ± 15.0	2.4 ± 0.8	65.8 ± 15.7
BPA	4.57 ± 1.1	8.86 ± 1.3	45.6 ± 11.4	201.9 ± 10.5	78.9 ± 15.2	5.3 ± 2.4	83.9 ± 17.3
Experiment 3:							
CON	2.71 ± 0.4	8.9 ± 1.1	39.1 ± 9.7	197.0 ± 12.4	85.6 ± 11.1	3.1 ± 1.2	88.8 ± 11.7
E2	5.13 ± 0.9	10.3 ± 0.8	69.4 ± 12.9	155.6 ± 9.4	97.0 ± 13.5	4.3 ± 2.2	101.3 ± 15.1
BPA	5.25 ± 0.9	10.6 ± 1.2	58.3 ± 11.2	179.5 ± 16.1	65.9 ± 9.7	2.4 ± 0.8	68.3 ± 10.3

Data is the average ± SEM number of entries made and the time spent in the open versus closed arms during a 5 min EPM trial and the number of grid crossings made during a 6 min OF trial. Adolescent hormone exposure did not alter any EPM or OF measures when measured in adolescence (Experiment 2) or adulthood (Experiment 3), all $p > 0.05$.

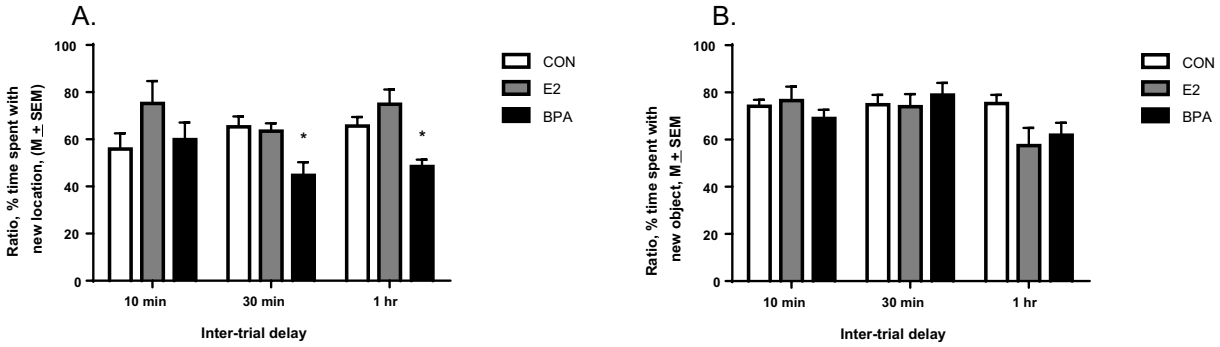

Fig. 3. OR and OP performance when measured in adolescence. Data are expressed as the percent of total T2 time spent with the object in the new location (OP, Panel A) and as the percent of total T2 time spent with the new object (OR, Panel B). All significant effects are $p < 0.05$ and significant differences between groups are denoted by *. Adolescent BPA exposure decreased object placement (Panel A), but not object recognition (Panel B) performance.

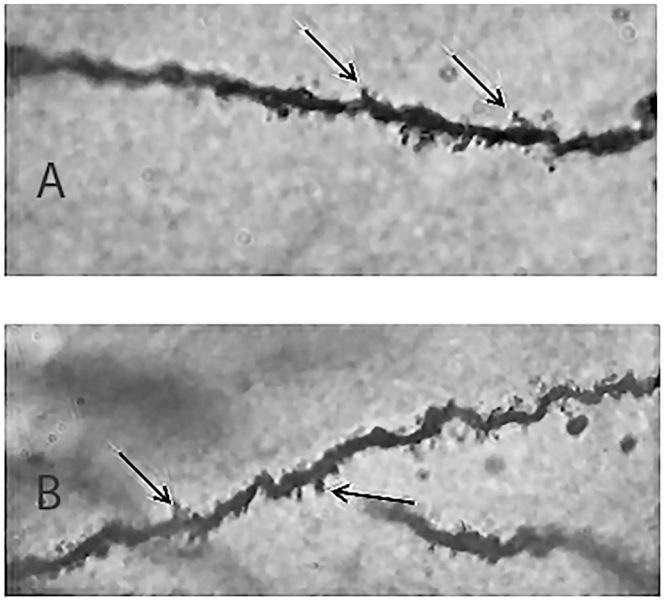

Fig. 4. Photomicrograph illustrating Golgi impregnated dendrites on granule cells from experiment 2. Top: BPA, Bottom: Control. Taken under oil at 100×. Arrows denote spines.

$p = 0.02$ $\eta^2 = 0.32$, and 1 h, $F(2,23) = 19.31$, $p = 0.0001$ $\eta^2 = 0.65$, inter-trial delays.

