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Sex-specific effects of developmental exposure to polychlorinated biphenyls on neuroimmune and dopaminergic endpoints in adolescent rats

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ABSTRACT

Exposure to environmental contaminants early in life can have long lasting consequences for physiological function. Polychlorinated biphenyls (PCBs) are a group of ubiquitous contaminants that perturb endocrine signaling and have been associated with altered immune function in children. In this study, we examined the effects of developmental exposure to PCBs on neuroimmune responses to an inflammatory challenge during adolescence. Sprague Dawley rat dams were exposed to a PCB mixture (Aroclor 1242, 1248, 1254, 1:1:1, 20 μg/kg/day) or oil control throughout pregnancy, and adolescent male and female offspring were injected with lipopolysaccharide (LPS, 50 μg/kg, *ip*) or saline control prior to euthanasia. Gene expression profiling was conducted in the hypothalamus, prefrontal cortex, striatum, and midbrain. In the hypothalamus, PCBs increased expression of genes involved in neuroimmune function, including those within the nuclear factor kappa b (NF-κB) complex, independent of LPS challenge. PCB exposure also increased expression of receptors for dopamine, serotonin, and estrogen in this region. In contrast, in the prefrontal cortex, PCB exposure blunted or induced irregular neuroimmune gene expression responses to LPS challenge. Moreover, neither PCB nor LPS exposure altered expression of neurotransmitter receptors throughout the mesocorticolimbic circuit. Almost all effects were present in males but not females, in agreement with the idea that male neuroimmune cells are more sensitive to perturbation and emphasizing the importance of studying both male and female subjects. Given that altered neuroimmune signaling has been implicated in mental health and substance abuse disorders that often begin during adolescence, these results highlight neuroimmune processes as another mechanism by which early life PCBs can alter brain function later in life.

1. Introduction

Polychlorinated biphenyls (PCBs) are environmental contaminants that were used in industry for decades before being banned in the United States in 1979 (Borja et al., 2005; Seegal, 2000; Tilson et al., 1990). However, they are still found in the environment and in tissues of virtually all humans due to their persistence and continued generation as an unintentional industrial byproduct (Borja et al., 2005; Dewailly et al., 1999; Seegal et al., 2011; Shain et al., 1991). In mammals, PCBs cross the placenta and are transferred from mother to infant during lactation (DeKoning and Karmaus, 2000; Grandjean et al., 1995; Heilmann et al., 2010; Tilson et al., 1990). This perinatal exposure can affect development of immune, endocrine, and nervous systems, and their interactions (Desaulniers et al., 2013; Weisglas-Kuperus, 1998).

The effects of PCBs on peripheral immune function are well-studied. In humans, PCB exposure has been linked to blunted adaptive immune responses in infants and children (Dewailly et al., 2000; Heilmann et al., 2010; Heilmann et al., 2006; Hochstenbach et al., 2012; Stolevik et al., 2013; Weisglas-Kuperus et al., 2000), and proinflammatory effects in adults (Perkins et al., 2016; Turunen et al., 2013). There are multiple potential mechanisms behind these effects, including production of reactive oxygen species and altered activity of the nuclear factor kappa b (NF-κB) complex (Abliz et al., 2016; Choi et al., 2010; Hennig et al., 2002; Kwon et al., 2002; Sipka et al., 2008; Wang et al., 2019), an important transcription factor for both inflammation and neural development and plasticity (Lawrence, 2009; O'Neill and Kaltschmidt, 1997). PCBs and their metabolites also disrupt steroid hormone activity (Abdelrahim et al., 2006; Bergeron et al., 1994; Grimm et al., 2015; Hamers et al., 2011; Layton et al., 2002; Matthews et al., 2007; Seegal

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et al., 2005; Takeuchi et al., 2017; Tavolari et al., 2006). For example, PCBs are known to act as both estrogen receptor agonists and antagonists (Gore et al., 2015; Hamers et al., 2011) which could indirectly alter immune function due to estrogen's complex but generally immunosuppressive properties (Bellavance and Rivest, 2012; Bruce-Keller et al., 2000; Klein and Flanagan, 2016; Loram et al., 2012; Villa et al., 2016). Given that most mechanistic studies of PCBs on immune function were performed in cell lines or only in male animals, continued investigation on the effects of PCBs on immune function in both males and females is warranted.

Far less is known about neuroimmune effects of PCBs. We previously reported effects of prenatal PCB exposure on the neuroimmune system of neonatal male and female rats (Bell et al., 2018), and (Hayley et al., 2011) described effects of PCBs on brain cytokines in adult females. Epidemiological and laboratory research studies have demonstrated that PCBs can induce neuronal dysfunction and death, especially of dopaminergic and serotonergic cells (Bell et al., 2018; Boix and Cauli, 2012; Dervola et al., 2015; Enayah et al., 2018; Kodavanti, 2006; Pessah et al., 2010; Pessah et al., 2019; Seegal et al., 1988; Seegal et al., 2002; Tilson et al., 1998). Dopamine and serotonin interact with neuroimmune systems in multiple ways. For example, neuroinflammation is associated with excitotoxicity, dopaminergic cell dysfunction and death, and altered serotonin metabolism and handling (Capuron et al., 2012; Dantzer, 2018; Felger et al., 2007; Felger et al., 2013; A.H. Miller et al., 2009; Qin et al., 2007; Robson et al., 2017). Conversely, dopamine, has anti-inflammatory effects (Sarkar et al., 2010; Shao et al., 2013; Yan et al., 2015) while serotonin has been shown to both inhibit and potentiate neuroimmune cell function (Glebov et al., 2015; Ledo et al., 2016).

