Published in final edited form as:

J Steroid Biochem Mol Biol. 2016 June ; 160: 148–159. doi:10.1016/j.jsbmb.2015.08.021.

Assessment of sex specific endocrine disrupting effects in the prenatal and pre-pubertal rodent brain

Meghan E. Rebuli^{a,b} and Heather B. Patisaul^{a,b,*}

^aNorth Carolina State University, Department of Biological Sciences, Raleigh, NC 27695

^bW. M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, NC 27695

Abstract

Background—Brain sex differences are found in nearly every region of the brain and fundamental to sexually dimorphic behaviors as well as disorders of the brain and behavior. These differences are organized during gestation and early adolescence and detectable prior to puberty. Endocrine disrupting compounds (EDCs) interfere with hormone action and are thus prenatal exposure is hypothesized to disrupt the formation of sex differences, and contribute to the increased prevalence of pediatric neuropsychiatric disorders that present with a sex bias.

Objective—Available evidence for the ability of EDCs to impact the emergence of brain sex differences in the rodent brain was reviewed here, with a focus on effects detected at or before puberty.

Methods—The peer-reviewed literature was searched using PubMed, and all relevant papers published by January 31, 2015 were incorporated. Endpoints of interest included molecular cellular and neuroanatomical effects. Studies on behavioral endpoints were not included because numerous reviews of that literature are available.

Results—The hypothalamus was found to be particularly affected by estrogenic EDCs in a sex, time, and exposure dependent manner. The hippocampus also appears vulnerable to endocrine disruption by BPA and PCBs although there is little evidence from the pre-pubertal literature to make any conclusions about sex-specific effects. Gestational EDC exposure can alter fetal neurogenesis and gene expression throughout the brain including the cortex and cerebellum. The available literature primarily focuses on a few, well characterized EDCs, but little data is available for emerging contaminants.

Conclusion—The developmental EDC exposure literature demonstrates evidence of altered neurodevelopment as early as fetal life, with sex specific effects observed throughout the brain even before puberty.

*Corresponding author at: Associate Professor, David Clark Labs 154, Box 7617, Department of Biological Sciences, North Carolina State University, Raleigh, NC 27695. Tel.: 001-919-513-7567.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Endocrine disrupting compounds; neurodevelopment; sex differences; developmental exposure; rodent; Bisphenol; BPA; genistein; xenoestrogen; EDC

1. Introduction

Brain sexual dimorphisms are fundamental to understanding neurophysiology and behavior (de Vries 1984, Becker, Berkley et al. 2007). These differences are present as early as gestation and found in nearly every region of the brain, particularly the hypothalamus (De Vries 2004, Ohtani-Kaneko 2006). Many neuropathological and neuropsychiatric disorders have a sex bias in their prevalence but it is unclear why (Paus, Keshavan et al. 2008). For example, attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) disproportionately affect boys while anxiety and depressive disorders are more common in girls (Costello, Mustillo et al. 2003, Ramtekkar, Reiersen et al. 2010, CDC 2012, Tang and Pinsky 2015). Some of these disorders emerge in early childhood, suggesting that sex differences in etiology and risk arise prenatally. This sex bias indicates that sex hormones are likely involved, but potential mechanisms remain unclear. ASD, for example, has been associated with prenatal androgen excess and attenuation of sex differences in some studies (Baron-Cohen, Knickmeyer et al. 2005, Baron-Cohen, Cassidy et al. 2014), but genome-wide studies have revealed that a complex mixture of gene by environment interactions likely contribute to risk (Merikangas, Nakamura et al. 2009). Rates of pediatric psychiatric disorders, most notably ADHD and ASD, are rapidly rising; further emphasizing that an environmental component must be contributing to their etiology. Because endocrine disrupting chemicals (EDCs) interfere with hormone action, EDCs may be one environmental factor contributing to greater prevalence of adolescent neuropsychiatric disorders (Brown 2009, Diamanti-Kandarakis, Bourguignon et al. 2009, Schug, Janesick et al. 2011, de Cock, Maas et al. 2012). One mechanism by which they may enhance risk is via developmental disruption of brain sexual differentiation. Thus understanding how EDC exposures alter brain sex differences is fundamental to assessing their potential risk. This review surveyed the available evidence regarding the ability of EDCs to impact the emergence of brain sexual dimorphisms, with a focus on effects detected at or before puberty in rodents. A primary question of interest was how well the EDC literature accounted for sex in the identified literature.

The Endocrine Society defines an EDC as an exogenous chemical substance or mixture that alters the structure or function(s) of the endocrine system (Diamanti-Kandarakis, Bourguignon et al. 2009). This can occur through a range of mechanisms including direct agonism/antagonism of sex hormone receptors (Gore and Dickerson 2012) and abrogation of sex differences in hormone receptor expression (Cao, Mickens et al. 2012, Cao, Rebuli et al. 2013, Cao, Joyner et al. 2014). Thus, we believe that accounting for sex and sexual dimorphisms in the developing (gestational through pubertal) brain is fundamental to EDC research. It is striking, however, how poorly sex is accounted for in the neuroscience literature, particularly the experimental literature. Although understanding how hormones and other factors shape neural sexual dimorphisms and sex-specific vulnerability to

neuropsychiatric illness, is clearly critical for understanding these disorders, sex differences are underreported in the animal literature used to study these differences, and biased towards males (Woodruff, Kibbe et al. 2014). The lack of emphasis on females in neurodevelopmental research leaves a blatant gap in understanding the ontogeny of neuropsychiatric disorders, especially those which are sex biased towards women such as anxiety and depression. Because a fundamental aspect of the endocrine disruption hypothesis is that developmental exposure may contribute to neurobehavioral effects by altering sexual differentiation, we hypothesized that animal-based EDC studies would do a better job of taking sex into account.

EDC effects on behavior, including sexually dimorphic behaviors have been comprehensively reviewed numerous times (Palanza, Morellini et al. 1999, Schantz and Widholm 2001, Lephart, Setchell et al. 2004, Palanza, Gioiosa et al. 2008, Ramos, Patricio et al. 2008, Patisaul and Adewale 2009, Frye, Bo et al. 2012), but fewer reviews have specifically focused on neural changes, particularly in pre-pubertal animals (Gore 2008, Patisaul and Polston 2008). Thus for the present evaluation we focused on molecular, cellular and neuroanatomical endpoints in sub-adult rodent models including gene expression, neurogenesis, neural plasticity and epigenetic changes. Effects reported prior to puberty were the primary focus of this review because pediatric psychiatric disorders, most notably ADHD and ASD, clearly have fetal origins and emerge in infancy and early childhood. A better understanding of how EDCs can affect sex differences in neurodevelopment can guide knowledge on their role in human health and the onset of adolescent neuropsychiatric disorders (Grandjean and Landrigan 2006). Accounting for sex is thus a critical aspect of EDC research, and the sex-specific outcomes of these studies are informative for the broader neuroscience community.

2. Methods

The review includes rodent studies (published by January 31, 2015) in which exposure was gestational and/or neonatal and assessment was made prior to pubertal maturation (before postnatal day (PND) 37). Studies were identified by searching PubMed using the keywords: endocrine disrupting compound, EDC, endocrine active compound, EAC, brain, neuro, hypothalamus, dimorphic and development. Because the list of potential EDCs is hundreds of compounds long, we did not use specific chemical names as search terms. While the search was intended to be thorough, it was unlikely to have been exhaustive. Additionally, although effects in adults are reported in numerous studies, they were considered beyond the scope of this limited, focused survey. Studies with adult animals were included only if animals younger than PND 37 were part of the experimental design. Studies on behavioral endpoints were not included. Details regarding the reviewed studies including the species, strain, sex, exposure route, exposure window, dose, endpoint, and age at testing are summarized in four tables grouped by brain region of interest: hypothalamus (Table 1); hippocampus (Table 2); cortex, cerebellum, and mid-brain (Table 3); and whole brain and embryonic brain regions (Table 4).

3. Results

3.1. Hypothalamus (Table 1)

The hypothalamus is the apical coordinator for homeostatic functions including stress, emotion, reproduction, feeding, and the regulation of sex hormones production and circulation (Campbell 2005). In rodents, sexual dimorphisms within hypothalamic regions are organized primarily during the perinatal period by aromatized testosterone (Lenz and McCarthy 2010); thus disruption of estrogen signaling is hypothesized to be a primary route in which ECDs alter neuroendocrine development. It is therefore not entirely surprising that most of the available literature obtained for this review focused on three of the most well-known estrogen-altering EDCs: Bisphenol A (BPA; exposures ranged from 2.5 µg/kg bw/day to 50 mg/kg bw/day), polychlorinated biphenyls (PCBs) (exposures ranged from 1 mg/kg to 10 mg/kg), and the phytoestrogen, genistein (exposures ranged from 250 µg to 10 mg/kg bw/day). Exposures were mostly gestational (7 studies), with several extending into the neonatal period (6 studies) and the remaining employing neonatal exposure.

Of the 19 studies identified (Table 1), 15 included both sexes, reported outcomes based upon sex, and reported sex-specific effects. Sex specific effects varied by sub-hypothalamic region of interest and compound investigated. For example, gestational exposure to the PCB mixture Aroclor 1221 (A1221) via subcutaneous injection to pregnant rat dams (1mg/kg), altered the expression of numerous estrogen-sensitive genes in the anteroventral periventricular nucleus (AVPV) of females, but not males (Dickerson, Cunningham et al. 2011, Walker, Goetz et al. 2014), while the arcuate nucleus (ARC) was found to be affected in males, but not females (Walker, Goetz et al. 2014). Affected genes included steroid hormone receptors (*Ar*, *Thra*, *Gper*), neuropeptide receptors (*Gnrh1* and *Kiss1r*), methyl transferase (*Dnmt1*), and clock genes (*Arntl* and *Per2*) (Walker, Goetz et al. 2014). Other studies have also identified the developing AVPV as having sex-specific vulnerability to EDCs. Neonatal genistein demasculinized (reduced) tyrosine hydroxylase immunoreactivity in the male AVPV (Patisaul, Fortino et al. 2006, Patisaul, Fortino et al. 2007) and defeminized (reduced) kisspeptin fiber density in the female AVPV and ARC (Losa, Todd et al. 2011). The AVPV is a highly sexually dimorphic region which coordinates the luteinizing hormone (LH) surge in females and is thus essential for ovulation (Simerly 2002). These observed impacts on AVPV sexual differentiation are consistent with the large body of literature reporting EDC-related effects on estrous cyclicity, fecundity, and ovulation (Crain, Janssen et al. 2008, Walshe, Connell et al. 2011).

