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Hypothalamic gene expression changes in F_1 California mice (*Peromyscus californicus*) parents developmentally exposed to bisphenol A or ethinyl estradiol

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Abstract

Bisphenol A (BPA) is a pervasive industrial chemical used in many common household items. To examine how early exposure to BPA and ethinyl estradiol (EE, estrogen in birth control pill) might affect biparental care, effects of these chemicals in male and female California mice (*Peromyscus californicus*), who are monogamous and biparental, were examined. California mice exposed during pre- and peri-natal life to BPA at an environmentally relevant concentration or EE show later disrupted biparental behaviors. The hypothalamus is an important brain region for regulating parental behaviors. Thus, it was hypothesized compromised biparental care might be partially due to hypothalamic gene alterations. To address this question, brains from F_1 parenting female and male

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California mice from controls, BPA- and EE-exposed groups were collected at postnatal day (PND) 2, and RNA was isolated from hypothalamic micropunches. Gene expression was examined in this brain region for genes affected by BPA exposure and attributed to governing parental care in rodents and humans. BPA-exposed California mice showed increased hypothalamic expression of *Kiss1, Esr1* and *Esr2* relative to AIN control and EE-exposed parents in the case of *Esr2*. Notably, current studies represent the first report to show that early exposure to BPA can induce longstanding effects on hypothalamic gene expression in parenting male and female rodents. Absence of such hypothalamic gene expression changes in EE-exposed parents indicates early BPA exposure may induce later transcriptomic effects through estrogen receptor-independent pathways. BPA-driven changes in hypothalamic function of California mice might contribute to decreased biparental investment, which could result in F_2 multigenerational effects.

Keywords: Molecular biology, Neuroscience, Toxicology

1. Introduction

Brain sexual differentiation is governed by rising concentrations in gonadal steroid hormones during early perinatal life followed by a second spike later in adulthood. This concept is considered "organization-activational programming" and occurs in a sex-specific manner (Phoenix et al., 1959; Arnold and Breedlove, 1985). In developing females, testosterone and estrogen concentrations remain low such that various brain regions are not sculpted to the male pathway and instead programmed to be female (Clarkson and Herbison, 2016). Early exposure to testosterone and estrogens are essential in guiding the architecture of the neural networks and connections (Beyer, 1999).

During early perinatal life, the brain is thus especially vulnerable to endocrine disrupting chemicals (EDCs). EDCs are compounds that interfere with physiological processes regulated by endogenous hormones (Gore et al., 2015). Such chemicals include plastics and plasticizers, pesticides, and other widely used industrial chemicals. Common products made with these chemicals include food and beverage packaging, toys, contraceptives, and medical supplies (Gore et al., 2015). One of the most abundant EDCs is bisphenol A (BPA) (vom Saal and Welshons, 2014). BPA is detected in the urine of 93% of individuals tested (Calafat et al., 2008). Because of a similar molecular structure to 17β-estradiol, BPA is considered a weakly estrogenic compound that can bind and activated estrogen receptor-α (ESR1) and estrogen receptor-β (ESR2), but it can also act through other steroidogenic and non-steroidogenic receptors (Vandenberg et al., 2012).

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BPA and other EDCs can disrupt later maternal care in various rodent species (Palanza et al., 2002; Cummings et al., 2005; Rosenfeld, 2015). However, it was not clear from these rodent studies whether BPA or other EDCs might affect biparental care. Paternal involvement in pup rearing has been examined in the wild and laboratory setting, for select rodent species, including California mice, *Peromyscus californicus*, which are monogamous and biparental (Rosenfeld et al., 2013). Notably, we have shown that California mice parents developmentally exposed to BPA or EE during gestation and lactation show compromised biparental care ability, as evidenced by reduced time spent nursing for the dams and in the nest with the pups for the dams and sires (Johnson et al., 2015). It is not clear though if these behavioral disturbances are due to persistent changes in neural gene expression changes in BPA- and EE-exposed individuals.