Neuroimmune systems are especially important during development, influencing sexual differentiation of the hypothalamus perinatally, glutamatergic synapses in the prefrontal cortex during adolescence, and dopaminergic synapses in the nucleus accumbens during adolescence (Kopec et al., 2018; Lenz et al., 2013; Mallya et al., 2018; Nissen, 2017; Schafer et al., 2012). Thus, disruption of neuroimmune function during development has been proposed as a factor prevalence of autism spectrum disorder, depression, schizophrenia, neurocognitive deficits, substance abuse, and later neurodegeneration in humans (Brenhouse and Schwarz, 2016; Greene et al., 2019; Hanamsagar et al., 2017; Hoops and Flores, 2017; Marín, 2016). Adolescence is also concurrent with the pubertal increase in gonadal hormones, thus making it a period of great interest (Brenhouse and Schwarz, 2016; Walker et al., 2017).

For all the above reasons, in this study we tested the hypothesis that developmental exposure to PCBs would alter basal and stimulated neuroimmune function and dopaminergic, serotonergic, and gonadal hormone signaling during adolescence in sex-specific ways.

2. Materials and methods

2.1. Experimental design

Pregnant Sprague Dawley rats were treated with either an oil control or PCB mixture throughout their pregnancy until parturition. In adolescence, one male and one female from each litter were injected with either saline or lipopolysaccharide (LPS) to provide an inflammatory challenge prior to euthanasia and tissue collection. Thus, this study uses a two (oil or PCB perinatal exposure) by two (sal or LPS adolescent challenge) design within each sex (Fig. 1).

2.2. Animals and husbandry

All animal protocols were approved by The University of Texas at Austin's Institutional Animal Care and Use Committee and done in accordance with the Guide for the Care and Use of Laboratory Animals and the ARRIVE guidelines (Kilkenny et al., 2010). Sprague Dawley rats were purchased from Harlan Laboratories (Houston, Texas) and were housed in a temperature-controlled room (21–23 °C) with a 12 h light/dark cycle with lights off at 2:00 pm. Rats were housed (2–3 animals per cage) in polycarbonate cages (43 × 21 × 25 cm) with aspen bedding (PJ Murphy Forest Products, Sani-Chip). Cages were changed weekly and were provided with a 5–10 cm long section of PVC pipe for habitat enrichment. Animals received a low phytoestrogen, fishmeal-free Global Diet (Harlan-Teklad 2019, Indianapolis, Indiana) and water from glass bottles and metal sippers ad libitum. Rats were acclimated to the laboratory through daily handling for at least two weeks prior to mating.

Virgin females (3–4 months old) were paired overnight with untreated male rats (~6 months old). Each male sired one litter that was treated with the oil vehicle and one with PCBs. Successful mating was determined via a sperm-positive vaginal smear, after which dams were singly housed, randomly assigned to treatment group in a counterbalanced design, and began receiving oil (n = 12) or PCB (n = 12) treatment as described below. Dams were provided nesting materials several days before the expected day of birth, postnatal day (P) 0, and oil/PCB treatment stopped the morning pups were observed. On P1, pups were weighed and individually identified with a black Sharpie brand permanent marker. In each litter, up to four pups were randomly assigned to provide P1 tissue in a companion study (Bell et al., 2018); if necessary, other randomly chosen pups were then culled such that litters had 6–8 pups with equal sex ratios beginning on P1.

From P7 on, pups were weighed, relabeled for identification, and handled (> 5 min) weekly. On P21, pups were weaned and housed with same-sex littermates (2–3 animals per cage). Animals were monitored daily for age at eye opening and puberty onset (vaginal opening or preputial separation). On P40–42, up to four randomly assigned pups per litter (one male and one female each exposed to saline or LPS) were used for adolescent tissue. The remaining offspring were monitored for body weight until adulthood for use in an ongoing study. Twenty-four total litters were split across three cohorts, separated by 1–8 months, and PCB treatment was evenly represented across the cohorts. Cohort did not affect expression of genes significantly altered by PCBs or LPS when tested as a fixed variable or covariate. The experimenters were blind to treatment throughout the duration of the experiment.

2.3. Treatments

A 1:1:1 ratio of Aroclor 1242, 1248, and 1254 was used as described in Bell et al., 2018. This mixture is predominately composed of non-coplanar congeners with 2–6 chlorine substitutions and was chosen to mimic the broad range of congeners present in the environment (Frame et al., 1996; Hites et al., 2004; Kostyniak et al., 2005). Aroclors were purchased from AccuStandard (New Haven, Connecticut) with the following identification numbers: C-242-N-50MG, Lot# 01141, CAS# 53469-21-9; C-248 N-50MG, Lot# F-110, CAS# 12672-29-6; C-254 N-50MG, Lot# 5428, CAS# 11097-69-1. PCBs were handled with necessary personal protective equipment in a chemical fume hood, and chemical and animal waste was disposed of with the Environmental Health and Safety office on campus.

The PCB mixture or Crisco vegetable oil vehicle was fed to the dams to mimic naturalistic exposure routes by applying approximately 100 ul of oil or PCB to a quarter of a palatable wafer (Nilla Wafer, Nabisco, ~0.9 g), with the volume adjusted such that dams were exposed daily to 20 μg/kg body weight. This dose was chosen to provide an exposure similar to that of infants in heavily exposed human populations. This was estimated using a) measures of PCBs in human breast milk, adipose tissue, and maternal to infant transmission (DeKoning and Karmaus, 2000; Dewailly et al., 1999; Grandjean et al., 1995; Lanting et al., 1998; Stellman et al., 1998) and b) relationships between exposure dose and resulting body burden in rats, both in adult males and in maternal transmittance to offspring (Hany et al., 1999; Kodavanti et al., 1998). Dams were fed the treated wafers every weekday morning beginning on