3.3.5. Golgi analysis

Fig. 7 shows the region-specific effects of adolescent hormone treatment exposure on dendritic spine density following behavior testing in adulthood. CA1 basal dendritic spine density was increased by E2 and decreased by BPA exposure compared to CON, $F(2,15) = 11.9$, $p < 0.001$ $\eta^2 = 0.65$. CA1 apical dendritic spine density was decreased by BPA, $F(2,15) = 5.59$, $p < 0.02$ $\eta^2 = 0.46$, compared to E2 but not CON. In the DG, the same effect in experiment 2 was observed, BPA decreased dendritic spine density on granule cells compared to CON, but not E2 groups, $F(2,22) = 4.3$, $p < 0.03$ $\eta^2 = 0.30$. No group differences in spine density where observed on mPFC basal dendrites ($p > 0.05$); however, BPA exposure decreased apical dendritic spine density, $F(2,17) = 9.4$, $p < 0.002$ $\eta^2 = 0.56$, compared to E2 but not CON.

4. Discussion

In the present study, OVX rats were used to assess the effects of adolescent BPA or E2 in three experiments. In the first experiment, spine density was assessed in the absence of behavior. In the next two experiments, the effects of adolescent BPA or E2 on behavior and dendritic spine density were analyzed at two developmental time points (adolescence versus adulthood). The overall results demonstrate that BPA administration in adolescence in the OVX rat has similar effects to those we have previously described for gonadally intact rats for

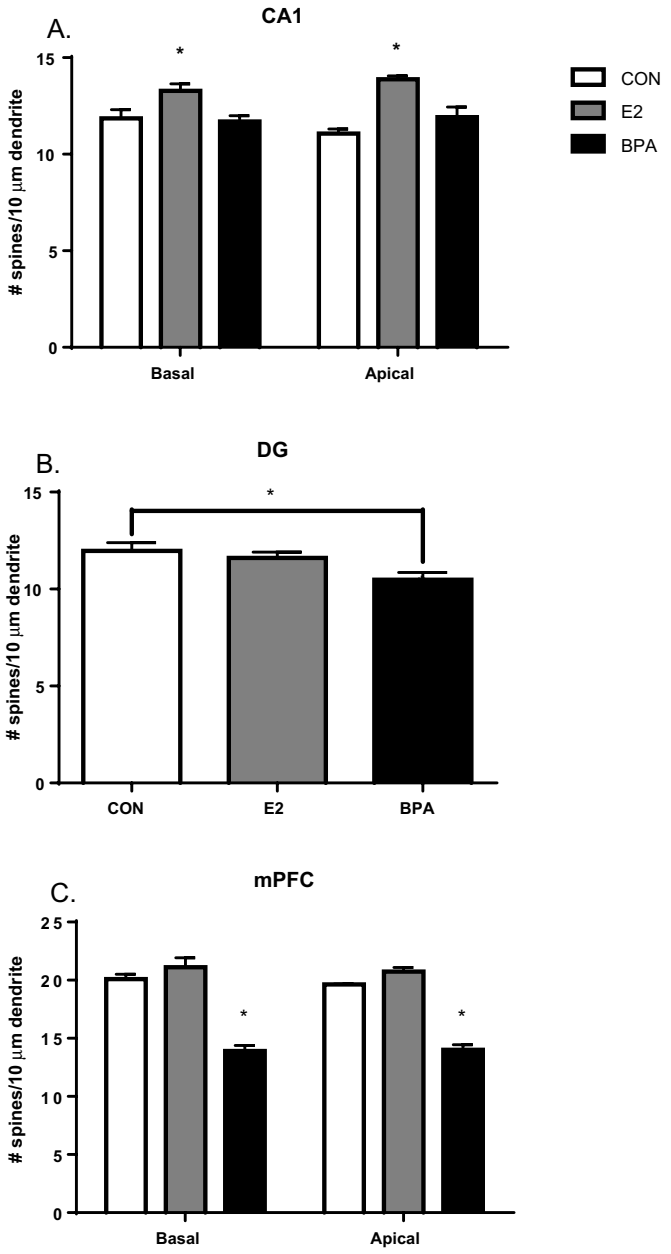

Fig. 5. Adolescent hormone exposure effects on dendritic spine density when measured following adolescent behavioral testing. Entries are the average # spines/10 μm ± SEM. All significant effects are $p < 0.05$ and group differences are denoted by *. (Panel A) E2 exposure increased CA1 basal and apical dendritic spine density. (Panel B) BPA decreased dendritic spines on granule cells compared to CON. (Panel C) Basal and apical mPFC dendritic spine density was decreased by BPA exposure.

memory and dendritic spine density (Bowman et al., 2015; Bowman et al., 2014; Diaz Weinstein et al., 2013) as well as some differences that are discussed below (see Table 2). As described in the Methods, behavioral testing was conducted during the light phase in order to remain consistent with all previously reported BPA data from our lab (Bowman et al., 2015; Bowman et al., 2014; Diaz Weinstein et al., 2013) and should be interpreted with this in mind. The present results also suggest that the interaction between E2 and BPA differs in the adolescent and adult brain.