The hypothalamus is the primary brain region regulating maternal and paternal behaviors in rodent species where the males exert an active role in caring for the pups (Wang et al., 2000; de Jong et al., 2009; Bales and Saltzman, 2016). Some of the genes and their protein products altered in the hypothalamus and other brain regions by BPA exposure include estrogen receptor α (*Esr1* and transcript variants), estrogen receptor β (*Esr2*), DNA methyltransferases (*Dnmt1*, *3a, 3b*), androgen receptor (*Ar*), brain derived neural factor (*Bdnf*), vasopression (*Avp*), oxytocin (*Oxt*), oxytocin receptor (*Oxtr*), gonadotropin releasing hormone (*GnRH*), kisspeptin (*Kiss1*) (Aloisi et al., 2001; Ceccarelli et al., 2007; Monje et al., 2007; Mahoney and Padmanabhan, 2010; Wolstenholme et al., 2011, 2012; Kundakovic et al. 2013, 2015; Warita et al., 2013; Chen et al., 2014; Goldsby et al., 2016; Johnson et al., 2017). Several of these genes and associated hormones/binding receptors are implicated in regulating maternal and paternal behaviors in California mice, other rodent species, and humans (Koch, 1990; Gubernick et al., 1995; Parker and Lee, 2001; Champagne et al., 2003; Bales et al., 2004; Song et al., 2010; Lambert et al., 2011; Wu et al., 2011; de Jong et al., 2012).

While these past studies have shown that early exposure to BPA can affect hypothalamic gene expression, especially in neonates, no previous studies have considered whether developmental exposure to this chemical could result in gene expression changes in this brain region for parenting animals. Based on our background study that showed developmental exposure to BPA or EE could affect biparental care in California mice (Johnson et al., 2015), we hypothesized that at least some of the parental care deficits observed in these groups might be due to EDC-induced gene expression changes in the hypothalamus.

Thus, the expression patterns of genes listed above that have either been shown to be affected by BPA exposure in non-parental animals and/or associated with parental care in California mice and other rodent models were measured in the hypothalamus of dams and sires on postnatal day two. At this postnatal age, we have determined

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previously that control dams and sires are both actively involved in pup rearing (35). However, biparental care deficits are already noted by this time period in BPA- and EE-exposed California mice (Johnson et al., 2015). Our paradigm offers a chance to examine whether perinatal exposure to EDCs might manifest at a critical time-point when resulting adults are raising the next generation.

2. Materials & methods

2.1. Animals and tissue collection

California mice raised in a pathogen free breeding colony at the Animal Sciences Research Center (ASRC) at the University of Missouri were used in this study. Founder mice were originally purchased from the *Peromyscus* Genetic Stock Center (PGSC) at the University of South Carolina. All experiments were approved by University of Missouri Animal Care and Use Committee and performed in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

To reduce BPA contamination from plastics, white polypropylene (27.8 × 7.5 × 12 cm) cages were used along with glass water bottles. Mice were maintained on a 12:12 hour light:dark cycle (lights on at 7:00, lights off at 19:00). Two weeks prior to breeding, female California mice (P_0) were randomly assigned to receive one of three diets: 1) AIN 93G (7% corn oil) control diet to minimize phytoestrogen contamination found in most rodent chow diet formulations (Envigo, TD. 95092), 2) AIN 93G control diet supplemented with 50 mg BPA/kg feed weight (Envigo, TD.09518a), as previously tested and shown to induce epigenetic and phenotypic effects and considered environmentally relevant (Dolinoy et al., 2007; Cox et al., 2010; Wolstenholme et al., 2011; Anderson et al., 2012), 3) AIN 93G control diet supplemented with 0.1 parts per billion (ppb) EE (Envigo, TD.09695), FDA required positive control for BPA studies (vom Saal et al., 2005). We have measured internal serum concentrations of BPA in laboratory mice and *Peromyscus* species provided this dietary dose of BPA (Sieli et al., 2011; Jasarevic et al., 2013). In *Peromyscus* species, consumption of this dose of BPA yields internal serum concentrations of 5.48 ± 2.07 ng/ml (Jasarevic et al., 2013). Such concentrations are similar to that which has identified in pregnant and non-pregnant women and men unknowingly exposed to this chemical (Padmanabhan et al., 2008; Vandenberg et al., 2010; Teeguarden et al., 2011). The P_0 dams remained on the assigned diets through gestation and lactation. After two weeks on the diet, females were singly paired with breeder males, and the pair remained together for the duration of the study.