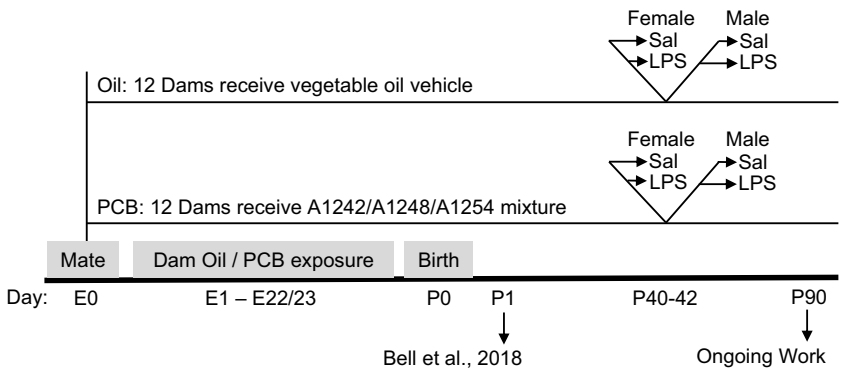

Fig. 1. Experimental timeline. Pregnant dams were orally exposed to PCBs or oil control, throughout gestation (embryonic day E1 – E22/23). The day after birth (P1), up to four pups per litter were used for a previous study (Bell et al., 2018), and then the litter was culled to four male and four female pups. Per litter, two males and two females were randomly assigned for use during adolescence (current study) or during adulthood (in a companion study, data not shown). Between P40–42, one male and one female were randomly assigned to receive an immune challenge (lipopolysaccharide, LPS, 50 μg/kg, i.p.) or saline vehicle control 2.5 h prior to tissue collection.

the day that a sperm-positive vaginal smear was detected, until the day of parturition, in the second half of their light phase. Animals were habituated to taking the wafer in advanced and were watched to confirm that the entire wafer was consumed. While the dams were only treated with PCBs while pregnant, pups were exposed via placental and lactational transfer until weaning (Takagi et al., 1986; Takagi et al., 1976).

LPS was used to stimulate a temporary and non-infectious inflammatory reaction (*E. coli* 0111:B4, Sigma, L4391, Lot 014M4019V). Between P40–42, animals were injected with LPS (50 μg/kg, ip) or sterile saline vehicle and immediately returned to their home cage. They were scored for sickness behaviors (piloerection, lethargy, and ptosis) on a 0–3 scale as in (Kentner et al., 2006) two hours after injection and sickness behaviors were summed for analysis (max of 9).

2.4. Tissue collection

Animals were euthanized on P40–42, 2.5 h after the LPS injection, in the first half of their dark phase. This chronological age was chosen to be mid-adolescent, a time when hormone- dependent and independent maturational processes occur in the brain. Males and females are at slightly different developmental phases at this age: females have completed vaginal opening whereas males have not yet completed preputial separation. Animals were kept in their home cage until just prior to rapid decapitation in an adjacent room. Brains were quickly removed from the skull, chilled on ice, and sectioned coronally using a rat brain matrix into 1 or 2 mm sections. From these sections, prefrontal cortex, striatum, hypothalamus, and midbrain were dissected out with razor blades and transferred into individual RNase-free microcentrifuge tubes, quickly frozen on dry ice, and stored at −80 °C. Trunk blood samples were collected and allowed to clot for 30 min before centrifugation (1500 ×*g* for 5 min). Sera were collected and stored at −80 °C until use. Adrenals and gonads were also dissected out and weighed to indicate gross organ function. Females were of different stages of their estrous cycles at time of tissue collection, according to postmortem vaginal cytology: proestrus (10–20%), estrus (10–30%), or diestrus (50–60%). These stages were equally represented across oil/PCB and sal/LPS groups.

2.5. RNA isolation and gene expression quantification

RNA from the hypothalamus, prefrontal cortex, and striatum were extracted as previously described (Bell et al., 2018) with Qiagen mini RNeasy or Invitrogen Purelink kit protocols and treated with associated DNase while on the column, per manufacturer's directions. RNA was extracted from midbrain samples using TRIzol™ (Cat # 15596026) according to manufacturer's directions and treated with TURBO™ DNase upon isolation (Cat # AM2238). The RNA yield was determined using a NanoDrop™ Spectrophotometer, and RNA quality was assessed by randomly selecting approximately 10% of the samples to run on a Bioanalyzer 2100 (Agilent Technologies); all tested samples had RNA integrity numbers of 9 and above. Isolated RNA samples (200 ng) were reverse transcribed to cDNA using a high capacity cDNA reverse transcriptase kit with RNase inhibitor (Cat # 4374967), according to manufacturer's protocol. Negative controls that did not receive reverse transcriptase during the cDNA conversion did not amplify during qPCR.

We initially focused on the hypothalamus because it is a hormone sensitive region that has an intrinsic dopaminergic cell population and is essential in regulating pyrogenic responses to pathogens. Here, 48 genes were selected for initial analysis, including those related to neuroimmune, hormone, and neuromodulator signaling (Table 1). Custom designed microfluidic Taqman Low Density Array (TLDA) cards (Applied Biosystems, Cat No 4342253) were used with Taqman Gene Expression Mastermix (Applied Biosystems, Cat No 4369016) according to manufacturer's directions; procedures were completed in consultation with the MIQE guidelines (Bustin et al., 2009) and gene assay details are included in (Bell et al., 2018). The samples were run at 50 °C for 2 min, 95 °C for 10 min, 45 cycles of 95 °C for 15 s, and 60 °C for 1 min using a ViiA 7 qPCR system (Applied Biosystems), which automatically determined the quantification cycle (Cq) of each sample. *Gapdh*, *Rpl3a*, and *18s* were included and their geometric mean was used to normalize sample Cq values to calculate the relative expression of target genes to same sex oil- and saline-control groups, as in (Bell et al., 2018). Four of 70 samples, each from different groups, were identified by a Grubbs test as within-group outliers in more than four genes; they were removed from analysis for all 48 genes.