In intact females, we have previously demonstrated that exposure to BPA during adolescence increased anxiety, impaired spatial memory in OP, and decreased dendritic spine density in the mPFC and CA1 when measured during adolescence (Bowman et al., 2015; Diaz Weinstein et al., 2013). Results in the OVX model (data from experiment 2) differ from the intact females in that OVX rats show no group differences on any measure of anxiety or activity (EPM and OF) after BPA. This suggests that the E2 present in intact animals might increase anxiety or that there is a complex interaction between E2 and adolescent brain development that is not clear. Studies by Juraska and colleagues (reviewed in Juraska and Willing, 2017) have shown that female rats exhibit a larger decrease in the number of neurons in the PFC, as well as dendritic complexity than male rats during puberty. Thus, in the present study, the fact that exogenous administration of E2 to OVX rats also had no effect on anxiety may result from a complex process of hormonal influences during puberty that cannot be replicated in the OVX model by simply replacing E2.

Unlike the measures of anxiety, spatial memory was impaired following adolescent BPA exposure at both the 30 min and 1 h inter-trial delays in the OVX rats, which is similar to the BPA effect observed in intact animals (Diaz Weinstein et al., 2013). Furthermore, the current results show that E2 treated OVX females have spatial memory performance that does not differ from controls. The importance of E2 in enhancing spatial memory is well documented (Luine et al., 2018; Luine, 2014) and the intact performance of spatial memory in E2 treated OVX subjects is indicative of this effect. In adult rats, OVX without E2 replacement impairs spatial memory (Wallace et al., 2006) and this differs from the results in the present study in which OVX rats without E2 replacement did not show any spatial memory impairments. It is interesting to speculate that differences between adolescent and adult females after OVX might be due to differences between organizational and activational effects of E2 (Koebele and Bimonte-Nelson, 2015), such that in intact rats there is a lasting organizational effect of estrogen on the brain. Alternatively, this may be an example of the complex interaction between E2 and the adolescent brain that requires further investigation.

In contrast to spatial memory, OR performance was intact for all treatment groups across all trials. This is a novel finding, as OR was not assessed in our previous study on intact female rats (Diaz Weinstein et al., 2013) and, to date, no other reports on adolescent BPA or E2 treatment effects on adolescent measures of OR have been reported. In general OR has been associated with the prefrontal cortex which underdoes plasticity for longer periods than other brain areas (Kolb et al., 2012) and it seems conceivable that the lack of group differences observed in adolescent rats may be due to the ongoing prefrontal cortex development. It is possible that the extensive neural plasticity during adolescence is potentially masking BPA or E2 effects. This possibility is supported by the results in experiment 3 where both the CON and E2 OVX groups performed even better than in adolescence. The average OR ratio across trials increased by 4% and 11%, for CON and E2 groups, respectively in adulthood (compared to average performance when measured in adolescence) and there was a significant BPA induced decrease in OR (see below).

As previously stated, spatial memory performance in adulthood continued to be impaired by adolescent BPA exposure when examined at the 10-min inter-trial delay, and while not significant ($p = 0.07$) the same trend was observed at longer delays (similar to experiment 2). Thus, adolescent BPA in OVX subjects impairs spatial memory in adolescence and, while modified, these impairments persist into adulthood. The effects of BPA on spatial memory in OVX adults are similar to those in adult intact animals in that the impairment of spatial memory is no longer significant in adulthood when measured at longer delays (e.g., 30 min) (Bowman et al., 2015). Overall, it appears that adolescent BPA exposure impairs spatial memory in adolescence and these impairments persist only at short inter-trial delays in adulthood, for both intact and OVX females. One possible explanation is that BPA's deleterious effects on spatial memory are declining over time and future studies might address this possibility by aging adolescent exposed subjects longer prior to behavioral testing to examine when/if BPA's effect attenuate