F_1 male and female offspring were weaned at 30 days of age, singly housed, and placed on the AIN 93G control diet. When offspring reached adulthood (~90 days), males and females were paired with unrelated individuals of the opposite

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sex who were exposed to the same P_0 maternal diet to result in three F_1 pairings (BPA-exposed ♂ to BPA-exposed ♀, EE-exposed ♂ to EE-exposed ♀, and AIN control ♂ to AIN control ♀). California mice were weighed biweekly to estimate parturition date. On PND 2, adult male and female F_1 breeding pairs ($n = 10$ to 22 individuals per F_1 treatment groups) were humanely euthanized, and whole brain was quickly excised and flash frozen on powdered dry ice and stored at −80 until processing. While no brain dissection guide for *Peromyscus* is available, the landmarks described in the Rat and Mouse Brain Dissection Guide Atlases (Franklin and Paxinos, 2008; Paxinos, 2013) can be used for California mice. A Harris Micro-Punch 2 mm in diameter and 2 mm in depth (Catalogue # 15093, Ted Pella, Redding, CA) was used to obtain 2 sequential punches that spanned the rostral to caudal medial regions of the hypothalamus, as we have done previously with juvenile California mice (Johnson et al., 2017). The experimental diagram is depicted in Fig. 1.

2.2. Quantitative real-time PCR

DNA, RNA, and miRNA were isolated using the AllPrep DNA/RNA/miRNA Universal Kit (Catalogue #80224; Qiagen, Hilden, Germany). RNA was quantified using a spectrophotometer (Nanodrop 2000, ThermoFisher Scientific). This kit was used to obtain DNA and miRNA for future epigenetic studies. Total RNA was reverse transcribed into cDNA using the QuantiTect Reverse Transcription Kit

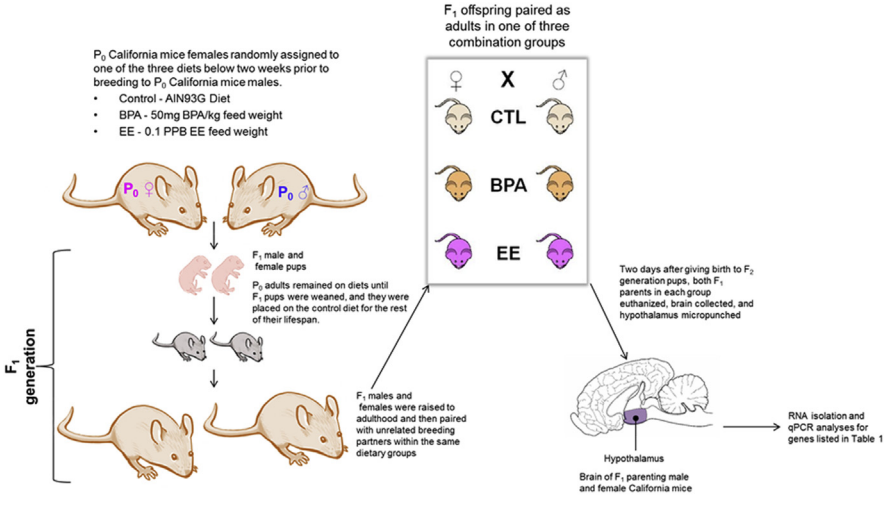

Fig. 1. Diagram of the studies showing generation of F_1 California mice for later collection of brains from parenting males and females. P_0 female California mice were placed on one of three diets two weeks prior to breeding to P_0 male California mice. The breeding pair was maintained on the respective diets until F_1 offspring were weaned. F_1 offspring were at this time placed on the AIN control diet and raised to adulthood, upon which they were paired with unrelated breeding partners within the same maternal/paternal dietary group. When their F_2 pups reached post-natal day (PND 2), the F_1 parenting males and females were euthanized, brains collected, and hypothalamic region micropunched for RNA isolation and qPCR analyses of the genes listed in Table 1.