When indicated by hypothalamic results and preliminary data from unpublished studies, additional targets were quantified and run independently in the prefrontal cortex (*Ikbkb*, *Nfkb1*, *Rela*, *Tlr4*, *Drd1*, *Drd2*), striatum (*Drd1*, *Drd2*, *Tlr4*), and midbrain (*Drd1*, *Drd2*, *Th*, *Tlr4*) to determine region-specific effects. Taqman Gene Expression Mastermix (Part No 4369016) and similar parameters were used on a QuantStudio 6 qPCR system and *Gapdh* was used to normalize sample Cq. One sample was an outlier in all of the prefrontal cortex analysis and so was removed.

2.6. Serum corticosterone quantification

Total serum corticosterone was determined via a radioimmunoassay (ImmuChem Double Antibody ^{125}I RIA Kit, MP Biomedicals LLC, Orangeburg NY). Samples were run across two assays in duplicate, according to manufacturer directions. Intra-assay CV was 1.99% and inter-assay CV of standards was 11.65%; minimum level of detection was 7.7 ng/ml. Two outliers from two different groups were identified via Grubb's test and so were removed.

2.7. Serum cytokine quantification

Serum samples were thawed on ice and diluted in assay buffer. The Milliplex Cytokine/Chemokine Hormone assay (RECYTMAG-65K, Cat

Table 1
Summary of effects of PCB exposure and/or LPS challenge on adolescent hypothalamic gene expression. Significant effects (*p < 0.05; **p < 0.01) are noted within each sex.

Gene		Effect of	Females		Males	
			PCB	LPS	PCB	LPS
Xenobiotic signaling						
AhR	Aryl hydrocarbon receptor					
Arnt	Aryl hydrocarbon receptor nuclear translocator					
Neuroimmune signaling						
Ccl22	Chemokine (C—C motif) ligand 22			Sal < LPS**		Sal < LPS**
Cxcl9	Chemokine (C-X-C motif) ligand 9			Sal < LPS**		Sal < LPS**
Cybb	Cytochrome *b*-245, beta polypeptide					
Ifna1	Interferon-alpha 1					
Ikbkb	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta		Oil < PCB*			
Il1a	Interleukin 1 alpha			Sal > LPS**		Sal > LPS**
Il1b	Interleukin 1 beta			Sal < LPS**		Sal < LPS**
Il7r	Interleukin 7 receptor			Sal < LPS*		Sal < LPS*
Itgam	Integrin, alpha M				Oil < PCB*	
Itgb2	Integrin, beta 2					
Myd88	Myeloid differentiation primary response gene 88					Sal < LPS**
Nfkb1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1			Sal < LPS*	Oil < PCB*	Sal < LPS*
Ptgs2	Prostaglandin-endoperoxide synthase 2			Sal < LPS**		Sal < LPS**
Ptges	Prostaglandin E synthase			Sal < LPS**		Sal < LPS**
Rela	v-rel reticuloendotheliosis viral oncogene homolog A (avian)				Oil < PCB*	Sal < LPS**
Tlr4	Toll-like receptor 4					
Tnf	Tumor necrosis factor					Sal < LPS**
Neuroimmune modulators						
Arrb1	Arrestin, beta 1					
Map3k7	Mitogen activated protein kinase kinase kinase 7					
Tgfb2	Transforming growth factor, beta 2				Oil < PCB*	
Hormones, enzymes, and receptors						
Ar	Androgen receptor					
Crh	Corticotropin releasing hormone					
Cyp19a1	Cytochrome P450, family 19, subfamily a, polypeptide 1 (aromatase)					
Esr1	Estrogen receptor 1					
Esr2	Estrogen receptor 2				Oil < PCB**	Sal < LPS*
Opioid precursors and receptors						
Oprk1	Opioid receptor, kappa 1			Sal < LPS*		
Oprm1	Opioid receptor, mu 1					Sal < LPS*
Pdyn	Prodynorphin					
Pomc	Proopiomelanocortin					
Dopamine enzymes, receptors, and transporters						
Drd1a	Dopamine receptor D1A				Oil < PCB**	
Drd2	Dopamine receptor D2				Oil < PCB**	
Th	Tyrosine hydroxylase					
Slc6a3	Solute carrier family 6, member 3 (dopamine transporter)					
Serotonin enzymes, receptors, and transporters						
Htr1a	5-Hydroxytryptamine receptor 1A					
Htr2a	5-Hydroxytryptamine receptor 2A				Oil < PCB**	
Tph1	Tryptophan hydroxylase 1					
Slc6a4	Solute carrier, family 6, member 4 (serotonin transporter)					

No) was run according to manufacturer directions. This assay contains interleukin (IL)- 1a, 1b, 4, 6, 10, interferon gamma (IFNγ), and tumor necrosis factor (TNF, also known as TNFα), which were selected based on literature and availability in the assay. Samples (25 μl) were run in duplicate across two plates and experimental groups were represented evenly between plates. Fewer than 30% of the samples within each group were above limits of detection for IFNγ and IL4 and were not analyzed further. Variability (%CV) of sample replicates within an assay and quality control values between assays were as follows, respectively: IL1a (15%, 19%), IL1b (13%, 18%), IL6 (12%, 3%), IL10 (11%, 11%), and TNF (10%, 7%). Three animals were removed from all assays because their replicate % CV values were unusually high. Limits of detection for IL1a, IL1b, IL6, IL10, and TNF were 45, 10.79, 235, 4.18, and 5.29 ng/ul, respectively.

2.8. Analysis and statistics

Effects and interactions between PCB exposure and LPS challenge (2 × 2 design) were determined within males and females independently because of known sex differences in neuroimmune outcomes. Each PCB or oil exposed litter provided no more than one animal per male/female, sal/LPS group, and individual animal was the unit of analysis. Number of adolescent pups collected per group were as follows: female oil-sal (n = 9); female oil-LPS (n = 9); female PCB-sal (n = 9); female PCB-LPS (n = 8); male oil-sal (n = 8); male oil-LPS (n = 9); male PCB-sal (n = 9); male PCB-LPS (n = 9). Grubbs tests were used to identify outliers within each group (described above) prior to analysis using SPSS and GraphPad Prism, with final ns and significant differences shown in figures and tables as *, $p < 0.05$ and **, $p < 0.01$.