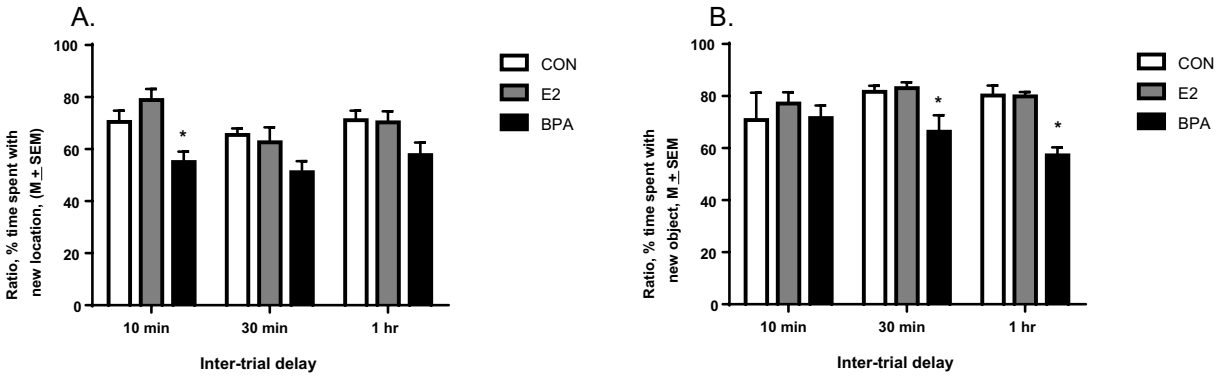

Fig. 6. OP and OR performance when measured in adulthood. Data are expressed as the percent of total T2 time spent with the object in the new location (OP, Panel A) and as the percent of total T2 time spent with the new object (OR, Panel B). All significant effects are $p < 0.05$ and significant differences between groups are denoted by *. Adolescent BPA exposure decreased object placement at short, but not long delays (Panel A), and decreased the percentage of time spent with the new object during both the 30 min and 1 h OR trials (Panel B).

across time.

Because E2 is known to enhance spatial memory, the finding that OVX controls continue to successfully discriminate between the old and new locations (in the long-term absence of E2) it is of particular interest. Future studies in our lab will consider the role of non-ovarian sources of E2, such as adipose tissue. Due to the sustained spatial memory performance of the controls, there is no enhancing effect of E2 and this is the same effect observed when measured in adolescence (experiment 2).

Adult OR performance was impaired by adolescent BPA treatment, an effect not seen when measured in adolescence (experiment 2). This BPA impairment in OVX subjects is different from that in intact adults in which BPA treated males, but not females, were unable to discriminate between the old and new objects at a 30 min inter-trial delay (Bowman et al., 2015). In the context of the present data, the difference between OVX female adolescent and adult OR performance supports the idea that maturation of the prefrontal cortex may be important for non-spatial working memory. The difference between OVX and intact females suggests that in adult intact female rats BPA and E2 interact differently than in adolescence.

The neural basis for this differential effect of BPA on memory performance in intact vs. OVX and in adolescence vs. adulthood is unknown, but changes in dendritic spine density may be a contributing factor. In all three experiments in this study, E2 increased dendritic spines on CA1 pyramidal cells. This E2 replacement effect in CA1 has been demonstrated in adult rats following OVX (Frankfurt and Luine, 2015; Gould et al., 1990) and therefore it is interesting to note the same effect when female rats are OVX at such an early age.

BPA had no effect on CA1 dendritic spine density in Experiment 1 or Experiment 2 (adolescence) but decreased spine density in CA1 in adulthood. The BPA induced decrease in adults OVX rats is consistent with earlier reports in adult intact male and female rats (Bowman et al., 2015; Bowman et al., 2014; Eilam-Stock et al., 2012) and OVX adult rats (Inagaki et al., 2012). BPA also induced decreases in synaptic density in CA1 in both adult rats (MacLusky et al., 2005) and monkeys (Elsworth et al., 2013) when administered in the prenatal period. Interestingly, BPA administration to juvenile monkeys did not alter synapse density in CA1 or the prefrontal cortex (Elsworth et al., 2013) supporting the idea that the interaction between development and gonadal state are critical factors to BPA effects. However, it is important to note that there may be other factors confounding the BPA results obtained during adolescent exposure after OVX. The process of ovariectomy at day 21 may have more profound effects on adolescence than simply removing estrogens. The stress of the surgical procedure and/or the exposure to anesthetic may alter spine density. In addition, the rats in the present experiment were fed rat chow that contains phytoestrogens which may counteract the effects of OVX. Thus, the actual effects of OVX on the adolescent brain require further investigation and may confound our BPA results.