Developmental EDC exposure also impacted neuropeptides and their receptors in other hypothalamic regions. For example, kisspeptin mRNA expression was observed to be reduced in hypothalamic micropunches from both sexes at PND 30 (Navarro, Sanchez-Garrido et al. 2009), and in the male preoptic area (POA) by the PCB mixture A1221 (Dickerson, Cunningham et al. 2011) on PND 1. In the periventricular and ventromedial (VMN) nuclei, increased levels of somatostatin receptor 2 were found with BPA exposure at PND 10, and in the periventricular nucleus at PND 23 (Facciolo, Alo et al. 2002). BPA-related Increases in the expression of somatostatin receptor 3 were also reported in the PND 7 VMN and ARC (Facciolo, Madeo et al. 2005). Dioxin reportedly reduced GAD 67

expression in the preoptic area (POA) at PND 3, thereby reducing the naturally occurring sex difference in the rostral portion of the POA (Hays, Carpenter et al. 2002). This effect is purportedly linked to disruption of sex-typical reproductive behavior. These examples illustrate the variety of ways EDCs can affect hypothalamic sexual differentiation and development. EDCs may alter both sexes (but not disrupt sex differences), reduce sex differences via impacts on both sexes, or reduce sex differences by only affecting one sex (Hays, Carpenter et al. 2002, Ceccarelli, Della Seta et al. 2007, Navarro, Sanchez-Garrido et al. 2009, Dickerson, Cunningham et al. 2011).

The available literature clearly reveals that the developing hypothalamus has sex-specific vulnerability to EDCs with the POA and mediobasal hypothalamus (MBH) being the most intensely studied and robustly affected. Estrogen receptor and aromatase expression are highly sexually dimorphic and altered by developmental EDC exposure; thus potentially serving as a central mechanism by which other aspects of hypothalamic sexual differentiation are altered by EDCs. Exposures encompassing both the gestational and postnatal period appear to be the most effective in altering steroid receptor expression, while exposures limited to later postnatal development seem to result in more transient effects. In humans, both of these rodent critical periods would be entirely prenatal.

Because the hypothalamus is critically important for the regulation of mood and emotion, as well as sociality (Engel, Miodovnik et al. 2010, Hoffman, Webster et al. 2010, Miodovnik, Engel et al. 2011, Eskenazi, Chevrier et al. 2013), the neural outcomes reviewed here are consistent with and supportive of epidemiological data in children associating BPA and PCBs with ASD, ADHD, and anxiety disorders (Eubig, Aguiar et al. 2010, Sagiv, Thurston et al. 2010, Braun, Kalkbrenner et al. 2011, Cheslack-Postava, Rantakokko et al. 2013). Interestingly, no immune related endpoints have been investigated to date in sub-adult animals, even though some immune cells like microglia (Wood 2011, Wu, Tan et al. 2013) and neutrophils (Molero, Garcia-Duran et al. 2002, Shindo, Moore et al. 2013) have been found to express estrogen receptors, impact sexually dimorphic development (O'Malley, Liston et al. 2011) and the immune system is reciprocally influenced by the hypothalamus. Interactions between endocrine disrupting compounds and neuroimmune cells, such as microglia, may be mechanisms by which developmental sexually dimorphic diseases, such as autism occur and thus an important area of focus for future studies. Sexually dimorphisms may be present in these and other pathways, organs and cell types which extend beyond the HPG axis and other "classical" hypothalamic pathways including the brain gut axis. To fully explain mechanisms by which these complex systems may be vulnerable to EDCs, fundamental understanding of their sex-specific characteristics will be required.

Notably, there was a paucity of phthalate and polybrominated diphenyl ethers (PBDE) studies identified by the search strategy, even though these chemicals have been associated with human mood and anxiety disorders such as ASD and ADHD (Crinnion 2010, Hoffman, Webster et al. 2010, Kim, Han et al. 2010, Gascon, Vrijheid et al. 2011) and well known to alter sexually dimorphic endpoints in adult animals (Grandjean and Landrigan 2006, Costa and Giordano 2007, Grandjean and Landrigan 2014). Although this may be an artifact of the search strategy to some degree, the need to more comprehensively examine impacts in peri-pubertal animals is clearly evident.

3.2. Hippocampus (Table 2)

The hippocampus is fundamental to learning and memory. Hippocampal cell proliferation and differentiation, as well as circuit development, are organizationally regulated by sex and thyroid hormones (Bourguignon, Franssen et al. 2013); making it a potentially prime target for endocrine disruption. In stark contrast to the identified studies on the hypothalamus, where the majority reported outcomes based on sex, none of the hippocampal studies investigated sex-specific outcomes.

For the scope of this review, data was identified from only two EDCs: BPA (exposures ranged from 40 µg/kg/day to 200 mg/kg/day) and PCBs (exposures ranged from 1mg/kg to 8 mg/kg). In all studies exposures began in gestation, and in all but two (Kim, Son et al. 2009) extended into the postnatal period. Endpoints investigated included neurogenesis, phosphorylation, and protein and hormone-sensitive (gonadal or thyroid hormone) gene expression. BPA was found to influence the formation of the dentate gyrus, causing it to delineate earlier in PND 1 mice (sex was not distinguished) compared to controls (Kim, Son et al. 2009). BPA was also found to reduce synaptic density, enlarge the synaptic cleft, and shorten the active zone in PND 14 and 21 male mice in region 1 of the hippocampus proper (CA1) (Xu, Xie et al. 2013). PCB mixture A1254 increased RC3/neurogranin mRNA expression in the dentate gyrus (DG) at PND 15 (sex not stated) compared to controls (Zoeller, Dowling et al. 2000). Overall, evidence for disruptive effects in the hippocampus is strongest for the PCBs (Morreale de Escobar, Obregon et al. 2004). Impacted processes include early delineation of the dentate gyrus, altered synapses in the CA1 and altered neurogenesis, all consistent with reports on children exposed to PCBs exhibiting cognitive, memory, and learning deficits (Jacobson and Jacobson 1996, Stewart, Lonky et al. 2008).

Although BPA and PCBs can clearly alter hippocampal neurodevelopment, results were not reported by sex in most cases but instead conducted only in males (6 studies), only in females (1 study), or with a group of animals where sex was not defined/distinguished in the analysis (4 studies). Thus it is not possible to ascertain which sex may be more vulnerable to effects of EDCs in this region. This lack of focus on sexual dimorphisms in the hippocampus is reflective of the hippocampal neuroscience literature as a whole which, despite the identification of numerous structural and functional sex differences, has traditionally has focused on one sex within a single study (Cryan and O'Mahony 2011, Doumy, Roedig et al. 2011, Foley, Clifford et al. 2011, Woodruff, Kibbe et al. 2014). Establishing how EDCs modulate sex differences, particularly those associated with memory deficits and other mental impairments, remains an area ripe for future investigation. Moreover, identifying specifically when the developing hippocampus is most susceptible to EDC exposures (most available studies exposed animals from mid-gestation to weaning), needs further assessment. Ideally, these studies would also include both sexes.

3.3. Cortex, Cerebellum, and Mid-brain (Table 3)

Compared to the hypothalamus and hippocampus, relatively few studies have examined developmental EDC related effects elsewhere in the brain, but we identified 3 relevant PCB studies (exposures ranging from 1–25 mg/kg), 7 BPA studies (exposures ranging from 2 – 20,000 µg/kg) and 4 dioxin studies (2,3,7,8– tetrachlorodibenzo-p-dioxin (TCDD; a class of

highly persistent environmental compounds formed in industrial processes such as waste combustion and the primary component of Agent Orange; exposures ranging from 0.2 – 2 µg/kg bw/day). Seven of 14 studies differentiated results by sex, but three failed to identify which sex was used. All but one study used an exposure window that began in gestation and five used a longer perinatal exposure window.

Evidence for sex-specific vulnerability within this literature was uneven with one study finding that females were more sensitive to PCB exposure than males, showing decreased forebrain thyroxine concentrations as well as decreased deiodination, but only in females (Morse, Wehler et al. 1996). Other studies reported that both males and females were affected by TCDD or BPA. For example, dioxin was found to lower *Bcl-2* expression in cortex and cerebellum at PND 0 in females but not males. In contrast, dioxin was found to increase *Bcl-xl* expression at PND 0 in the cortex and cerebellum in males but not females (Chang, Sun et al. 2005). BPA has also been shown to alter expression of genes such as *S100b*, *Slc6a4*, *Htr1b*, *Maoa*, *Scia3*, and *Gap43*, at PND 1, 4, 8 in a sex and spatiotemporal manner in the cortex, thalamus, and pons (Itoh, Yaoi et al. 2012). One study investigating BPA-related effects on extracellular signal-regulated kinase (ERK) 1/2 signaling in the cerebellum via intracerebellar injection, reported that BPA and ethinyl estradiol (EE) increased ERK cell numbers and signaling at low and high, but not intermediate, exposures (Zsarnovszky, Le et al. 2005). This effect was not sex-specific, which is not entirely surprising given that the cerebellum is not considered a highly sexually dimorphic structure. In general, this literature revealed that sex specific effects are observable as early as the late gestational period (Morse, Wehler et al. 1996, Nishizawa, Manabe et al. 2003, Nishizawa, Imanishi et al. 2005, Nishizawa, Morita et al. 2005, Itoh, Yaoi et al. 2012) indicating that maternal exposure to EDCs can have a significant effect on fetal brain development, even in regions not typically considered to be sexually dimorphic.

Collectively, the TCDD studies found decreased expression of myelin basic protein (Fernandez, Paradisi et al. 2010), increased expression of apoptotic genes (Chang, Sun et al. 2005), and inhibited acetylcholinesterase activity (Ahmed 2011), suggesting impaired cerebellar development and function. These studies help reveal the neurologic sources of motor and learning disabilities seen in dioxin-exposed children (Vreugdenhil, Lanting et al. 2002). An extensive literature outside the scope of this review has identified interactions with the aryl hydrocarbon receptor (AhR) as the primary mode of action for TCDD neurotoxicity (Mimura and Fujii-Kuriyama 2003, Patel, Kim et al. 2006, Tijet, Boutros et al. 2006). Dioxin is known to bind to AhR with high affinity and when AhR is hyper-activated during embryogenesis it can be teratogenic (Peters, Narotsky et al. 1999). AhR is also thought to aid in the metabolism of xenobiotic compounds, especially polycyclic aromatic hydrocarbons. Interestingly none of the dioxin studies identified for this review examined effects on AhR activity or expression. By contrast, increased expression of AhR was reported in the BPA studies in both males and females, all investigating similar gestational periods of exposure in the ICR mouse (outbred strain selected for high fertility and used in cancer research) (Nishizawa, Manabe et al. 2003, Nishizawa, Imanishi et al. 2005, Nishizawa, Morita et al. 2005). Although there was no report of birth defects in late gestation (when the animals were evaluated), like abnormal liver or kidney function, cleft palate, reduced litter size, etc. typically seen with altered AhR function in dioxin exposure,

increased AhR expression could potentially result in altered neural differentiation, resulting in inappropriate catecholaminergic differentiation of Neuro2a cells (Akahoshi, Yoshimura et al. 2006).