Body weight was analyzed with a repeated measures analysis of variance tests (ANOVA), with age as a within-subject variable and PCB

treatment as the between-subject variable; follow-up *t*-tests were performed within an age. Age at eye opening, vaginal opening, and preputial separation were analyzed with t-tests to determine effects of PCB exposure prior to any LPS challenge. Relative expression of genes and concentrations of serum corticosterone were analyzed using two-way ANOVA to determine main effects of PCB exposure and LPS challenge, and any interactions between these two variables. Any significant interaction effects were followed-up by independent t-tests: effects of PCB within saline-control and LPS-challenged groups, and effects of LPS within oil-control and PCB-treated groups, were determined within each sex. Any groups that failed to meet parametric assumptions by failing Levene's test were analyzed using a Mann-Whitney *U* test (MW), including *Ptgs2*, *Crh*, *Cybb*, and *Ptges* for both sexes, *Tnf* and *Map3k7* in females, and *Arrb1*, *Ar*, and *Htr1a* in males. Serum cytokine values also did not meet parametric assumptions and were analyzed with a Mann-Whitney *U* test.

For six genes (*Il1b*, *Slc6a4*, *Ifna1*, *Tph1*, *Ccl22*, and *Cxcl9*), at least one group had fewer than 30% of its samples fail to amplify (defined as Cq > 35) or reach detectable levels. As such, differences in the percent of samples that amplified within a group were identified with a χ^2 goodness of fit test. Effects of PCB within saline-control and LPS-challenged groups, and effects of LPS within oil-control and PCB-treated groups, were determined within each sex to identify effects that are analogous to main effects of each treatment, or an interaction when the effect of one variable depended on the level of the other. Cqs for *Cyp1a1*, *Ido1*, *Il4*, *Il6*, and *O3far1* were all either above 35 or undetermined and could not be analyzed further.

3. Results

3.1. Physiological development

When analyzed with age as a repeated measure, PCB exposure was associated with greater body weight in males ($F_{(1,33)} = 4.27$, $p < 0.05$), independent of PCB exposure x age interactions. However, when follow-up t-tests were performed at each age, significant effects were only present from P28–35 in males, who were 5–10% heavier when PCB-exposed throughout this period (Fig. 2).

PCBs also advanced the age at eye opening by approximately half a day, in both females (MW, $p < 0.01$, age at eye opening for oil: 15.31 days, PCB: 14.94) and males ($F_{(68)} = 8.83$, $p < 0.01$, oil: 15.47, PCB: 14.97). This effect was no longer significant when the age at eye opening was normalized to body weight at P14. No significant effects of PCBs on age at pubertal onset, adrenal or gonad weight (relative to body weight), and sickness behavior responses to LPS were observed. As expected, summed sickness behavior was increased by LPS challenge in both females (MW, $p < 0.01$, from 0.22 to 1.76) and males (MW, $p < 0.01$, from 0.06 to 2.55), but this was not significantly altered by PCB exposure.

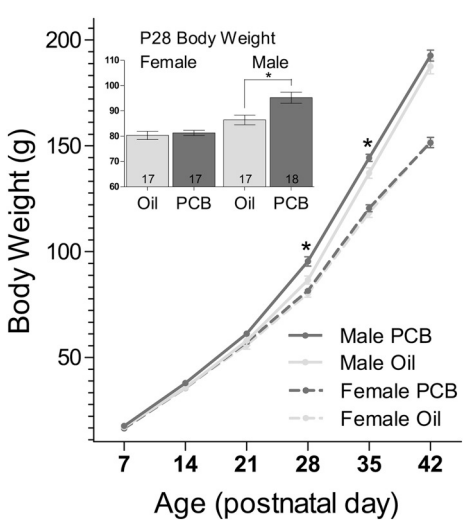

Fig. 2. Male animals exposed to PCBs had significantly greater body weights. This effect was present from P28–35. Data are presented as mean body weight values ± SEM across time, and data for P28 are shown in the top left inset. Final n per group are shown within insert bar graph and represent final group counts for all days analyzed. Significant effects ($p < 0.05$) are noted (*).

3.2. Gene expression in the hypothalamus

The effects of PCB exposure and LPS challenge on relative expression of 48 genes were analyzed in the hypothalamus (Table 1).

In the hypothalamus, all effects of PCBs were sex-specific and independent of LPS exposure. Animals exposed to PCBs had greater expression of five immune-signaling genes compared to those exposed to oil (Fig. 3A-E). These genes included *Ikbkb* ($F_{(1,29)} = 5.23$, $p < 0.05$) in females and *Rela* ($F_{(1,29)} = 4.37$, $p < 0.05$), *Nfkb1* ($F_{(1,29)} = 4.35$, $p < 0.05$), *Itgam* ($F_{(1,29)} = 4.24$, $p < 0.05$), and *Tgfb2* ($F_{(1,29)} = 5.66$, $p < 0.05$) in males. In both sexes, exposure to PCBs had no effect on the relative expression of *Tlr4* in the hypothalamus (Fig. 3F). Independent of PCB exposure, LPS increased expression of *Nfkb1* ($F_{(1,30)} = 5.08$, $p < 0.05$) in females (Fig. 3B), and of *Nfkb1* ($F_{(1,30)} = 5.09$, $p < 0.05$) and *Rela* ($F_{(1,30)} = 8.28$, $p < 0.01$) in males (Fig. 3B-C).