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(Catalogue #205310, Qiagen). The qPCR procedure was performed on the Applied Biosystems 7500 Real-Time PCR System (Carlsbad, CA) using the QuantiTect SYBR Green PCR Kit (Catalogue #204143; Qiagen). Primers were designed based on our California mice reference transcriptomic library derived from the hypothalamus region, as reported in Johnson et al. (2017). The sequences are publicly available on this site (http://genomics.ircf.missouri.edu/cgi-psd/ppsd.cgi?db=681). Primer sequences for the genes examined are listed in Table 1 and were purchased from IDT (Coralville, IA). All primer sequences were confirmed beforehand to ensure that only a single product was obtained and the amplicon was the correct size. We also validated the primers based on obtained dissociation curves. For each individual, replicate cDNA samples were tested and results then averaged together. The qPCR conditions employed were 1) 15 minutes at 95 °C to activate the HotStarTaq DNA polymerase, as per the manufacturer's instructions; 2) 35−40 cycles of: denaturation 15 seconds at 94 °C, annealing 30 seconds at 56−57 °C, and extension 72 °C for 30 seconds; 3) Dissociation melt curve analysis

Table 1. Primer sequences and corresponding expected product sizes.

Gene		Primer Sequence	Expected Product Size
B2m1	Forward	TCTAGTGGGAGGTCCTGTGG	86
	Reverse	TGCGTTAGACCAGCAGAAGG	
Esr1	Forward	GTGCAATGACTACGCCTCTG	197
	Reverse	CCTTCATCATGCCCACTTCG	
Esr2	Forward	AGTCTCGGTGTCTGATTGCA	178
	Reverse	TGACCAAGGCAGCCATAAGA	
Kiss1	Forward	GGCTTCTCTTGGTGTGTTCC	115
	Reverse	TCATTCTGGCAGGAAGAGGC	
Lepr	Forward	GAGCAGCCTGTATTGTTCCG	179
	Reverse	ACGTTGGTGGAGAGTCAAGT	
Dnmt3a	Forward	TCTTGAGTCCAACCCCGTGATG	156
	Reverse	CCTCACTTTGCTGAACTTGGCT	
Dnmt3b	Forward	GCTGTCAAAGAGGGAGGTCT	229
	Reverse	CTCACTTTGCTGAACTTGGCT	
Bdnf	Forward	GAAGAGCTGCTGGATGAGGA	182
	Reverse	CGCCGGACTCTCATAGACAT	
Gnrh	Forward	GCAGCACTCAACTCACCAAA	211
	Reverse	ACGTCCTCACCCATCTCTTG	
Avp	Forward	TGGCCTAAACTTCCCCTAGC	216
	Reverse	TCACCCACATGAGAACCGAA	
Oxtr	Forward	CTCAGCCATCAGAAGGTTGC	200
	Reverse	TCCCATTCCTTGTCCCAGAC	

https://doi.org/10.1016/j.heliyon.2018.e00672

from 60 °C to 90 °C. The internal control primer was β2 microglobulin (*B2m1*) and test genes included: *Esr1*, *Esr2*, *Dnmt3a*, *Dnmt3b*, *Avp*, *Oxtr*, *Kiss1*, *Gnrh*, and *Bdnf*. In pilot studies, we had tested several possible internal control genes, and *B2m1* provided the most consistent result and was not affected by any of the treatments, as we have shown previously (Wright et al., 2017).

2.3. Statistics

Data were analyzed using SAS version 9.2 Software (SAS Institute, Cary, NC). ANOVA was done using a PROC GLIMMIX procedure to analyze the dCT values based on main effects in the P_0 maternal treatment group (AIN, BPA, and EE) and F_1 parental sex and their potential interaction. However, in Table 2, data are expressed as relative fold change based on the $2^{-\Delta\Delta CT}$ method.

3. Results

When sex of each F_1 parent was considered alone, there were no interactive effects of P_0 maternal chemical exposure and F_1 parental sex, i.e. no specific maternal or paternal effects alone were observed. There were, however, changes in gene expression due to perinatal EDC exposure when both male and female results were combined (Fig. 2). *Esr1* was significantly increased in the hypothalamus of F_1 parents developmentally exposed to BPA (Fig. 2A, $p = 0.008$). A similar increase was found in *Esr2* for F_1 BPA-exposed parents when compared with control (Fig. 2B, $p = 0.02$) and EE-exposed (Fig. 2B, $p = 0.008$) parents.