The reviewed PCB studies primarily investigated thyroid hormone related endpoints in the forebrain and cerebellum, including brain thyroid hormone concentration and metabolism (Morse, Wehler et al. 1996) as well as thyroid hormone responsive gene expression (Zoeller, Dowling et al. 2000, Takahashi, Negishi et al. 2009). Thyroid hormone is crucial for neurodevelopment and alteration of gene expression and disrupted thyroid action likely contributes to the cognitive disorders seen in PCB exposed children (Jacobson and Jacobson 1996, Vreugdenhil, Lanting et al. 2002, Stewart, Lonky et al. 2008).

In general the reviewed studies found spatiotemporal, sex, and exposure related effects on gene expression related to neurogenesis, aryl hydrocarbon receptor expression and related genes, and apoptosis regulation (Nishizawa, Manabe et al. 2003, Chang, Sun et al. 2005, Nishizawa, Imanishi et al. 2005, Nishizawa, Morita et al. 2005, Itoh, Yaoi et al. 2012). Altered neuronal differentiation, along with altered receptor signaling (Zsarnovszky, Le et al. 2005) and changes in neuronal migration (Itoh, Yaoi et al. 2012) could be a mechanism by which EDCs, including BPA, alter hallmark behaviors of ADHD, ASD and other psychosocial disorders (Eubig, Aguiar et al. 2010, de Cock, Maas et al. 2012), as reported in children (Braun, Kalkbrenner et al. 2011, Harley, Gunier et al. 2013) and numerous animal studies (Wolstenholme, Rissman et al. 2011, Landrigan, Lambertini et al. 2012, Grandjean and Landrigan 2014).

3.4 Whole Brain and Embryonic Brain Regions (Table 4)

Because it is logistically difficult to differentiate brain regions in the pre-term rodent brain, some analyses have focused on whole brain (4 of 9 studies) or large subregions including the ventricular zone, cortical plate, telencephalon or diencephalon (5 of 9 studies). Because sex differences emerge during embryonic development, examination of the fetal brain for EDC effects yields critical insight as to the mechanisms by which EDCs manipulate sexual differentiation, and identifies critical windows during which the developing brain is most vulnerable.

Only 2 studies differentiated results by sex, and, of the remaining, only 1 reported the sex examined. Expression of thyroid hormone responsive, estrogen receptor related, myelination related, and oxytocin and vasopressin related genes, as well as neurogenesis were the most common endpoints investigated, with BPA (4 studies with exposures ranging from 20 µg/kg to 5 mg/kg), PCBs (3 studies with exposures ranging from 20 µg/kg – 6 mg/kg), and dioxin exposure (2 studies with exposure ranging from 0.7–20 µg/kg TCDD). Expression of myelination (Fernandez, Paradisi et al. 2010), organo- and neuro-genesis (Fujita, Samejima et al. 2006, Komada, Asai et al. 2012), and thyroid hormone related genes (Morse, Groen et al. 1993, Gauger, Kato et al. 2004), as well as neuronal differentiation and migration (Itoh, Yaoi et al. 2012) and cell cycle exit (Nakamura, Itoh et al. 2006, Naveau, Pinson et al. 2014) were all found to be altered by EDC exposure. Some of these studies evaluated similar endpoints later in development, such as myelination levels in the cerebellum (Fernandez, Paradisi et al. 2010) and whole brain thyroxine deiodinase activity (Morse, Groen et al.

1993), and confirm that many of the effects may persist into adolescence and beyond. A sex difference in the expression of *Gper* and *Esrrg* not present in controls was induced in whole brain gene expression on GD 18.5 in BPA exposed animals, with males having higher concentrations than females (Wolstenholme, Edwards et al. 2012). These two genes are estrogen receptor related genes and may indicate that males are more sensitive to BPA exposure. With only 2 studies on whole or embryonic brain regions reporting results by sex, the induction of sex differences (not typically present) by EDC exposures remains difficult to interpret. Whether or not these changes are indicative of sex-specific, long term outcomes on behavior, neuroendocrine function, or cognition (for example) is not resolved. Collectively these findings reinforce the hypothesis that EDCs can have organizational effects on brain structure and suggest that gestational exposure alone has the ability to forever alter neurodevelopment including myelination.

3.5 Summary of Effects

Evidence of altered neurodevelopment and sexual differentiation in pre-pubertal animals was found in each region examined, emphasizing that endocrine disrupting effects can be detected throughout the brain, even before birth. That these effects are persistent and functionally significant is supported by a much larger and robust literature assessing neural and behavioral outcomes post-puberty. Consistent evidence of altered neurogenesis across a spectrum of chemicals and brain regions is particularly concerning and supports the hypothesis that EDC exposure may contribute to rapidly growing rates of pediatric neurobehavioral disorders. Remaining critical goals include identifying which EDCs, over which doses, and in which exposure window(s) are likely to contribute to altered brain development and how those changes are associated with specific neuropsychiatric disorders. With the growing barrage of exogenous chemicals humans are exposed to on a daily basis (Skakkebaek, Toppari et al. 2011, Grandjean and Landrigan 2014), deciphering precisely when and how much of an EDC (or combination of EDCs) can amplify risk of neuropsychological effects is a challenge, but one which requires substantive attention and an eye towards sex differences.

3.5 Sex differences

Of the 46 studies included, 49% included both males and females and looked for potential sex differences as part of their analysis (Figure 1). 26% included males and/or females, but did not separate them in the analysis or did not state which sexes were included. 17% of studies only included males and 8% only included females. This is considerably better than the neuroscience literature in general. A survey of 1200 neuroscience papers from 2011 to 2012 (Woodruff, Kibbe et al. 2014) found outcome by sex was reported only 36% of the time, and studies listed the sex of the animals only 42% of the time. Thus, the number of studies identifying sex, and the number of studies reporting outcome by sex was 32% and 13% higher, respectively, in the EDC literature reviewed here. The more frequent consideration of sex in the EDC literature, compared to the neuroscience literature, is logical considering their mechanisms of action. It was recognized quite early in the genesis of the field that EDCs have the potential to alter sex-specific physiology, and thus effects by sex have historically been considered an important element to report in the EDC literature. This work has significantly contributed to, and advanced basic knowledge of, the

neuroendocrinology of sexual differentiation (Gore 2008, Patisaul and Polston 2008), and demonstrates that EDC researchers embraced the inclusion of sex in study design long before the NIH mandated inclusion of sex differences in preclinical research (Clayton and Collins 2014). Just as sex may be a factor in calculating appropriate doses of drugs like zolpidem (used in Ambien) and acetaminophen (Whitley and Lindsey 2009, FDA 2013), effects of EDCs are likely also sex-specific and should be taken into consideration when determining susceptibility and potential mechanisms of action, even in pre-pubertal animals.

4. Conclusion

The reviewed data reveal that developmental EDC exposure can alter neurodevelopment across the brain beginning early in fetal life, with sex specific effects observable throughout the brain even before puberty. As anticipated, the hypothalamus was found to be particularly affected by estrogen-active EDCs in a sex, time, and exposure dependent manner. This finding was anticipated given the fundamental importance of gonadal hormones for masculinizing the developing rodent brain. These data clearly demonstrate that EDCs are capable of interfering with neural estrogen signaling, but whether or not the resulting phenotypic effects will be observable humans is unclear. There are key species differences in the developing rodent and human brain. Most significantly, androgens appear to be fundamental to primate brain masculinization while the role of estrogen is less well understood (Wallen 2005, Alexander 2014). It is reasonable to conclude, however, that EDCs are capable of perturbing steroid hormone function in the developing human brain, particularly the hypothalamus, and thus potentially altering sexually dimorphic development. Accordingly, evidence from human literature indicates that EDC exposure may be contributing to escalating rates of sex-biased mood and affective disorders in humans, especially children (de Cock, Maas et al. 2012, Grandjean and Landrigan 2014). Better understanding those potential relationships is a priority.

The hippocampus also appears particularly vulnerable to endocrine disruption by estrogenic compounds such as BPA and PCBs although there is not enough available evidence from this specific data set to make any conclusions about sex-specific effects. The mechanisms by which BPA and PCBs alter hippocampal organization likely differ from each other and include mechanisms other than disruption of estrogen signaling. PCBs are well characterized thyroid hormone disruptors (Winneke 2011), for example, and emerging evidence suggests BPA may be an androgen disrupter in the developing brain (Shoyele, Sivadas et al. 2011). Because the hippocampus is critical for learning and memory, further investigation into EDC effects on the organization of this region may help to elucidate potential linkages between EDC exposure and sex-biased deficits in IQ, memory and related cognitive functions in humans (Grandjean and Landrigan 2014).

Analyses of whole brain or large subregions in EDC-exposed embryos are limited but reveal evidence of effects primarily related to neurogenesis, altered neuropeptide expression, and other effects which may involve mechanisms other than disruption of steroid hormone activity. Moreover, these data suggest that EDC exposure prior to established hormone-dependent critical windows of sexual differentiation could result in broad, fundamental changes to brain organization, particularly in cortex and cerebellum. These changes may

include alteration of neuronal connections fundamental to complex behaviors such as activity, sociality, and executive function (Diamanti-Kandarakis, Bourguignon et al. 2009, Shoyele, Sivadas et al. 2011, Wolstenholme, Goldsby et al. 2013). Evidence from the cortex, cerebellum, and mid brain also emphasize that effects from gestational exposures might be exacerbated by additional exposures later in development, including the postnatal period.

Information on how exposures to emerging EDCs including fire retardants, surfactants, and other persistent contaminants now recognized to be ubiquitous in household environments (Stapleton, Klosterhaus et al. 2009, Lawlor, Kelly et al. 2011) impacts synaptogenesis, neural migration, and other developmental processes within the brain, particularly the cortex, cerebellum, and mid brain are critically needed and likely to reveal new mechanisms of sex-specific vulnerability. More comprehensive sex-specific comparisons of exposure-related effects in rodents at ages near neurodevelopmental critical periods like the pre- and postnatal testosterone surge, as well as the brain growth spurt at PND 10, are also needed to provide further insight into how chemically-induced alteration of brain organization might contribute to diseases such as ADHD, schizophrenia, and autism.

A limitation of the present review was the broad search terms used to identify relevant papers. Not using chemical names as search terms means it is likely that some papers were missed. This experience should serve as a critical warning for the EDC community. Not including broader search terms such as “endocrine disrupting compound” in the abstract or keyword list may limit the ability of systematic reviews to identify them, particularly reviews which are not chemical specific. For example, as a follow-up exercise, a search using the terms “PFOA” and “hypothalamus” turned up only three papers, none of which were salient for the present review, but a search of “phthalate” and “hypothalamus” turned up 29, two of which had data on the pre-pubertal brain but were not identified in the initial search conducted for this review ((Ramos, Coesel et al. 2008, Ramos, Masuet et al. 2009); the first of which examined sex-specific effects and the second looked only at males). For the present review it was not feasible to search for papers chemical by chemical as there are literally hundreds of EDCs and searching for the most well-known ones (eg. BPA, PBDEs, phthalates, genistein) would have biased the overall outcome. Identifying a standard set of 3–4 keywords would make papers on EDCs easier to identify and provide cohesion across the wide scope of papers in this field.