PCB exposure also altered neuromodulating endpoints in the hypothalamus. Males exposed to PCBs had greater expression of four receptors (Fig. 4A-D): *Esr2* ($F_{(1,29)} = 14.28$, $p < 0.01$), *Htr2a* ($F_{(1,29)} = 8.03$, $p < 0.01$), *Drd1a* ($F_{(1,29)} = 9.82$, $p < 0.01$) and *Drd2* ($F_{(1,29)} = 8.80$, $p < 0.01$) but PCBs did not alter the relative expression of the rate limiting enzyme in catecholamine production, *Th* (Fig. 4E). Males challenged with LPS also had increased expression of *Esr2* ($F_{(1,30)} = 5.72$, $p < 0.05$, Fig. 4A).

LPS also altered hypothalamic expression of several genes related to inflammation, independent of PCB exposure. Data are shown collapsed across oil and PCB groups (Table 2). In both males and females, those challenged with LPS had lower expression of *Il1a* and greater expression of *Il7r*, *Nfkb1*, *Ptgs2*, and *Ptges* than saline exposed animals. In addition, LPS-challenged females had greater relative expression of *Oprk1* than saline controls. LPS-challenged males had greater relative expression of *Esr2*, *Myd88*, *Oprm1*, *Rela* and *Tnf* than saline controls. LPS also altered the proportion of samples that were reliably quantified via qPCR ($C_q < 35$), as determined by χ^2 analysis: LPS exposure increased amplification of *Ccl22*, *Cxcl9*, and *Il1b* in both males and females.

3.3. Gene expression in the mesocorticolimbic regions

To follow up on effects of PCBs in the hypothalamus, genes associated with the NF-κB complex were analyzed in the prefrontal cortex, and *Tlr4* and dopaminergic genes were analyzed throughout the mesocorticolimbic region (Table 3).

Three of the four neuroimmune signaling genes in the prefrontal cortex showed significant interaction effects of PCBs and LPS in males but not in females (Fig. 5): *Ikbkb* ($F_{(1,25)} = 5.88$, $p < 0.05$), *Tlr4* ($F_{(1,26)} = 5.33$, $p < 0.05$), and *Rela* ($F_{(1,29)} = 5.73$, $p < 0.05$). More specifically, oil-exposed animals showed no response to LPS, but males exposed to PCBs showed a decrease in expression of *Ikbkb* ($F_{(1,13)} = 4.73$, $p < 0.05$) and *Tlr4* ($F_{(1,13)} = 7.89$, $p < 0.05$) in response to LPS. A main effect of LPS on *Rela* expression was observed ($F_{(1,29)} = 7.85$, $p < 0.05$), however this was likely driven by increased *Rela* expression in response to LPS in oil-exposed animals ($F_{(1,14)} = 13.80$, $p < 0.01$), but not in PCB-exposed animals; in addition, males exposed to PCBs had greater expression of *Rela*, but only in those not exposed to LPS ($F_{(1,14)} = 4.79$, $p < 0.05$). Exposure to PCBs and/or LPS did not significantly affect the relative expression of *Nfkb1* in the prefrontal cortex in either sexes (Table 3; Fig. 5C).