BPA decreased dendritic spine density in CA1 in intact rats during adolescence (Bowman et al., 2014) but not in OVX rats in adolescence. In contrast, BPA decreased spine density in both OVX and intact adults (Bowman et al., 2015) suggesting that development may be more important than gonadal state with respect to BPA. In support of this, Chen et al. (2018) recently found that extensive dendritic remodeling occurs in CA1 pyramidal cells from day 35 to 55 in intact female rats. In addition, although the number of spines was unchanged, there were more mature spines later in later adolescence than early adolescence. Another important consideration is that BPA effects may be mediated by other endocrine systems such as the thyroid axis (Moriyama et al., 2002) Taken together it appears that development of CA1 during adolescence may not be dependent on E2 and that the effects of BPA in the adolescent brain are not necessarily E2 dependent in every situation. Future studies will address the potential interaction between E2 and BPA in OVX adolescent rats.

Results in the mPFC are somewhat different to those in CA1. In the current study E2 had no effect on dendritic spine density in mPFC when measured immediately in experiment 1 and decreased dendritic spine density in both adolescence and adulthood when behavior was tested (experiments 2 and 3, respectively). The decreased spine density after BPA in the PFC in OVX rats is similar to what was seen in adolescent intact rats (Bowman et al., 2014) but differs from our results in adult intact rats where BPA had no effect (Bowman et al., 2015). In apparent contrast to the present BPA induced decrease in spine density in the adolescent mPFC, no change in synaptic density was seen in the prefrontal cortex in juvenile monkeys treated with BPA (Elsworth et al., 2013). This may reflect differences between assessing spine and synapse density or may be related to evolutionary differences in prefrontal cortex circuitry.

Observed differences between adolescent and adult effects of BPA on dendritic spines may be due to the ongoing pruning that occurs in the prefrontal cortex (Kolb et al., 2012). Indeed, Shapiro et al. (2017) have recently reviewed the changes that occur in several cytoskeletal proteins associated with synaptic plasticity in the prefrontal cortex in female mice. During adolescence there were differential changes in TRKb receptors, BDNF, PSD -95 and synaptophysin in different prefrontal cortex sub regions. This is of particular interest because many cytoskeletal proteins have been shown to be altered by estrogens (Frick, 2015) and therefore one may speculate that differences between BPA's effects on intact and OVX rats may be due to gonadal steroid interaction with pruning in the mPFC during adolescence.

Unlike CA1 and the mPFC, spine density in the DG following BPA

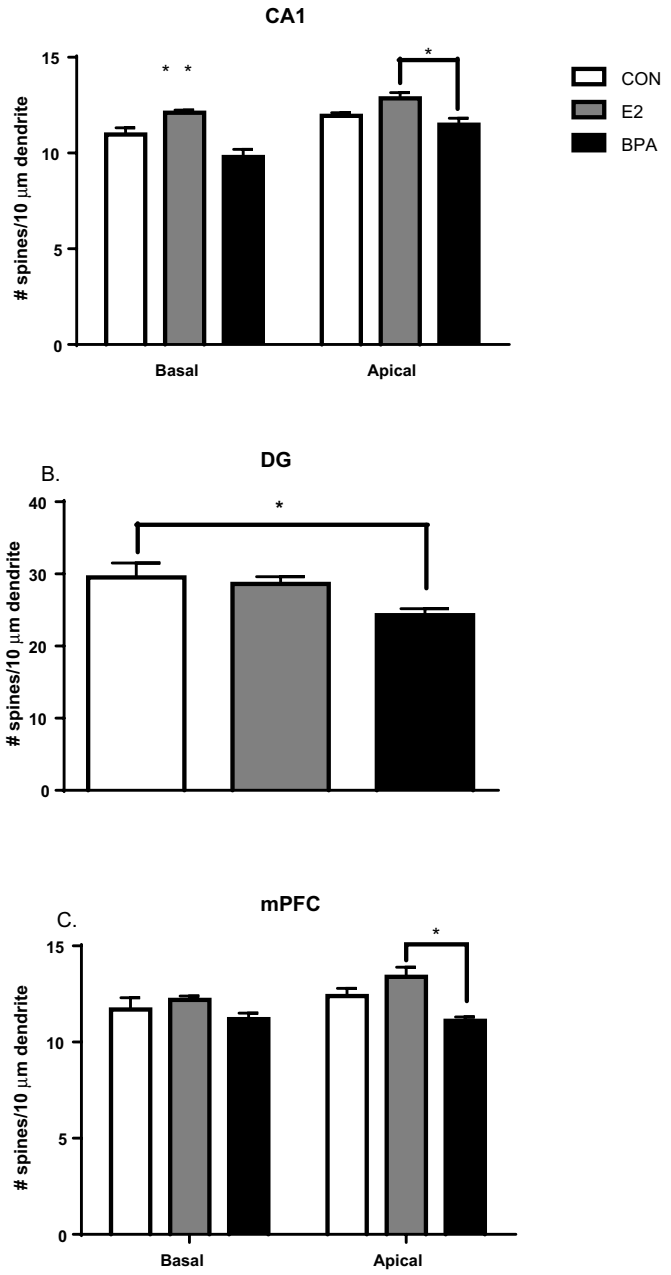

Fig. 7. Adolescent hormone exposure effects on dendritic spine density when measured following behavioral testing in adulthood. Entries are the average # spines/10 μm ± SEM. All significant effects are $p < 0.05$ and group differences are denoted by *. (Panel A) E2 increased and BPA decreased CA1 basal dendritic spine density compared to CON and BPA decreased CA apical compared to E2. (Panel B) BPA decreased dendritic spines on granule cells compared to CON. (Panel C) Apical dendritic spine density was decreased by BPA compared to E2 and there were no effects on basal dendrites.