Compared to the neuroscience field as a whole, the neurodevelopmental EDC literature reported information about sex and potential sex differences more frequently. That neurodevelopmental effects of EDC exposure included alteration of sex differences was a common finding, and these observations will ultimately result in a better understanding of how male and female brains differ in their development and vulnerability to environmental insult. The results of this review clearly demonstrate the importance of including and analyzing both sexes in any experimental design, even *in vitro* approaches when possible. The endocrine disruption field has done a reasonably good job to date and should be considered a model for the larger neuroscience community.

Acknowledgements

The authors would like to thank Roberto C. Melcangi and Giancarlo Panzica for their invitation to write this review and its incorporation in their Special Issue. We also thank Robert Anholt and John Godwin for their editorial comments. This work was funded by NIEHS U01ES020929 and NIEHS R21ES021233 to HBP.

Abbreviations

AXXXX	Aroclor XXXX
ADHD	Attention-deficit/hyperactivity disorder
AhR	aryl hydrocarbon receptor
ARC	arcuate nucleus
ASD	Autism spectrum disorder
AVPV	anteroventral periventricular nucleus
BPA	bisphenol A
bw/day	by weight per day
CA1	region I of hippocampus proper
DES	diethylstilbestrol
DG	dentate gyrus
EAC	endocrine active compound
EB	estradiol benzoate
EDCs	endocrine disrupting compounds
EE	ethinyl estradiol
ER	estrogen receptor
ERK	extracellular signal-related kinases
GD	gestational day
LH	luteinizing hormone
MBH	mediobasal hypothalamus
PBDE	polybrominated diphenyl ethers
PCB	polychlorinated biphenyl
PFOA	perfluorooctanoic acid
PND	postnatal day
POA	preoptic area
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin, dioxin
VMN	ventromedial nucleus

References

- Ahmed RG. Perinatal TCDD exposure alters developmental neuroendocrine system. *Food Chem Toxicol.* 2011; 49(6):1276–1284. [PubMed: 21414370]
- Akahoshi E, Yoshimura S, Ishihara-Sugano M. Over-expression of AhR (aryl hydrocarbon receptor) induces neural differentiation of Neuro2a cells: neurotoxicology study. *Environ Health.* 2006; 5:24. [PubMed: 16956419]
- Alexander GM. Postnatal Testosterone Concentrations and Male Social Development. *Front Endocrinol (Lausanne).* 2014; 5:15. [PubMed: 24600437]
- Baron-Cohen S, Cassidy S, Auyeung B, Allison C, Achoukhi M, Robertson S, Pohl A, Lai MC. Attenuation of Typical Sex Differences in 800 Adults with Autism vs. 3,900 Controls. *PLoS One.* 2014; 9(7):e102251. [PubMed: 25029203]
- Baron-Cohen S, Knickmeyer RC, Belmonte MK. Sex differences in the brain: implications for explaining autism. *Science.* 2005; 310(5749):819–823. [PubMed: 16272115]
- Becker, JB.; Berkley, KJ.; Geary, N.; Hampson, E.; Herman, JP.; Young, EA. Sex Differences in the Brain : From Genes to Behavior: From Genes to Behavior. USA: Oxford University Press; 2007.
- Bourguignon JP, Franssen D, Gerard A, Janssen S, Pinson A, Naveau E, Parent AS. Early neuroendocrine disruption in hypothalamus and hippocampus: developmental effects including female sexual maturation and implications for endocrine disrupting chemical screening. *J Neuroendocrinol.* 2013; 25(11):1079–1087. [PubMed: 24028442]
- Braun JM, Kalkbrenner AE, Calafat AM, Yoltan K, Ye X, Dietrich KN, Lanphear BP. Impact of early-life bisphenol a exposure on behavior and executive function in children. *Pediatrics.* 2011; 128(5): 873–882. [PubMed: 22025598]
- Brown JS Jr. Effects of bisphenol-A and other endocrine disruptors compared with abnormalities of schizophrenia: an endocrine-disruption theory of schizophrenia. *Schizophr Bull.* 2009; 35(1):256–278. [PubMed: 18245062]
- Campbell I. Hypothalamic and pituitary function. *Anaesthesia and Intensive Care Medicine.* 2005; 6(10):324–326.
- Cao J, Joyner L, Mickens JA, Leyrer SM, Patisaul HB. Sex-specific Esr2 mRNA expression in the rat hypothalamus and amygdala is altered by neonatal bisphenol A exposure. *Reproduction.* 2014; 147(4):537–554. [PubMed: 24352099]
- Cao J, Mickens JA, McCaffrey KA, Leyrer SM, Patisaul HB. Neonatal Bisphenol A exposure alters sexually dimorphic gene expression in the postnatal rat hypothalamus. *Neurotoxicology.* 2012; 33(1):23–36. [PubMed: 22101008]
- Cao J, Rebuly ME, Rogers J, Todd KL, Leyrer SM, Ferguson SA, Patisaul HB. Prenatal bisphenol A exposure alters sex-specific estrogen receptor expression in the neonatal rat hypothalamus and amygdala. *Toxicol Sci.* 2013; 133(1):157–173. [PubMed: 23457122]
- Cao J, Rebuly ME, Rogers J, Todd KL, Leyrer SM, Ferguson SA, Patisaul HB. Prenatal bisphenol A exposure alters sex-specific estrogen receptor expression in the neonatal rat hypothalamus and amygdala. *Toxicological sciences.* 2013; 133(1):157–173. [PubMed: 23457122]
- CDC. Prevalence of autism spectrum disorders--Autism and Developmental Disabilities Monitoring Network, 14 sites, United States, 2008. *MMWR Surveill Summ.* 2012; 61(3):1–19.
- Ceccarelli I, Della Seta D, Fiorenzani P, Farabollini F, Aloisi AM. Estrogenic chemicals at puberty change ERalpha in the hypothalamus of male and female rats. *Neurotoxicol Teratol.* 2007; 29(1): 108–115. [PubMed: 17157476]
- Chang SF, Sun YY, Yang LY, Hu SY, Tsai SY, Lee WS, Lee YH. Bcl-2 gene family expression in the brain of rat offspring after gestational and lactational dioxin exposure. *Ann N Y Acad Sci.* 2005; 1042:471–480. [PubMed: 15965093]
- Cheslack-Postava K, Rantakokko PV, Hinkka-Yli-Salomaki S, Surcel HM, McKeague IW, Kiviranta HA, Sourander A, Brown AS. Maternal serum persistent organic pollutants in the Finnish Prenatal Study of Autism: A pilot study. *Neurotoxicol Teratol.* 2013; 38:1–5. [PubMed: 23591055]
- Clayton JA, Collins FS. Policy: NIH to balance sex in cell and animal studies. *Nature.* 2014; 509(7500):282–283. [PubMed: 24834516]

- Colciago A, Casati L, Mornati O, Vergoni AV, Santagostino A, Celotti F, Negri-Cesi P. Chronic treatment with polychlorinated biphenyls (PCB) during pregnancy and lactation in the rat Part 2: Effects on reproductive parameters, on sex behavior, on memory retention and on hypothalamic expression of aromatase and 5 α -reductases in the offspring. *Toxicol Appl Pharmacol*. 2009; 239(1):46–54. [PubMed: 19464308]
- Costa LG, Giordano G. Developmental neurotoxicity of polybrominated diphenyl ether (PBDE) flame retardants. *Neurotoxicology*. 2007; 28(6):1047–1067. [PubMed: 17904639]
- Costello EJ, Mustillo S, Erkanli A, Keeler G, Angold A. Prevalence and development of psychiatric disorders in childhood and adolescence. *Arch Gen Psychiatry*. 2003; 60(8):837–844. [PubMed: 12912767]
- Crain DA, Janssen SJ, Edwards TM, Heindel J, Ho SM, Hunt P, Iguchi T, Juul A, McLachlan JA, Schwartz J, Skakkebaek N, Soto AM, Swan S, Walker C, Woodruff TK, Woodruff TJ, Giudice LC, Guillette LJ Jr. Female reproductive disorders: the roles of endocrine-disrupting compounds and developmental timing. *Fertil Steril*. 2008; 90(4):911–940. [PubMed: 18929049]
- Crinnion WJ. Toxic effects of the easily avoidable phthalates and parabens. *Altern Med Rev*. 2010; 15(3):190–196. [PubMed: 21155623]
- Cryan JF, O'Mahony SM. The microbiome-gut-brain axis: from bowel to behavior. *Neurogastroenterol Motil*. 2011; 23(3):187–192. [PubMed: 21303428]
- de Cock M, Maas YG, van de Bor M. Does perinatal exposure to endocrine disruptors induce autism spectrum and attention deficit hyperactivity disorders? Review. *Acta Paediatr*. 2012; 101(8):811–818. [PubMed: 22458970]
- de Vries GJ. SEX DIFFERENCES IN THE BRAIN: THE RELATION BETWEEN STRUCTURE AND FUNCTION, jElsevier Science. 1984
- De Vries GJ. Minireview: Sex differences in adult and developing brains: compensation, compensation, compensation. *Endocrinology*. 2004; 145(3):1063–1068. [PubMed: 14670982]
- Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, Zoeller RT, Gore AC. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. *Endocr Rev*. 2009; 30(4):293–342. [PubMed: 19502515]
- Dickerson SM, Cunningham SL, Gore AC. Prenatal PCBs disrupt early neuroendocrine development of the rat hypothalamus. *Toxicol Appl Pharmacol*. 2011; 252(1):36–46. [PubMed: 21277884]
- Doumy G, Roedig C, Son SK, Blaga CI, DiChiara AD, Santra R, Berrah N, Bostedt C, Bozek JD, Bucksbaum PH, Cryan JP, Fang L, Ghimire S, Glowina JM, Hoener M, Kanter EP, Krassig B, Kuebel M, Messerschmidt M, Paulus GG, Reis DA, Rohringer N, Young L, Agostini P, DiMauro LF. Nonlinear atomic response to intense ultrashort x rays. *Phys Rev Lett*. 2011; 106(8):083002. [PubMed: 21405568]
- Engel SM, Miodovnik A, Canfield RL, Zhu C, Silva MJ, Calafat AM, Wolff MS. Prenatal phthalate exposure is associated with childhood behavior and executive functioning. *Environ Health Perspect*. 2010; 118(4):565–571. [PubMed: 20106747]
- Eskenazi B, Chevrier J, Rauch SA, Kogut K, Harley KG, Johnson C, Trujillo C, Sjodin A, Bradman A. In utero and childhood polybrominated diphenyl ether (PBDE) exposures and neurodevelopment in the CHAMACOS study. *Environ Health Perspect*. 2013; 121(2):257–262. [PubMed: 23154064]
- Eubig PA, Aguiar A, Schantz SL. Lead and PCBs as risk factors for attention deficit/hyperactivity disorder. *Environ Health Perspect*. 2010; 118(12):1654–1667. [PubMed: 20829149]
- Facciolo RM, Alo R, Madeo M, Canonaco M, Dessi-Fulgheri F. Early cerebral activities of the environmental estrogen bisphenol A appear to act via the somatostatin receptor subtype sst(2). *Environ Health Perspect* 110 Suppl. 2002; 3:397–402.
- Facciolo RM, Madeo M, Alo R, Canonaco M, Dessi-Fulgheri F. Neurobiological effects of bisphenol A may be mediated by somatostatin subtype 3 receptors in some regions of the developing rat brain. *Toxicol Sci*. 2005; 88(2):477–484. [PubMed: 16162846]
- FDA. Risk of next-morning impairment after use of insomnia drugs. FDA requires lower recommended doses for certain drugs containing zolpidem (Ambien, Ambien CR, Edluar, and Zolpimist). 2013
- Fernandez M, Paradisi M, D'Intino G, Del Vecchio G, Sivilia S, Giardino L, Calza L. A single prenatal exposure to the endocrine disruptor 2,3,7,8-tetrachlorodibenzo-p-dioxin alters developmental

myelination and remyelination potential in the rat brain. *J Neurochem.* 2010; 115(4):897–909. [PubMed: 20807317]