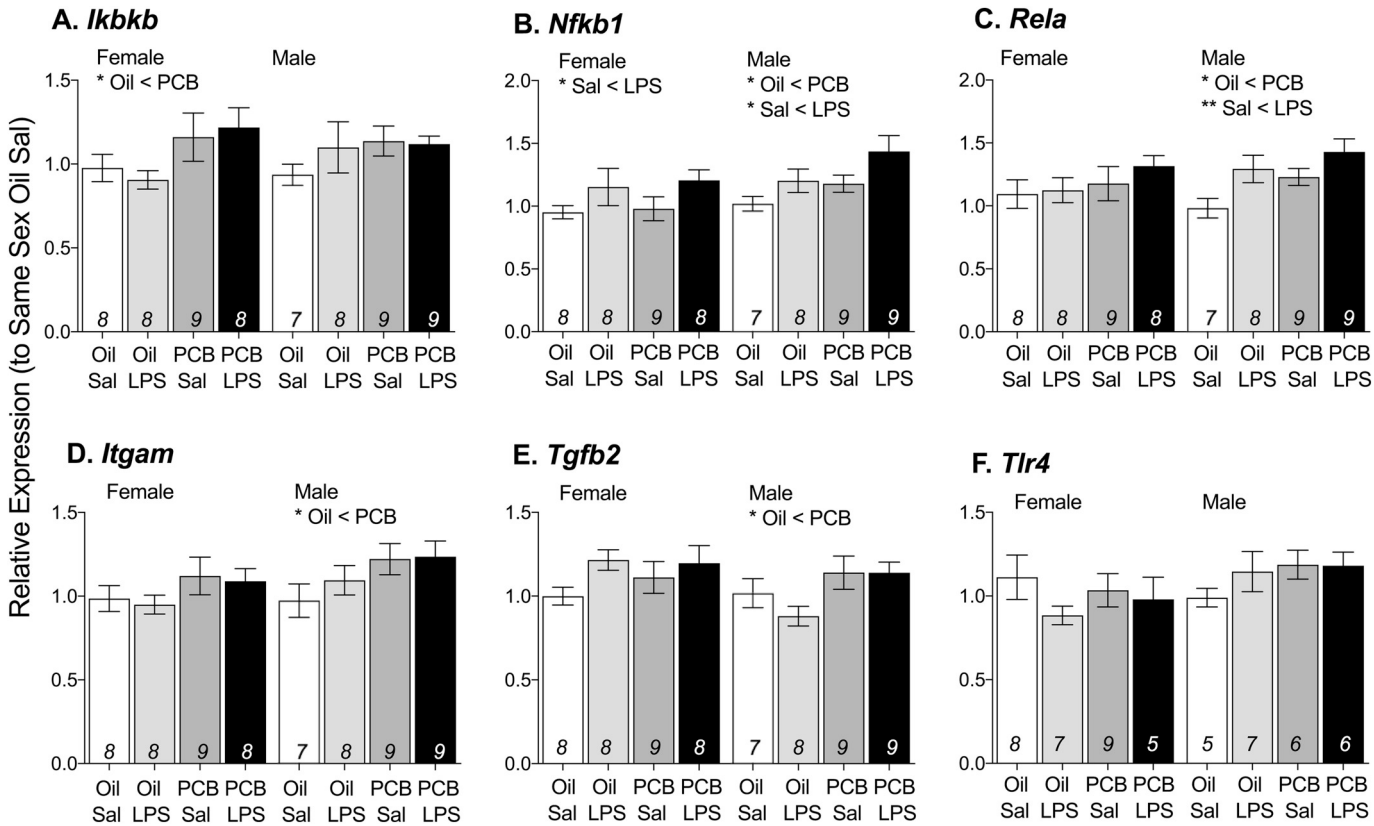

Fig. 3. Animals exposed to PCBs had greater expression of immune-related genes in the hypothalamus. In response to PCB exposure, females had greater expression of *Ikbkb* (A) and males had greater expression of *Rela* (B), *Nfkb1* (C), *Itgam* (D), and *Tgfb2* (E). In both sexes, PCB exposure did not affect the relative expression of *Tlr4*. LPS also increased expression of *Nfkb1* (B) and *Rela* (C) Data are presented as mean values ± SEM with final n per group, after removing outliers and samples that failed to amplify, shown within bars. Significant effects (*$p < 0.05$) are noted within sex.

In contrast to the hypothalamus, expression of dopaminergic signaling genes *Drd1a*, *Drd2*, and *Th* in mesocorticolimbic system was not altered by PCB exposure or the LPS challenge (Table 3).

3.4. Serum corticosterone

No main effects of PCBs or interactions between PCBs and LPS on serum corticosterone concentrations were found (Fig. 6). Animals exposed to LPS had higher concentrations of corticosterone than those exposed to saline, in females ($F_{(1,33)} = 113.31$, $p < 0.01$) and in males ($F_{(1,35)} = 53.99$, $p < 0.01$).

3.5. Serum cytokines

LPS challenge was associated with higher concentrations of serum IL1b (MW, $p < 0.01$) and IL10 (MW, $p < 0.01$) in both males and females (Fig. 7A-B). The effect of LPS on IL10 was independent of PCB exposure, whereas the effect of LPS on IL1b concentration was present only in oil controls (MW, $p < 0.01$). PCB exposure was also associated with lower concentrations of IL1b in LPS challenged females (MW, $p < 0.05$). In both males and females, LPS challenge was associated with a greater number of samples with detectable concentrations of IL6 and TNF, independent of PCB exposure (χ^2, $p < 0.01$, Fig. 7C-D). No effects of PCB exposure or LPS challenge were found on concentrations of IL1a (data not shown).

4. Discussion

As a whole, our results show significant effects of perinatal PCB exposure on neuroimmune and dopaminergic measures in adolescent rats. A major finding was the extent of differences between the sexes: in the hypothalamus, extensive gene expression profiling showed that while both sexes responded to LPS, only males showed effects of PCBs, with the exception of *Ikbkb*. Further work done in the prefrontal cortex, midbrain, and striatum revealed region-specificity, with effects of PCBs in the mesocorticolimbic region limited to neuroimmune signaling genes in the male prefrontal cortex. LPS also affected peripheral serum cytokines in both sexes, with an effect of PCBs found only in females for IL1b; however, given the variability and the relatively low sample size, these data need to be interpreted with caution. These findings suggest that while prior PCB exposure does not cause wholesale changes to the neuroimmune system, it affects specific aspects, in a sex-specific and brain region-specific manner. These alterations could shift the developmental trajectory of the brain, potentially altering risk for mental health and substance abuse disorders that are regulated by neuroimmune signaling.

Sex-specific or sexually differentiated effects of environmental contaminants on neural outcomes are common, as described in reviews of endocrine disrupting compounds (EDCs) (Gore et al., 2019; Rebuli and Patisaul, 2016) and other studies in this special issue; indeed, sex differences in responses are present in a range of species, from zebrafish (Wang et al., 2015) to humans (Braun et al., 2017). However, there are several salient themes to highlight. 1) Sex-specific effects of PCBs have been detected not only in vivo, but also in the dendritic arborization and axon growth in neurons grown and treated in vitro (Keil et al., 2018; Sethi et al., 2018). 2) While the majority of basic PCB studies that include and analyze both males and females use a developmental exposure model (likely because both sexes are present in resulting litter), sex differences in neural, endocrine, and behavioral outcomes are also found in response to juvenile and adult exposures (Bell et al., 2016a; Bell et al., 2016b; Jackson et al., 2019; Viluksela et al., 2014). 3) Sex-specific effects extend beyond the usual EDC suspects, including lead