Developmental exposure to BPA altered a few other genes in the hypothalamus of parenting California mice. *Kiss1* was elevated in BPA-exposed parents relative to AIN control parents (Fig. 2C, $p = 0.05$). There was also a trend toward

Table 2. Expression of other genes in the hypothalamus of F_1 exposed parental brains collected at PND 2.

Transcript	Relative Fold Change*		p-value		
	BPA	EE	Vehicle vs. BPA	Vehicle vs. EE	EE vs. BPA
Dnmt3a	2.16 ± 0.6	1.77 ± 0.8	0.2	0.5	0.8
Dnmt3b	0.69 ± 0.7	0.91 ± 0.9	0.6	0.9	0.7
Bdnf	1.58 ± 0.5	1.06 ± 0.7	0.4	0.9	0.5
Gnrh	1.76 ± 0.4	1.07 ± 0.6	0.2	0.7	0.3
Avp	5.19 ± 0.9	2.59 ± 1.3	0.1	0.4	0.5
Oxtr	2.43 ± 1.2	8.59 ± 1.5	0.5	0.2	0.3

*The relative fold change is compared to vehicle control ± standard error of the mean (SEM) and calculated based on $2^{-\Delta\Delta CT}$ method.

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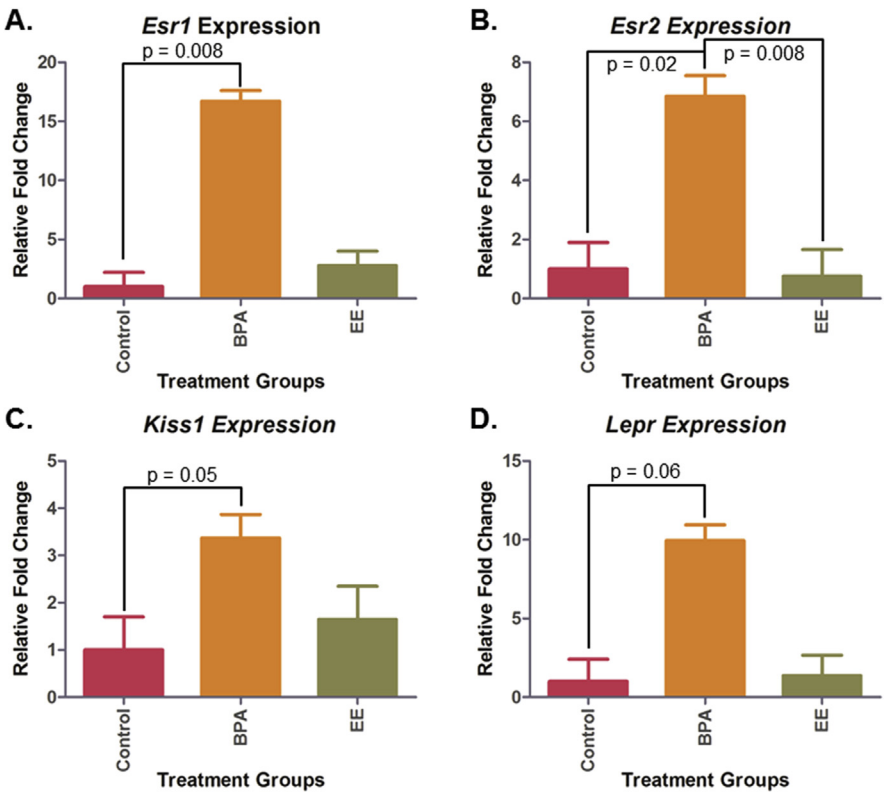

Fig. 2. Expression of hypothalamic genes that were altered in BPA-exposed F_1 California mice parents. A) *Esr1*, B) *Esr2*, C) *Kiss1*, and D) *Lepr*. The fold change is relative to vehicle control (which was set to 1) ± standard error of the mean (SEM) and calculated based on the $2^{-\Delta\Delta CT}$ method (n = 10 to 22 individuals were tested per F_1 treatment groups). Statistical differences were calculated based on dCt values, and p value differences are listed on the individual graphs.