Foley J, Clifford D, Castle K, Cryan P, Ostfeld RS. Investigating and managing the rapid emergence of white-nose syndrome, a novel, fatal, infectious disease of hibernating bats. *Conserv Biol.* 2011; 25(2):223–231. [PubMed: 21284732]

Frye CA, Bo E, Calamandrei G, Calza L, Dessi-Fulgheri F, Fernandez M, Fusani L, Kah O, Kajta M, Le Page Y, Patisaul HB, Venerosi A, Wojtowicz AK, Panzica GC. Endocrine disruptors: a review of some sources, effects, and mechanisms of actions on behaviour and neuroendocrine systems. *J Neuroendocrinol.* 2012; 24(1):144–159. [PubMed: 21951193]

Fujita H, Samejima H, Kitagawa N, Mitsuhashi T, Washio T, Yonemoto J, Tomita M, Takahashi T, Kosaki K. Genome-wide screening of dioxin-responsive genes in fetal brain: bioinformatic and experimental approaches. *Congenit Anom (Kyoto).* 2006; 46(3):135–143. [PubMed: 16922920]

Gascon M, Vrijheid M, Martinez D, Forns J, Grimalt JO, Torrent M, Sunyer J. Effects of pre and postnatal exposure to low levels of polybromodiphenyl ethers on neurodevelopment and thyroid hormone levels at 4 years of age. *Environ Int.* 2011; 37(3):605–611. [PubMed: 21237513]

Gauger KJ, Kato Y, Haraguchi K, Lehmler HJ, Robertson LW, Bansal R, Zoeller RT. Polychlorinated biphenyls (PCBs) exert thyroid hormone-like effects in the fetal rat brain but do not bind to thyroid hormone receptors. *Environ Health Perspect.* 2004; 112(5):516–523. [PubMed: 15064154]

Gore AC. Developmental programming and endocrine disruptor effects on reproductive neuroendocrine systems. *Frontiers in Neuroendocrinology.* 2008; 29(3):358–374. [PubMed: 18394690]

Gore, AC.; Dickerson, S. *Endocrine Disruptors and The Developing Brain.* Biota Publishing; 2012.

Grandjean P, Landrigan PJ. Developmental neurotoxicity of industrial chemicals. *Lancet.* 2006; 368(9553):2167–2178. [PubMed: 17174709]

Grandjean P, Landrigan PJ. Neurobehavioural effects of developmental toxicity. *Lancet neurology.* 2014; 13(3):330–338. [PubMed: 24556010]

Harley KG, Gunier RB, Kogut K, Johnson C, Bradman A, Calafat AM, Eskenazi B. Prenatal and early childhood bisphenol A concentrations and behavior in school-aged children. *Environ Res.* 2013; 126:43–50. [PubMed: 23870093]

Hays LE, Carpenter CD, Petersen SL. Evidence that GABAergic neurons in the preoptic area of the rat brain are targets of 2,3,7,8-tetrachlorodibenzo-p-dioxin during development. *Environ Health Perspect* 110 Suppl. 2002; 3:369–376.

He Z, Paule MG, Ferguson SA. Low oral doses of bisphenol A increase volume of the sexually dimorphic nucleus of the preoptic area in male, but not female, rats at postnatal day 21. *Neurotoxicol Teratol.* 2012; 34(3):331–337. [PubMed: 22507915]

Hobler C, Andrade AJ, Grande SW, Gericke C, Talsness CE, Appel KE, Chahoud I, Grote K. Sex-dependent aromatase activity in rat offspring after pre- and postnatal exposure to triphenyltin chloride. *Toxicology.* 2010; 276(3):198–205. [PubMed: 20708649]

Hoffman K, Webster TF, Weisskopf MG, Weinberg J, Vieira VM. Exposure to polyfluoroalkyl chemicals and attention deficit/hyperactivity disorder in U.S. children 12–15 years of age. *Environ Health Perspect.* 2010; 118(12):1762–1767. [PubMed: 20551004]

Itoh K, Yaoi T, Fushiki S. Bisphenol A, an endocrine-disrupting chemical, and brain development. *Neuropathology.* 2012; 32(4):447–457. [PubMed: 22239237]

Jacobson JL, Jacobson SW. Intellectual impairment in children exposed to polychlorinated biphenyls in utero. *N Engl J Med.* 1996; 335(11):783–789. [PubMed: 8703183]

Kawai K, Murakami S, Senba E, Yamanaka T, Fujiwara Y, Arimura C, Nozaki T, Takii M, Kubo C. Changes in estrogen receptors alpha and beta expression in the brain of mice exposed prenatally to bisphenol A. *Regul Toxicol Pharmacol.* 2007; 47(2):166–170. [PubMed: 17222491]

Khurana S, Ranmal S, Ben-Jonathan N. Exposure of newborn male and female rats to environmental estrogens: delayed and sustained hyperprolactinemia and alterations in estrogen receptor expression. *Endocrinology.* 2000; 141(12):4512–4517. [PubMed: 11108262]

Kim K, Son TG, Park HR, Kim SJ, Kim HS, Kim HS, Kim TS, Jung KK, Han SY, Lee J. Potencies of bisphenol A on the neuronal differentiation and hippocampal neurogenesis. *J Toxicol Environ Health A.* 2009; 72(21–22):1343–1351. [PubMed: 20077206]

- Kim SM, Han DH, Lyoo HS, Min KJ, Kim KH, Renshaw P. Exposure to environmental toxins in mothers of children with autism spectrum disorder. *Psychiatry Investig.* 2010; 7(2):122–127.
- Komada M, Asai Y, Morii M, Matsuki M, Sato M, Nagao T. Maternal bisphenol A oral dosing relates to the acceleration of neurogenesis in the developing neocortex of mouse fetuses. *Toxicology.* 2012; 295(1–3):31–38. [PubMed: 22426297]
- Kunz N, Camm EJ, Somme E, Lodyginsky G, Darbre S, Aubert ML, Huppi PS, Sizonenko SV, Gruetter R. Developmental and metabolic brain alterations in rats exposed to bisphenol A during gestation and lactation. *Int J Dev Neurosci.* 2011; 29(1):37–43. [PubMed: 20955774]
- Landrigan PJ, Lambertini L, Birnbaum LS. A research strategy to discover the environmental causes of autism and neurodevelopmental disabilities. *Environ Health Perspect.* 2012; 120(7):a258–a260. [PubMed: 22543002]
- Lawlor C, Kelly C, O’Leary S, O’Sullivan MP, Gallagher PJ, Keane J, Cryan SA. Cellular targeting and trafficking of drug delivery systems for the prevention and treatment of MTb. *Tuberculosis (Edinb).* 2011; 91(1):93–97. [PubMed: 21237714]
- Lenz KM, McCarthy MM. Organized for sex - steroid hormones and the developing hypothalamus. *Eur J Neurosci.* 2010; 32(12):2096–2104. [PubMed: 21143664]
- Lephart ED, Setchell KD, Handa RJ, Lund TD. Behavioral effects of endocrine-disrupting substances: phytoestrogens. *Ilar J.* 2004; 45(4):443–454. [PubMed: 15454683]
- Lichtensteiger W, Bassetti-Gaille C, Faass O, Axelstad M, Boberg J, Christiansen S, Rehrauer H, Georgijevic JK, Hass U, Kortenkamp A, Schlumpf M. Differential Gene Expression Patterns in Developing Sexually Dimorphic Rat Brain Regions Exposed to Anti-androgenic, Estrogenic, or Complex Endocrine Disruptor Mixtures: Glutamatergic Synapses as Target. *Endocrinology.* 2015 en20141504.
- Losa SM, Todd KL, Sullivan AW, Cao J, Mickens JA, Patisaul HB. Neonatal exposure to genistein adversely impacts the ontogeny of hypothalamic kisspeptin signaling pathways and ovarian development in the peripubertal female rat. *Reprod Toxicol.* 2011; 31(3):280–289. [PubMed: 20951797]
- Merikangas KR, Nakamura EF, Kessler RC. Epidemiology of mental disorders in children and adolescents. *Dialogues Clin Neurosci.* 2009; 11(1):7–20. [PubMed: 19432384]
- Mimura J, Fujii-Kuriyama Y. Functional role of AhR in the expression of toxic effects by TCDD. *Biochim Biophys Acta.* 2003; 1619(3):263–268. [PubMed: 12573486]
- Miodovnik A, Engel SM, Zhu C, Ye X, Soorya LV, Silva MJ, Calafat AM, Wolff MS. Endocrine disruptors and childhood social impairment. *Neurotoxicology.* 2011; 32(2):261–267. [PubMed: 21182865]
- Molero L, Garcia-Duran M, Diaz-Recasens J, Rico L, Casado S, Lopez-Farre A. Expression of estrogen receptor subtypes and neuronal nitric oxide synthase in neutrophils from women and men: regulation by estrogen. *Cardiovasc Res.* 2002; 56(1):43–51. [PubMed: 12237165]
- Morreale de Escobar G, Obregon MJ, Escobar del Rey F. Role of thyroid hormone during early brain development. *Eur J Endocrinol* 151 Suppl. 2004; 3:U25–U37.
- Morse DC, Groen D, Veerman M, van Amerongen CJ, Koeter HB, Smits van Prooije AE, Visser TJ, Koeman JH, Brouwer A. Interference of polychlorinated biphenyls in hepatic and brain thyroid hormone metabolism in fetal and neonatal rats. *Toxicol Appl Pharmacol.* 1993; 122(1):27–33. [PubMed: 8378931]
- Morse DC, Wehler EK, Wesseling W, Koeman JH, Brouwer A. Alterations in rat brain thyroid hormone status following pre- and postnatal exposure to polychlorinated biphenyls (Aroclor 1254). *Toxicol Appl Pharmacol.* 1996; 136(2):269–279. [PubMed: 8619235]
- Nakamura K, Itoh K, Yaoi T, Fujiwara Y, Sugimoto T, Fushiki S. Murine neocortical histogenesis is perturbed by prenatal exposure to low doses of Bisphenol A. *J Neurosci Res.* 2006; 84(6):1197–1205. [PubMed: 16902998]
- Navarro VM, Sanchez-Garrido MA, Castellano JM, Roa J, Garcia-Galiano D, Pineda R, Aguilar E, Pinilla L, Tena-Sempere M. Persistent impairment of hypothalamic KiSS-1 system after exposures to estrogenic compounds at critical periods of brain sex differentiation. *Endocrinology.* 2009; 150(5):2359–2367. [PubMed: 19106226]