has not been well studied. In all three experiments in the present study, adolescent BPA decreased spine density compared to both CON and E2 groups. This decrease in spine density is consistent with the finding that perinatal BPA decreases synaptic density in a dose dependent manner in both intact adult male and female rats (Liu et al., 2016). However, to our knowledge, this is the first report of BPA induced decreases in spine density in the DG in the adolescent brain. Moreover, the BPA effect on the DG was consistent with and without behavior in all three experiments. The DG is a region that is known to exhibit active neurogenesis which may explain why it is more vulnerable to BPA.

Lastly the potential effects of behavior on spine density, independent of BPA must be addressed. In both the DG and CA1 spine density was the same independent of behavior. Not so in the PFC, where decreases in spine density were only seen after behavior. Although this may be due to regional differences in behavior mediation it may also be further evidence that the maturation of the mPFC during adolescence makes it particularly sensitive to stimuli.

Table 2
Comparison of BPA effects on intact and OVX females in adolescence and adulthood.

Behavior	Adolescent	Adult	Spine density	Adolescent	Adult	No behavior
OVX						
EPM	NC	↑	CA1	NC	↓	NC
OF	NC	NC	PFC	↓	↓	NC
OP	↓ (30 and 60 min)	↓ (10 min)	DG	↓	↓	↓
OR	NC	↓ (30 and 60 min)				
Intact						
EPM	↑ anxiety[a]	NC[c]	CA1	↓[b]	↓[c]	NA
OF	↑ anxiety[a]	NC[c]	PFC	↓[b]	NC[c]	NA
OP	↓[a]	NC[c]	DG	NA[b]	NA	NA
OR	NA	NC[c]				

NC = no change, NA = not assessed.
[a] Diaz Weinstein et al., 2013.
[b] Bowman et al., 2014.
[c] Bowman et al., 2015.

5. Conclusion

Together, adolescent BPA appears to have differential effects on spatial versus non-spatial working memory and these effects are maintained in intact and OVX rats. In addition, BPA exposure has differential effects on spatial memory in adolescence and adulthood. While it is difficult to reconcile alterations in spine density with every behavioral effect, spine density in key regions is decreased by adolescent BPA but the decreases in spine density seem to be tempered during the complex process of neural plasticity that occurs though adolescence. As recent studies show that urinary BPA is detectable in > 95% of sampled Americans (Lehmler et al., 2018) it seems imperative to better understand the behavioral and neuronal implications of low-dose exposure during adolescence when the brain is particularly developmentally vulnerable.

Declaration of interests

The authors have none.

Acknowledgements

This work was supported by Sacred Heart University Undergraduate Research Initiative grants to J. Hagedorn and E. Madden.

References

Ahmed, R.G., Walaa, G.H., Asmaa, F.S., 2018. Suppressive effects of neonatal bisphenol A on the neuroendocrine system. Toxicol. Ind. Health 36 (6), 397–407.
Bowman, R.E., Luine, V., Khandaker, H., Villafane, J.J., Frankfurt, M., 2014. Adolescent bisphenol-A exposure decreases dendritic spine density: role of sex and age. Synapse 68, 498–507.
Bowman, R.E., Luine, V., Diaz Weinstein, S., Khandaker, H., DeWolf, S., Frankfurt, M., 2015. Bisphenol-A exposure during adolescence leads to enduring alterations in cognition and dendritic spine density in adult male and female rats. Horm. Behav. 69, 89–97.
Chen, Y.W., Akad, A., Aderogba, R., Chowdhury, T.G., Aoki, C., 2018. Dendrites of the dorsal and ventral hippocampal CA1 pyramidal neurons of singly housed female rats exhibit lamina-specific growths and retractions during adolescence that are responsive to pair housing. Synapse 72, e22034.