BPA-exposed parents having an upregulation in *Lepr* expression compared with the AIN group (Fig. 2D, $p = 0.06$). None of the other genes, however, examined showed expression differences between the various parental groups (Table 2).

4. Discussion

The primary objective of the current studies was to examine whether perinatal exposure to BPA at an environmentally relevant concentration or EE could manifest in later expression changes for candidate genes in the hypothalamus of parenting male and female California mice. Such disruptions in the hypothalamus might at least partially account for compromised biparental care previously observed in F_1 male and female California mice exposed early on to these EDCs (Johnson et al., 2015). The hypothalamic expression of *Esr1*, *Esr2*, and *Kiss1* was increased in BPA-exposed parents, who also showed a trend for elevated gene expression of *Lepr*. However, similar changes were not evident in EE-exposed parents. This finding is somewhat surprising, as both EDC-exposed groups showed parental

https://doi.org/10.1016/j.heliyon.2018.e00672

deficits, although some behavioral differences between the two groups were noted (Johnson et al., 2015). EE-exposed parents tended to groom excessively their pups but such responses were not observed in BPA-exposed parents. While BPA can act as a weak estrogen, it can also induce effects through other steroidogenic and non-steroidogenic receptors, which might account for these neurobehavioral differences observed between these two groups (Vandenberg et al., 2012).

Our prediction at the outset was that parenting California mice males and females would demonstrate reduced expression of *Avp* and *Oxtr*, as these have been linked with at least paternal care in California mice and prairie voles (Gubernick and Nelson, 1989; Parker and Lee, 2001; Bales et al., 2004; Wynne-Edwards and Timonin, 2007; Lambert et al., 2011), and increased signaling through the OXTR is correlated with high degree of maternal behavior (Champagne et al., 2006). However, as detailed further below, elevated expression of *Esr1* in the hypothalamus might affect oxytocin receptor pathways (Champagne et al., 2006).

Other studies that examined effects of gestational exposure of BPA in rodents and sheep indicate that BPA can alter *Esr1* and *Esr2* expression in various regions of the hypothalamus, including the anterioventral periventricular nucleus (AVPV), mediobasal portion, and medial preoptic area (MPOA), but the directionality of these changes varied across studies (Mahoney and Padmanabhan, 2010; Kundakovic et al., 2013).

Similarly, experiment testing the effects of neonatal or pubertal exposure of rodents to BPA on expression of ESR1 and ESR2 in the hypothalamus have provided mixed results with some studies reporting an increase in one or both ESR or their splice variants (Ceccarelli et al., 2007; Monje et al., 2007; Yu et al., 2015). In contrast, others suggest the effects of this chemical on ESR expression in this brain region differs depending on PND examined and offspring sex (Yu et al., 2010). Exposure of pregnant/lactating and estrous cycling rats to BPA reveals that lactating females have fewer ESR-immunoreactive cells in the MPOA and ventromedial nucleus (VMN) than non-lactating females (Aloisi et al., 2001). Taken together, the collective findings suggest that the effects of BPA on hypothalamic *Esr1* and *Esr2* expression can vary according to when the exposure occurs during the lifespan, dose and duration tested, offspring sex, region of the hypothalamus examined, species studied, and reproductive status.

Hypothalamic ESR expression has been associated with parental care in a variety of rodents, including male mandarin voles (*Microtus mandarinus*), who are also monogamous (Koch, 1990; Gubernick et al., 1995; Champagne et al., 2003; Song et al., 2010; Wu et al., 2011; de Jong et al., 2012). In Long-Evans (LE) hooded rats, increase *Esr1* expression is detected in the MPOA in dams that exhibited increased pup licking and grooming (LG) responses relative to those who showed low LG behaviors, suggesting that natural variations in maternal care are linked

https://doi.org/10.1016/j.heliyon.2018.e00672

with *Esr1* expression in this brain region (Champagne et al., 2003). Notably, two polymorphisms in *ESR1* gene are linked with negative human maternal behaviors (Lahey et al., 2012).