- Naveau E, Pinson A, Gerard A, Nguyen L, Charlier C, Thome JP, Zoeller RT, Bourguignon JP, Parent AS. Alteration of rat fetal cerebral cortex development after prenatal exposure to polychlorinated biphenyls. *PLoS One*. 2014; 9(3):e91903. [PubMed: 24642964]
- Nayyar T, Zawia NH, Hood DB. Transplacental effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the temporal modulation of Sp1 DNA binding in the developing cerebral cortex and cerebellum. *Exp Toxicol Pathol*. 2002; 53(6):461–468. [PubMed: 11926288]
- Nishizawa H, Imanishi S, Manabe N. Effects of exposure in utero to bisphenol a on the expression of aryl hydrocarbon receptor, related factors, and xenobiotic metabolizing enzymes in murine embryos. *J Reprod Dev*. 2005; 51(5):593–605. [PubMed: 16284450]
- Nishizawa H, Manabe N, Morita M, Sugimoto M, Imanishi S, Miyamoto H. Effects of in utero exposure to bisphenol A on expression of RARalpha and RXRalpha mRNAs in murine embryos. *J Reprod Dev*. 2003; 49(6):539–545. [PubMed: 14967906]
- Nishizawa H, Morita M, Sugimoto M, Imanishi S, Manabe N. Effects of in utero exposure to bisphenol A on mRNA expression of arylhydrocarbon and retinoid receptors in murine embryos. *J Reprod Dev*. 2005; 51(3):315–324. [PubMed: 15738621]
- O'Malley D, Liston M, Hyland NP, Dinan TG, Cryan JF. Colonic soluble mediators from the maternal separation model of irritable bowel syndrome activate submucosal neurons via an interleukin-6-dependent mechanism. *Am J Physiol Gastrointest Liver Physiol*. 2011; 300(2):G241–G252. [PubMed: 21109592]
- Ohtani-Kaneko R. Mechanisms underlying estrogen-induced sexual differentiation in the hypothalamus. *Histol Histopathol*. 2006; 21(3):317–324. [PubMed: 16372252]
- Palanza P, Gioiosa L, vom Saal FS, Parmigiani S. Effects of developmental exposure to bisphenol A on brain and behavior in mice. *Environ Res*. 2008; 108(2):150–157. [PubMed: 18949834]
- Palanza P, Morellini F, Parmigiani S, vom Saal FS. Prenatal exposure to endocrine disrupting chemicals: effects on behavioral development. *Neurosci Biobehav Rev*. 1999; 23(7):1011–1027. [PubMed: 10580314]
- Patel RD, Kim DJ, Peters JM, Perdew GH. The aryl hydrocarbon receptor directly regulates expression of the potent mitogen epiregulin. *Toxicol Sci*. 2006; 89(1):75–82. [PubMed: 16192470]
- Patisaul HB, Adewale HB. Long-term effects of environmental endocrine disruptors on reproductive physiology and behavior. *Front Behav Neurosci*. 2009; 3:10. [PubMed: 19587848]
- Patisaul HB, Fortino AE, Polston EK. Neonatal genistein or bisphenol-A exposure alters sexual differentiation of the AVPV. *Neurotoxicol Teratol*. 2006; 28(1):111–118. [PubMed: 16427766]
- Patisaul HB, Fortino AE, Polston EK. Differential disruption of nuclear volume and neuronal phenotype in the preoptic area by neonatal exposure to genistein and bisphenol-A. *Neurotoxicology*. 2007; 28(1):1–12. [PubMed: 17109964]
- Patisaul HB, Polston EK. Influence of endocrine active compounds on the developing rodent brain. *Brain Res Rev*. 2008; 57(2):352–362. [PubMed: 17822772]
- Paus T, Keshavan M, Giedd JN. Why do many psychiatric disorders emerge during adolescence? *Nat Rev Neurosci*. 2008; 9(12):947–957. [PubMed: 19002191]
- Peters JM, Narotsky MG, Elizondo G, Fernandez-Salguero PM, Gonzalez FJ, Abbott BD. Amelioration of TCDD-induced teratogenesis in aryl hydrocarbon receptor (AhR)-null mice. *Toxicol Sci*. 1999; 47(1):86–92. [PubMed: 10048156]
- Ramos A, Coesel S, Marques A, Rodrigues M, Baumgartner A, Noronha J, Rauter A, Brenig B, Varela J. Isolation and characterization of a stress-inducible *Dunaliella salina* Lcy-beta gene encoding a functional lycopene beta-cyclase. *Appl Microbiol Biotechnol*. 2008; 79(5):819–828. [PubMed: 18461318]
- Ramos R, Masuet C, Badia M, Perna V, Macia I, Escobar I, Villalonga R, Moya J. Quantification of eccrine sweat glands with acetylcholine sweat-spot test and anatomical redistribution of sweating after T2-T3 thoracoscopic sympathectomy. *Surg Endosc*. 2009; 23(2):321–326. [PubMed: 18461392]
- Ramos R, Patricio L, Abreu A, Soares C, Mamede A, Fernandes R, Gamboa P, Timoteo A, Sousa L, Cacela D, Bernardes L, Ferreira RC. Left main percutaneous coronary intervention in ST elevation myocardial infarction. *Rev Port Cardiol*. 2008; 27(7–8):965–973. [PubMed: 18959093]

- Ramtekkar UP, Reiersen AM, Todorov AA, Todd RD. Sex and age differences in attention-deficit/hyperactivity disorder symptoms and diagnoses: implications for DSM-V and ICD-11. *J Am Acad Child Adolesc Psychiatry*. 2010; 49(3):217–228. e211–e213. [PubMed: 20410711]
- Rebuli ME, Cao J, Sluzas E, Delclos KB, Camacho L, Lewis SM, Vanlandingham MM, Patisaul HB. Investigation of the effects of subchronic low dose oral exposure to bisphenol A (BPA) and ethinyl estradiol (EE) on estrogen receptor expression in the juvenile and adult female rat hypothalamus. *Toxicol Sci*. 2014; 140(1):190–203. [PubMed: 24752507]
- Rubin BS, Lenkowski JR, Schaeberle CM, Vandenberg LN, Ronsheim PM, Soto AM. Evidence of altered brain sexual differentiation in mice exposed perinatally to low, environmentally relevant levels of bisphenol A. *Endocrinology*. 2006; 147(8):3681–3691. [PubMed: 16675520]
- Sagiv SK, Thurston SW, Bellinger DC, Tolbert PE, Altshul LM, Korrick SA. Prenatal organochlorine exposure and behaviors associated with attention deficit hyperactivity disorder in school-aged children. *Am J Epidemiol*. 2010; 171(5):593–601. [PubMed: 20106937]
- Schantz SL, Widholm JJ. Cognitive effects of endocrine-disrupting chemicals in animals. *Environ Health Perspect*. 2001; 109(12):1197–1206. [PubMed: 11748026]
- Schug TT, Janesick A, Blumberg B, Heindel JJ. Endocrine disrupting chemicals and disease susceptibility. *J Steroid Biochem Mol Biol*. 2011; 127(3–5):204–215. [PubMed: 21899826]
- Shindo S, Moore R, Flake G, Negishi M. Serine 216 phosphorylation of estrogen receptor alpha in neutrophils: migration and infiltration into the mouse uterus. *PLoS One*. 2013; 8(12):e84462. [PubMed: 24386386]
- Shoyele SA, Sivadas N, Cryan SA. The effects of excipients and particle engineering on the biophysical stability and aerosol performance of parathyroid hormone (1–34) prepared as a dry powder for inhalation. *AAPS PharmSciTech*. 2011; 12(1):304–311. [PubMed: 21271316]
- Simerly RB. Wired for reproduction: organization and development of sexually dimorphic circuits in the mammalian forebrain. *Annu Rev Neurosci*. 2002; 25:507–536. [PubMed: 12052919]
- Skakkebaek NE, Toppari J, Soder O, Gordon CM, Dival S, Draznin M. The exposure of fetuses and children to endocrine disrupting chemicals: a European Society for Paediatric Endocrinology (ESPE) and Pediatric Endocrine Society (PES) call to action statement. *J Clin Endocrinol Metab*. 2011; 96(10):3056–3058. [PubMed: 21832106]
- Stapleton HM, Klosterhaus S, Eagle S, Fuh J, Meeker JD, Blum A, Webster TF. Detection of organophosphate flame retardants in furniture foam and U.S. house dust. *Environ Sci Technol*. 2009; 43(19):7490–7495. [PubMed: 19848166]
- Stewart PW, Lonky E, Reihman J, Pagano J, Gump BB, Darvill T. The relationship between prenatal PCB exposure and intelligence (IQ) in 9-year-old children. *Environ Health Perspect*. 2008; 116(10):1416–1422. [PubMed: 18941588]
- Takahashi M, Negishi T, Imamura M, Sawano E, Kuroda Y, Yoshikawa Y, Tashiro T. Alterations in gene expression of glutamate receptors and exocytosis-related factors by a hydroxylated-polychlorinated biphenyl in the developing rat brain. *Toxicology*. 2009; 257(1–2):17–24. [PubMed: 19114083]
- Tang MH, Pinsky EG. Mood and affect disorders. *Pediatr Rev*. 2015; 36(2):52–61. [PubMed: 25646309]
- Tijet N, Boutros PC, Moffat ID, Okey AB, Tuomisto J, Pohjanvirta R. Aryl hydrocarbon receptor regulates distinct dioxin-dependent and dioxin-independent gene batteries. *Mol Pharmacol*. 2006; 69(1):140–153. [PubMed: 16214954]
- Vreugdenhil HJ, Lanting CI, Mulder PG, Boersma ER, Weisglas-Kuperus N. Effects of prenatal PCB and dioxin background exposure on cognitive and motor abilities in Dutch children at school age. *J Pediatr*. 2002; 140(1):48–56. [PubMed: 11815763]
- Walker DM, Goetz BM, Gore AC. Dynamic postnatal developmental and sex-specific neuroendocrine effects of prenatal polychlorinated biphenyls in rats. *Mol Endocrinol*. 2014; 28(1):99–115. [PubMed: 24284824]
- Wallen K. Hormonal influences on sexually differentiated behavior in nonhuman primates. *Front Neuroendocrinol*. 2005; 26(1):7–26. [PubMed: 15862182]