Diaz Weinstein, S., Villafane, J.J., Juliano, N., Bowman, R.E., 2013. Adolescent exposure to bisphenol-A increases anxiety and sucrose preference but impairs spatial memory in rats independent of sex. Brain Res. 1529, 56–65.
Eilam-Stock, T., Serrano, P., Frankfurt, M., Luine, V., 2012. Bisphenol-A impairs memory and reduces dendritic spine density in adult male rats. Behav. Neurosci. 126, 175–185.
Elsworth, J.D., Jentsch, J.D., Vandevoort, C.A., Roth, R.H., Redmond Jr., D.E., Leranth, C., 2013. Prenatal exposure to bisphenol A impacts midbrain dopamine neurons and hippocampal spine synapses in non-human primates. Neurotoxicology 35, 113–120.
Elsworth, J.D., Jentsch, J.D., Groman, S.M., Roth, R.H., Redmond, E.D., Leranth, C., 2015. Low circulating levels of bisphenol-A induce cognitive deficits and loss of asymmetric spine synapses in dorsolateral prefrontal cortex and hippocampus of adult male monkeys. J. Comp. Neurol. 523, 1248–1257.
Frankfurt, M., Luine, V., 2015. The evolving role of dendritic spines and memory: interaction(s) with estradiol. Horm. Behav. 74, 28–36.
Frankfurt, M., Salas-Ramirez, K., Friedman, E., Luine, V., 2011. Cocaine alters dendritic spine density in cortical and subcortical brain regions of the postpartum and virgin female rat. Synapse 65, 955–961.
Frick, K.M., 2015. Molecular mechanisms underlying the memory-enhancing effects of estradiol. Horm. Behav. 74, 4–18.
Frick, K.M., Kim, J., Tuscher, J.J., Fortress, A.M., 2015. Sex steroid hormones matter for learning and memory: estrogenic regulation of hippocampal function in male and female rodents. Learn. Mem. 22, 472–493.
Galea, L.A.M., Frick, K.M., Hampson, E., Sohrabji, F., Choleris, E., 2017. Why estrogens matter for behavior and brain health. Neurosci. Biobehav. Rev. 76, 363–379.
Goncalves, C.R., Cunha, R.W., Barros, D.M., Martinez, P.E., 2010. Effects of prenatal and postnatal exposure to a low dose of bisphenol A on behavior and memory in rats. Environ. Toxicol. Pharmacol. 30, 195–201.
Gould, E., Woolley, C.S., Frankfurt, M., McEwen, B.S., 1990. Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood. J. Neurosci. 10, 1286–1291.
Hajszan, T., Leranth, C., 2010. Bisphenol A interferes with synaptic remodeling. Front. Neuroendocrinol. 31, 519–530.
Hu, F., Li, T., Gong, H., Chen, Z., Jin, Y., Xu, G., Wang, M., 2017. Bisphenol A impairs synaptic plasticity by both pre- and postsynaptic mechanisms. Adv. Sci. (Weinh) 4, 1600493.
Inagaki, T., Frankfurt, M., Luine, V., 2012. Estrogen-induced memory enhancements are blocked by acute bisphenol A in adult female rats: role of dendritic spines. Endocrinology 153, 3357–3367.
Juraska, J.M., Willing, J., 2017. Pubertal onset as a critical transition for neural development and cognition. Brain Res. 1654, 87–94.
Juraska, J.M., Sisk, C.L., DonCarlos, L.L., 2013. Sexual differentiation of the adolescent rodent brain: hormonal influences and developmental mechanisms. Horm. Behav. 64, 203–210.
Koebele, S.V., Bimonte-Nelson, H.A., 2015. Trajectories and phenotypes with estrogen exposures across the lifespan: what does Goldilocks have to do with it? Horm. Behav. 74, 86–104.
Kolb, B., Mychasiuk, R., Muhammad, A., Li, Y., Frost, D.O., Gibb, R., 2012. Experience and the developing prefrontal cortex. Proc. Natl. Acad. Sci. U. S. A. 109 (Suppl. 2), 17186–17193.
Lehmler, H.J., Liu, B., Gadogbe, M., Bao, W., 2018. Exposure to bisphenol A, bisphenol F, and bisphenol S in U.S. adults and children: the National Health and Nutrition Examination Survey 2013–2014. ACS Omega 3, 6523–6532.
Leranth, C., Hajszan, T., Szigeti-Buck, K., Bober, J., MacLusky, N.J., 2008a. Bisphenol A prevents the synaptogenic response to estradiol in hippocampus and prefrontal cortex of ovariectomized nonhuman primates. Proc. Natl. Acad. Sci. U. S. A. 105, 14187–14191.
Leranth, C., Szigeti-Buck, K., Maclusky, N.J., Hajszan, T., 2008b. Bisphenol A prevents the synaptogenic response to testosterone in the brain of adult male rats. Endocrinology 149, 988–994.