BPA-induced overexpression of *Esr1* and *Esr2* in the hypothalamus of parenting California mice males and females might contribute to parental care deficiencies. Alternatively, increase in hypothalamic *Esr1* and Esr2 expression in BPA-exposed parents might represent an attempt to compensate for other gene expression disruptions directly resulting in the compromised parental behaviors. To sort out these two possibilities, future studies should be designed to treat adult male and female California mice before and after birth with an ESR1/ESR2 antagonist, such as ICI 182,780 to determine if this treatment reduces parental care behaviors. Another approach that might be considered is to test whether adenovirus-induced overexpression of ESR1 in the MPOA affects biparental behaviors in this species. Such an approach was done into the MPOA of LE rat pups subjected to low levels of maternal care in terms of licking/grooming (LG). LG/ESR1 overexpression rat pups had persistent increase in ESR1-immunoreactivity in this region, and as juveniles, they showed decreased latency to engage in maternal behavior toward donor pups (Pena and Champagne, 2015).

It is unclear how to interpret the increased expression of *Kiss1* and *Lepr* in BPA-exposed California mice parents. Exposure of neonatal LE rats to BPA reduced *Kiss1* expression in the anterior hypothalamus when assessed at PND 10 (Cao et al., 2012). Analogous results were found in rats neonatally treated with BPA and then assessed at the prepubertal period (Navarro et al., 2009). On the other hand, a single injection of BPA at proestrus enhanced *Kiss1* expression in the AVPV of female mice (Wang et al., 2014). In male Sprague-Dawley rats exposed during prenatal life to BPA and then examined at PND1, *Lepr* is increased in the hypothalamus (Arambula et al., 2016). This finding concurs with our current results but any connection with parental behaviors requires further analyses. It is possible that the expression pattern of this gene can vary according to dose, duration of BPA exposure, age, physiological state (non-parenting vs. parenting). Thus, further work is needed to characterize the significance of these two gene alterations in BPA-exposed parenting California mice.

There could also be inter-relationships between *Esr1* and other transcripts examined. For instance, estrogen stimulates *Lepr* expression via ESR in ATDC5 cells (Wang et al., 2012). In certain breast cancer cells, there is crosstalk between LEPR and ESR1 (Fusco et al., 2010). In the arcuate nucleus of rats, estrogen regulates the expression of *Kiss1* (Kanaya et al., 2017). In the medial amygdala of mice, ESR1 but not ESR2 regulates the estrogen-induced upregulation of *Kiss1* expression (Stephens et al., 2016).

 https://doi.org/10.1016/j.heliyon.2018.e00672

While the work herein show that select candidate genes are altered in the hypothalamus of parenting California mice, causation cannot be established from the current studies. In this sense, siRNA or CRIPSR/Cas9 technology might be considered in future studies to determine whether suppression of these hypothalamic genes results in decreased biparental care in California mice. Based on the limited amount of tissue and animals available, we chose to examine candidate genes previously been shown to be affected by early BPA exposure. Follow-up studies should use other approaches, such as in Northern and Western blots, *in situ* hybridization, and immunohistochemistry to confirm and localize the transcript and potential protein expression changes. RNAseq could be used to examine for global gene expression changes in BPA- or EE-exposed parenting California mice that might also contribute to decreased parental care provided by these animals. As parenting males and females were collected on PND 2, we did not have an opportunity to measure and correlate their parental care to the identified gene expression changes.

5. Conclusions

Our current studies importantly show that perinatal exposure to BPA at an environmentally relevant concentration can induce longstanding effects that manifest in later hypothalamic gene expression in changes in parenting California mice. Overexpression of *Esr1*, *Esr2*, *Kiss1*, and to a lesser extent *Lepr* in parenting California mice developmentally exposed to BPA might contribute to compromised parental care behaviors previously observed in these groups (Johnson et al., 2015). It is also interesting to note that even though both BPA and EE-exposed California mice show reduced parental investment, similar gene expression changes in the hypothalamus were not evident in EE-exposed individuals. The fact that such changes were not observed in EE-exposed parents indicates that early BPA-exposure may cause later transcriptomic alterations in the hypothalamus through estrogen receptor-independent pathways. BPA-induced alterations in hypothalamic gene expression with ensuing decreased parental investment could result in F_2 multigenerational effects.

Declarations

Author contribution statement

Sarah Johnson: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mark Ellersieck: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Cheryl Rosenfeld: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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