- Walshe TE, Connell P, Cryan L, Ferguson G, Gardiner T, Morrow D, Redmond EM, O'Brien C, Cahill PA. Microvascular retinal endothelial and pericyte cell apoptosis in vitro: role of hedgehog and Notch signaling. *Invest Ophthalmol Vis Sci*. 2011; 52(7):4472–4483. [PubMed: 21498615]
- Whitley H, Lindsey W. Sex-based differences in drug activity. *Am Fam Physician*. 2009; 80(11):1254–1258. [PubMed: 19961138]
- Winneke G. Developmental aspects of environmental neurotoxicology: Lessons from lead and polychlorinated biphenyls. *J Neurol Sci*. 2011; 308(1–2):9–15. [PubMed: 21679971]
- Wolstenholme JT, Edwards M, Shetty SR, Gatewood JD, Taylor JA, Rissman EF, Connelly JJ. Gestational Exposure to Bisphenol A Produces Transgenerational Changes in Behaviors and Gene Expression. *Endocrinology*. 2012; 153(8):3828–2838. [PubMed: 22707478]
- Wolstenholme JT, Goldsby JA, Rissman EF. Transgenerational effects of prenatal bisphenol A on social recognition. *Hormones and behavior*. 2013; 64(5):833–839. [PubMed: 24100195]
- Wolstenholme JT, Rissman EF, Connelly JJ. The role of Bisphenol A in shaping the brain, epigenome and behavior. *Horm Behav*. 2011; 59(3):296–305. [PubMed: 21029734]
- Wood H. Neuroimmunology: estrogen receptor ligands suppress inflammatory responses in astrocytes and microglia. *Nat Rev Neurol*. 2011; 7(7):355. [PubMed: 21691333]
- Woodruff TK, Kibbe MR, Paller AS, Turek FW, Woolley CS. Commentary: Leaning in to support sex differences in basic science and clinical research. *Endocrinology*. 2014; 155(4):1181–1183. [PubMed: 24506076]
- Wu WF, Tan XJ, Dai YB, Krishnan V, Warner M, Gustafsson JA. Targeting estrogen receptor beta in microglia and T cells to treat experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci U S A*. 2013; 110(9):3543–3548. [PubMed: 23401502]
- Xu X, Xie L, Hong X, Ruan Q, Lu H, Zhang Q, Zhang G, Liu X. Perinatal exposure to bisphenol-A inhibits synaptogenesis and affects the synaptic morphological development in offspring male mice. *Chemosphere*. 2013; 91(8):1073–1081. [PubMed: 23490186]
- Xu XB, He Y, Song C, Ke X, Fan SJ, Peng WJ, Tan R, Kawata M, Matsuda K, Pan BX, Kato N. Bisphenol A regulates the estrogen receptor alpha signaling in developing hippocampus of male rats through estrogen receptor. *Hippocampus*. 2014; 24(12):1570–1580. [PubMed: 25074486]
- Xu XH, Wang YM, Zhang J, Luo QQ, Ye YP, Ruan Q. Perinatal exposure to bisphenol-A changes N-methyl-D-aspartate receptor expression in the hippocampus of male rat offspring. *Environ Toxicol Chem*. 2010; 29(1):176–181. [PubMed: 20821433]
- Zoeller RT, Bansal R, Parris C. Bisphenol-A, an environmental contaminant that acts as a thyroid hormone receptor antagonist in vitro, increases serum thyroxine, and alters RC3/neurogranin expression in the developing rat brain. *Endocrinology*. 2005; 146(2):607–612. [PubMed: 15498886]
- Zoeller RT, Dowling AL, Vas AA. Developmental exposure to polychlorinated biphenyls exerts thyroid hormone-like effects on the expression of RC3/neurogranin and myelin basic protein messenger ribonucleic acids in the developing rat brain. *Endocrinology*. 2000; 141(1):181–189. [PubMed: 10614638]
- Zsarnovszky A, Le HH, Wang HS, Belcher SM. Ontogeny of rapid estrogen-mediated extracellular signal-regulated kinase signaling in the rat cerebellar cortex: potent nongenomic agonist and endocrine disrupting activity of the xenoestrogen bisphenol A. *Endocrinology*. 2005; 146(12):5388–5396. [PubMed: 16123166]

Highlights

- Evidence of EDC effects is greatest for hypothalamic areas and endpoints.
- Greater focus on extrahypothalamic areas is needed to understand age and sex dependent vulnerabilities.
- Neurogenesis can be disrupted by gestational EDC exposure.
- Accounting for sex is higher in the EDC literature than the neuroscience literature.

Figure 1.

Assessment of sex in the EDC studies reviewed here. Nearly half of the studies looked for effects in both sexes but more than a quarter did not distinguish between the sexes at all. Consistent with the literature in general, studies considering only one sex disproportionately focus on males.

Table 1

EDC Effects on the Hypothalamus

(Cao, Mickens et al. 2012)	Rat, Long Evans	M/F	Subcutaneous injection	PND 0–2	10 µg EB 50 µg/kg, 50 mg/kg BPA	ERα, ERβ, and Kiss1 mRNA expression in the anterior and mediobasal hypothalamus	PND 4 and 10
(Cao, Rebuli et al. 2013)	Rat, Sprague Dawley	M/F	Oral gavage to dam	GD 6–21	2.5, 25 µg/kg bw/day BPA 5, 10 µg/kg bw/day EE	ER mRNA expression in the hypothalamus and amygdala	PND 1
(Ceccarelli, Della Seta et al. 2007)	Rat, Sprague Dawley	M/F	Oral	PND 23–30	40 µg/kg BPA 0.4 µg/kg EE	Hypothalamic ERα expression	PND 37 and 90
(Colciago, Casati et al. 2009)	Rat, Sprague Dawley	M/F	Subcutaneous injection to dam	GD 15–19	10 mg/kg/day mixture of equal parts PCB 138, 153, 180 and 10 ⁴ lower concentration of PCB 126	Enzyme expression in hypothalamus via RT-PCR	GD 20, PND 12, 21, 60
(Dickerson, Cunningham et al. 2011)	Rat, Sprague Dawley	M/F	i.p. injection, 0.1 ml DMSO 99.5%	GD 16 and 18	1 mg/kg Aroclor 1221 (industrial PCB mixture) 50 µg/kg EB	Hypothalamic ERα expression, apoptotic cell numbers, gene expression	PND 1
					1 mg/kg mixture of PCB138, 153, 180 at equimolar concentration		
(Facciolo, Alo et al. 2002)	Rat, Sprague Dawley	M/F, not distinguished	Oral, to dam	GD 0 – PND 23	400 µg/kg/day BPA	stt2 expression and binding in presence of α containing GAB A type A receptor agonists in the hypothalamus and hippocampus	PND 10, 23
(Facciolo, Madeo et al. 2005)	Rat, Sprague Dawley	F	Oral, to dam	GD 0 – PND 23	40 and 400 µg/kg/day BPA	stt3 expression and binding in presence of α containing GAB A type A receptor agonists in extrahypothalamic structures, hippocampus, and cortex	PND 7, 55

(Hays, Carpenter et al. 2002)	Rat, Holtzman Sprague Dawley	M/F	Oral, to dam	GD 15	1 µg/kg TCDD	GAD 67 expression and progesterin receptor (PR) expression in the preoptic area of the hypothalamus. Ahr and GAD dual label in preoptic area	PND 3
(He, Paule et al. 2012)	Rat, Sprague Dawley	M/F	Oral gavage, to dam	GD 6–21	2.5, 25 µg/kg BPA 5.0, 10.0 µg/kg EE	SDN-POA volume	PND 21
(Hobler, Andrade et al. 2010)	Rat, Wistar	M/F	Oral, to dam	GD 6 – PND 58	2 mg/kg/day TPTCl	Aromatase activity in the preoptic area of the hypothalamus	PND 1, 21, 58
(Khurana, Rammal et al. 2000)	Rat, Fischer 344	M/F	Subcutaneous injection	PND 1–5	100 µg day BPA 100 µg day octylphenol 5 µg day DES	ER expression in MBH and anterior pituitary	PND 30
(Lichtensteiger, Bassetti-Gaille et al. 2015)	Rat, Wistar	M/F	Oral gavage, to dam	GD 7 – PND 21 GD 13–19 for groups containing paracetamol	100, 200, 450 mg/kg/day mixtures A-mix (di-n-butylphthalate, diethylhexylphthalate, vinclozolin, prochloraz, procymidone, linuron, epoxiconazole, p,p'-DDE) E-mix (bisphenol A, 4-methylbenzylidene camphor, 2-ethylhexyl 4-methoxycinnamate, butylparaben) AEP-mix (A-mix and E-mix plus paracetamol) Paracetamol alone -For more details about amounts of each chemical, see paper	Gene expression in POA and VMN by exon-microarray and rt-PCR	PND 6
(Losa, Todd et al. 2011)	Rat, Long Evans	F	Subcutaneous injection	PND 0–3	1, 10 mg/kg bw Genistein 10 µg/kg bw EB	Kiss fiber density in the hypothalamus	PND 21, 24, 28, 33
(Navarro, Sanchez-Garrido et al. 2009)	Rat, Wistar	M/F	Subcutaneous injection	PND 1 and PND 1–5	1, 10, 100, 500 µg EB to males 0.1, 1, 10, 100 µg EB to females	Brain circulating hormone levels, hypothalamic mRNA expression	PND 30

(Patisaul, Fortino et al. 2006)	Rat, Sprague Dawley	M/F	Subcutaneous injection	PND 1, 1.5, 2, 2.5	100, 500 µg BPA	TH and ERα immunoreactivity in hypothalamic AVPV	PND 19
(Patisaul, Fortino et al. 2007)	Rat, Sprague Dawley	M/F	Subcutaneous injection	PND 1, 1.5, 2, 2.5	50 µg EE 250 µg BPA 250 µg genistein	AVPV and SDN volume, calbindin labeled neuron numbers, GnRH neuronal	PND 19 and 19
				PND 85	Gonadectomized adults with 10 µg EB and 48 hours later 500 µg progesterone	activation	PND 98
(Rebuli, Cao et al. 2014)	Rat, Sprague Dawley	F	Oral gavage, to the dam	GD 6 – PND 90	2.5, 25, 260, or 2700 µg/kg bw/day BPA 0.5, 5.0 µg/kg bw/day EE	Hypothalamic estrogen receptor expression	PND 21, 90
(Rubin, Lenkowski et al. 2006)	Mouse, CD-1	M/F	Osmotic pump to dam	GD 8 – PND 16	25, 250 ng/kg bw per day BPA	TH neuron number in preoptic area	PND 22–24, 27–29, post natal week 6–9
(Walker, Goetz et al. 2014)	Rat, Sprague Dawley	M/F	Subcutaneous injection to dam	GD 16, 18	1 mg/kg A1221 50 µg/kg EB	Gene expression and DNA methylation in the AVPV and ARC	PND 15, 30, 45, 90