Liu, Z.H., Ding, J.J., Yang, Q.Q., Song, H.Z., Chen, X.T., Xu, Y., Xiao, G.R., Wang, H.L., 2016. Early developmental bisphenol-A exposure sex-independently impairs spatial memory by remodeling hippocampal dendritic architecture and synaptic transmission in rats. Sci. Rep. 6, 32492.
Luine, V.N., 2014. Estradiol and cognitive function: past, present and future. Horm. Behav. 66, 602–618.
Luine, V., 2016. Estradiol: mediator of memories, spine density and cognitive resilience to stress in female rodents. J. Steroid Biochem. Mol. Biol. 160, 189–195.
Luine, V., Frankfurt, M., 2013. Interactions between estradiol, BDNF and dendritic spines in promoting memory. Neuroscience 239, 34–45.
Luine, V., Serrano, P., Frankfurt, M., 2018. Rapid effects on memory consolidation and spine morphology by estradiol in female and male rodents. Horm. Behav. https://doi.org/10.1016/j.yhbeh.2018.04.007. [Epub ahead of print].
MacLusky, N.J., Hajszan, T., Leranth, C., 2005. The environmental estrogen bisphenol a inhibits estradiol-induced hippocampal synaptogenesis. Environ. Health Perspect. 113, 675–679.
Mhaouty-Kodja, S., Belzunces, L.P., Canivenc, M.C., Schroeder, H., Chevrier, C., Pasquier, E., 2018. Impairment of learning and memory performances induced by BPA: evidences from the literature of a MoA mediated through an ED. Mol. Cell. Endocrinol. 475, 54–73.
Moriyama, K., Tagami, T., Akamizu, T., Usui, T., Saijo, M., Kanamoto, N., Hataya, Y., Shimatsu, A., Kuzuya, H., Nakao, K., 2002. Thyroid hormone action is disrupted by bisphenol A as an antagonist. J. Clin. Endocrinol. Metab. 87, 5185–5190.
Negri-Cesi, P., 2015. Bisphenol A interaction with brain development and functions. Dose Response 13 (1559325815590394).
Nesan, D., Sewell, L.C., Kurrasch, D.M., 2018. Opening the black box of endocrine disruption of brain development: lessons from the characterization of bisphenol A. Horm. Behav. 101, 50–58.
Ogiue-Ikeda, M., Tanabe, N., Mukai, H., Hojo, Y., Murakami, G., Tsurugizawa, T., Takata, N., Kimoto, T., Kawato, S., 2008. Rapid modulation of synaptic plasticity by estrogens as well as endocrine disrupters in hippocampal neurons. Brain Res. Rev. 57, 363–375.
Scharfman, H.E., Hintz, T.M., Gomez, J., Stormes, K.A., Barouk, S., Malthankar-Phatak, G.H., McCloskey, D.P., Luine, V.N., Maclusky, N.J., 2007. Changes in hippocampal function of ovariectomized rats after sequential low doses of estradiol to simulate the preovulatory estrogen surge. Eur. J. Neurosci. 26, 2595–2612.
Schneider, M., 2013. Adolescence as a vulnerable period to alter rodent behavior. Cell Tissue Res. 354, 99–106.
Shapiro, L.P., Parsons, R.G., Koleske, A.J., Gourley, S.L., 2017. Differential expression of cytoskeletal regulatory factors in the adolescent prefrontal cortex: implications for cortical development. J. Neurosci. Res. 95, 1123–1143.
Taylor, J.A., Welshons, W.V., Vom Saal, F.S., 2008. No effect of route of exposure (oral; subcutaneous injection) on plasma bisphenol A throughout 24h after administration in neonatal female mice. Reprod. Toxicol. 25, 169–176.
Tovote, P., Fadok, J.P., Luthi, A., 2015. Neuronal circuits for fear and anxiety. Nat. Rev. Neurosci. 16, 317–331.
Tuscher, J.J., Fortress, A.M., Kim, J., Frick, K.M., 2015. Regulation of object recognition and object placement by ovarian sex steroid hormones. Behav. Brain Res. 285, 140–157.
United States Environmental Protectional Agency, 1993. Bisphenol A, CASRN 80-05-7. Integrated Risk Information System. U.S. Environmental Protection Agency, Washington, DC Available at: http://www.epa.gov/iris/subst/0356.htm.
Wallace, M., Luine, V., Arellanos, A., Frankfurt, M., 2006. Ovariectomized rats show decreased recognition memory and spine density in the hippocampus and prefrontal cortex. Brain Res. 1126, 176–182.
Wang, C., Niu, R., Zhu, Y., Han, H., Luo, G., Zhou, B., Wang, J., 2014. Changes in memory and synaptic plasticity induced in male rats after maternal exposure to bisphenol A. Toxicology 322, 51–60.