Table 2

EDC Effects on the Hippocampus

(Facciolo, Alo et al. 2002)	Rat, Sprague Dawley	M/F, not distinguished	Oral, to dam	GD 0 – PND 23	400 µg/kg/day BPA	st2 expression and binding in presence of α A receptor agonists in the hypothalamus and hippocampus	PND 10, 23
(Facciolo, Madeo et al. 2005)	Rat, Sprague Dawley	F	Oral, to dam	GD 0 – PND 23	40 and 400 µg/kg/day BPA	st3 expression and binding in presence of α A receptor agonists in extrahypothalamic structures, hippocampus, and cortex	PND 7, 55
(Kim, Son et al. 2009)	Mouse, ICR	M/F, not distinguished	Subcutaneous injection	GD 14.5–18.5 and Postnatal week 8	5, 10, 20 mg/kg BPA	neonatal hippocampal development and adult hippocampal neurogenesis	PND 1, 22, Postnatal week 8
(Kunz, Camm et al. 2011)	Rat, Sprague Dawley	M/F, not distinguished	Oral, to dam	GD 6 – PND 20	1 mg/L BPA	Hippocampal histology, growth, morphology (hippocampus, motor and somatosensory cortices), glial and neuronal development (cingulum), myelination (cortex and corpus callosum)	PND 20
(Takahashi, Negishi et al. 2009)	Rat, F344/N	M	Subcutaneously injected osmotic pump to dam	GD 7 – PND 1	1 mg/kg PCB 106	Gene expression in cerebral cortex, hippocampus, and striatum	PND 1
(Xu, He et al. 2014)	Rat, Sprague Dawley	M	Oral, to dam	GD 21 – PND 21	0.1 mg/L BPA 500 µg/kg ER antagonist ICI 182,780	Hippocampal mRNA, phosphorylation, and total protein expression for ERα	PND 7, 11, 21
(Xu, Wang et al. 2010)	Rat, Sprague Dawley	M	Oral gavage, to dam	GD 7 – PND 21	0.05, 0.5, 5, 50, 200 mg/kg/day	Hippocampal NMDAR subunit, ERβ and aromatase cytochrome	PND 4, 7, 14, 21,
	Dawley				BPA	P450 protein expression	56

(Xu, Xie et al. 2013)	Mouse, ICR	M	Oral, to dam	GD 7 – PND 21	4, 0.4 or 0.04 mg/kg/day BPA	Pyramidal cell synaptic density in the CA1 region of the hippocampus, Synaptic structural modification in pyramidal cells of the CA1 region of the hippocampus, hippocampal western blot	PND 14, 21 and 56
(Xu, Xie et al. 2013)	Mouse, ICR	M	Oral, to dam	GD 7 – PND 21	0.04, 0.4, 40 mg/kg/day BPA	Synaptic density and structural modification of pyramidal cells in CA1 region of hippocampus, synaptic protein and receptor expression	PND 14, 21, 56
(Zoeller, Bansal et al. 2005)	Rat, Sprague Dawley	M	Oral, to dam	GD 6 – PND 15	1, 10, 50 mg/kg BPA	Hippocampal mRNA expression of TH responsive genes	PND 15
(Zoeller, Dowling et al. 2000)	Rat, Sprague Dawley	M/F, not distinguished	Oral, to dam	GD 6 – PND 21	1, 4, 8 mg/kg A1254	Hippocampal and cerebellar mRNA expression of TH responsive genes	PND 5, 15, 30

Table 3

EDC Effects on Cortex, Cerebellum, and Mid-brain

(Ahmed 2011)	Rat, Wistar	M/F, not distinguished	Gastric intubation, to dam	GD 1 – PND 30	0.2 or 0.4 µg/kg bw/ day TCDD	Cerebellar monoamine and GABA concentrations and acetylcholinesterase activity (AChE)	GD 16, 19, PND 10, 20, 30
(Chang, Sun et al. 2005)	Rat, Sprague Dawley	M/F	Oral, to dam	GD 15	2 µg/kg TCDD	Expression of cytochrome P450 1A1 (CYP1A1), Bax, Bcl-2, Bcl-xl using western blot and RT-PCR in cortex and cerebellum	PND 0 and PND115– 125
(Facciolo, Madeo et al. 2005)	Rat, Sprague Dawley	F	Oral, to dam	GD 0 – PND 23	40 and 400 µg/kg/day BPA	st3 expression and binding in presence of α containing GABA type A receptor agonists in extrahypothalamic structures, hippocampus, and cortex	PND 7, 55
(Fernandez, Paradisi et al. 2010)	Mouse, Dark- Agouti	M	Oral, to dam	GD 18	0.7 µg/kg TCDD	Myelin protein expression, morphological analysis of myelination, expression of myelination related genes in medulla oblongata, cerebellum, telencephalon, diencephalon	PND 2/3, 14, 30, 135
(Itoh, Yaoi et al. 2012)	Mouse, ICR/Icl	M/F	Subcutaneous injection, to the dam	GD 0.5– postnatal week 3	20 µg/kg bw/day BPA	neuronal migration in the ventricular zone, gene expression changes in embryonic telencephalon, DiI labeled cortical neurons in somatosensory cortex at postnatal week 3 and 12, gene expression in cortex, thalamus and pons	GD 10.5– 18.5, Postnatal weeks 3 and 12 and PND 1, 4, 8
(Kawai, Murakami et al. 2007)	Mouse, ICR	M	Oral, to dam	GD 11–17	2 ng/g bw/day BPA	ERα, ERβ, and serotonin immunoreactivity in the dorsal raphe nucleus	PND 35, 63, 91

(Morse, Wehler et al. 1996)	Rat, Wistar	M/F	Oral, to dam	GD 10–16	5, 25 mg/kg Aroclor 1254	Forebrain and cerebellum thyroid hormone concentrations and thyroid hormone metabolism	GD 20, PND 21 and 90
(Nayyar, Zawia et al. 2002)	Rat, Sprague Dawley	M/F, not distinguished	Oral, to dam	GD 15	0.25, 0.5, 1.0 µg/kg bw dioxin	Electrophoretic mobility shift assay of nuclear extract from cortex and cerebellum	PND 3, 5, 10, 15, 20, 30
(Nishizawa, Imanishi et al. 2005)	Mouse, ICR	M/F	Oral, to dam	GD 6.5–17.5	0.02, 2, 200, 20,000 µg/kg day BPA 5 µg/kg EE	AhR, AhRR, Amt expression in cortex and cerebellum, regulation of CYP1A1 and GST	GD 14.5 and 18.5
(Nishizawa, Manabe et al. 2003)	Mouse, ICR	M/F	Oral, to dam	GD 6.5–17.5	2 µg/kg day BPA	RAR α , and RXR α expression in cortex and cerebellum	GD 12.5, 14.5, 16.5, and 18.5
(Nishizawa, Morita et al. 2005)	Mouse, ICR	M/F	Oral, to dam	GD 6.5–17.5	0.02, 2, 200, 20,000 µg/kg day BPA	AhR, RAR α , and RXR α expression in cortex and cerebellum	GD 14.5 and 18.5
(Takahashi, Negishi et al. 2009)	Rat, F344/N	M	Subcutaneous injection, to dam	GD 7 – PND 1	1 mg/kg PCB 106	Gene expression in cerebral cortex, hippocampus, and striatum	PND 1
(Zoeller, Dowling et al. 2000)	Rat, Sprague Dawley	M/F, not distinguished	Oral, to dam	GD 6 – PND 21	1, 4, 8 mg/kg A1254	Hippocampal and cerebellar mRNA expression of TH responsive genes	PND 5, 15, 30
(Zsarnovszky, Le et al. 2005)	Rat, Sprague Dawley	M/F	Intracerebellar injection	PND 4–12	10 ⁻¹² – 10 ⁻¹⁰ M and 10 ⁻⁷ – 10 ⁻⁶ M BPA 10 ⁻¹⁰ M EE	Activated ERK1/2 signaling in cerebellum	PND 4–12

Table 4

EDC Effects on Whole Brain and Embryonic Brain Regions

(Fernandez, Paradisi et al. 2010)	Mouse, Dark-Agouti	M	Oral, to dam	GD 18	0.7 µg/kg TCDD	Myelin protein expression, morphological analysis of myelination, expression of myelination related genes in medulla oblongata, cerebellum, telencephalon, diencephalon	PND 2/3, 14, 30, 135
(Fujita, Samejima et al. 2006)	Mouse, C57BL/6	M/F, not distinguished	Oral, to dam	GD 7	20 µg/kg TCDD	Genes expression in whole fetal brain	GD 12
(Gauger Kato et al. 2004)	Rat, Sprague Dawley	M/F, not distinguished	Oral, to dam	GD 6–16	1, 4 mg/kg A1254	TH responsive gene expression in fetal cortex	GD 16
(Itoh, Yaoi et al. 2012)	Mouse, ICR/Icl	M/F	Subcutaneous injection, to dam	GD 0.5–postnatal week 3	20 µg/kg bw/day BPA	neuronal migration in the ventricular zone, gene expression changes in embryonic telencephalon, Dtl labeled cortical neurons in somatosensory cortex at postnatal week 3 and 12, gene expression in cortex, thalamus and pons	GD 10.5–18.5, Postnatal weeks 3 and 12 and PND 1, 4, 8
(Komada, Asai et al. 2012)	Mouse, C57BL/6J	F and M/F-not distinguished	Oral	GD 8.5–13.5	20 and 200 µg/kg BPA	fetal neurogenesis of the cortical plate of the	GD 14.5
						dorsal telencephalon	

(Morse, Groen et al. 1993)	Rat, Wistar	M/F, not distinguished	Oral, to dam	GD 1 or GD 1–18	0.2, 0.6, 1.8 mg/kg bw hexachlorobiphenyl (HCB) 0.6 mg/kg bw Tetrachlorobiphenyl (TCB)	Activity of type II thyroxine – 5' deiodinase in the whole brain	GD 20, PND 7 and 21
(Nakamura, Itoh et al. 2006)	Mouse, ICR/Jcl	M/F, not distinguished	Subcutaneous injection to dam	GD 0– 10.5, 12.5, 14.5, or 16.5	20 µg/kg BPA	cell proliferation, neuronal differentiation and migration in the ventricular zone and cortical plate, gene expression in embryonic telencephalon	GD 10.5, 12.5, 14.5 and 16.5
(Naveau, Pinson et al. 2014)	Rat, Wistar	M/F, not distinguished	Oral, to dam	GD 6–17, 19, or 20	6 mg/kg/day Aroclor 1254	cell proliferation, S phase and cell cycle exit in the cortex, ventricular zone, and cortical plate, cell death and neuronal differentiation rate, neuronal migration	GD 17, 19, 20
(Wolstenholme, Edwards et al. 2012)	Mouse, C57BL/6J	M/F	Oral, to dam	GD 0 – 18.5	5 mg BPA/kg diet	whole brain gene expression by microarray and rtPCR	GD 18.5