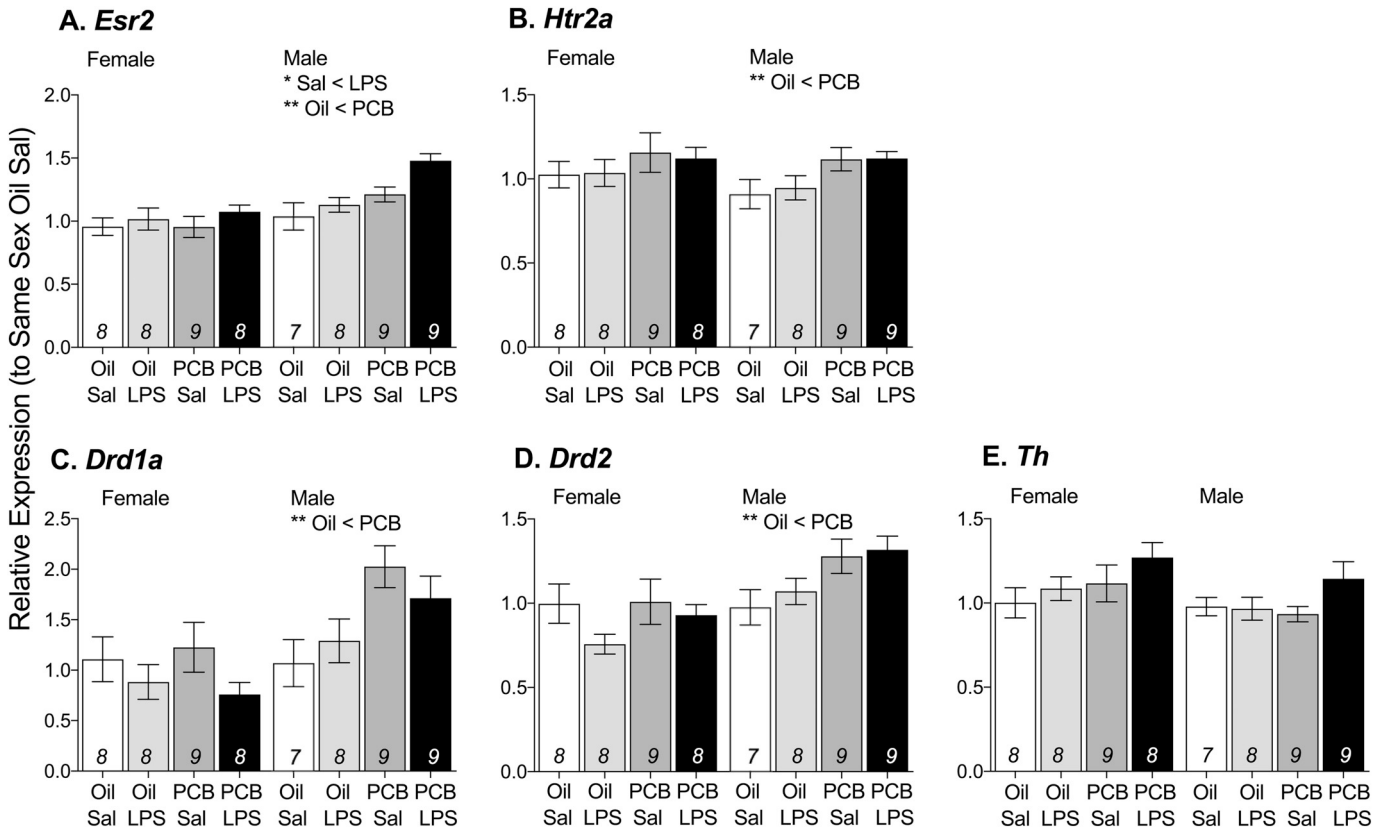

Fig. 4. Males exposed to PCBs had greater expression of neuromodulator receptors in the hypothalamus. In response to PCB exposure, males had greater expression of *Esr2* (*A*), *Htr2a* (*B*), *Drdla* (*C*), and *Drd2* (*D*). In both sexes, PCB exposure did not change the relative expression of *Th*. Data are presented as mean values ± SEM with final n per group, after removing outliers and samples that failed to amplify, shown within bars. Significant effects (**p < 0.01) are noted within sex.

and methylmercury (Kasten-Jolly and Lawrence, 2017; Ruszkiewicz et al., 2016). As brain maturation is sex-differentiated due to differences in hormone production or sensitivity, one obvious mechanism behind these sex-specific effects is that EDCs alter hormone signaling and disrupt these developmental processes. However, it is also possible that pre-existing differences in male and female neural, endocrine, or immune systems could make them differentially responsive to non-hormonal mechanisms of toxicity. Finally, sexes may also differ in their body burdens, as is sometimes found in wildlife (Hitchcock et al., 2019; Keogh et al., 2020) and humans (Yang et al., 2018). All possibilities emphasize the need for including both sexes in analysis and considering sex as a biological variable.

4.1. Perinatal PCB exposure is associated with greater expression of neuroimmune factors in the adolescent male hypothalamus

While PCBs are known to acutely alter activity of the NF-κB complex in a range of peripheral cells (Abliz et al., 2016; Gourronc et al., 2018; Hennig et al., 2002; Kwon et al., 2002; Phillips et al., 2018; Waugh et al., 2018), this is the first study to demonstrate that the NF-κB complex appears particularly sensitive to long-term effects of developmental PCB exposure in the brain. Specifically, in the male

Table 2
LPS challenge altered expression of immune and neuromodulator receptor expression in the hypothalamus, independent of PCB exposure. Data are shown as relative expression (mean ± SEM) or the percent of samples that amplified within a group. They were analyzed via a two-way ANOVA or Mann-Whitney test, or a χ^2 test, respectively. Statistics indicate effects of LPS, *p < 0.05 or **p < 0.01 within sex.

Gene	Females			Males		
	Sal	LPS	Statistics	Sal	LPS	Statistics
Ccl22	0%	81.25%	** χ^2 (1) = 22.79	0%	87.50%	** χ^2 (1) = 25.84
Cxcl9	5.88%	93.75%	** χ^2 (1) = 25.48	5.88%	93.75%	** χ^2 (1) = 25.48
Esr2	0.96 ± 0.05	1.05 ± 0.05	ns, $F_{(1,29)}$ = 1.43	1.14 ± 0.06	1.32 ± 0.06	* $F_{(1,29)}$ = 6.68
Il1a	0.84 ± 0.06	0.54 ± 0.04	** $F_{(1,29)}$ = 15.62	0.93 ± 0.06	0.65 ± 0.05	** $F_{(1,28)}$ = 12.00
Il1b	11.76%	81.25%	** χ^2 (1) = 16.05	11.76%	93.75%	** χ^2 (1) = 22.18
Il7r	1.05 ± 0.09	1.37 ± 0.09	* $F_{(1,29)}$ = 6.76	0.97 ± 0.07	1.22 ± 0.07	* $F_{(1,29)}$ = 6.65
Myd88	1.05 ± 0.07	1.20 ± 0.06	ns, $F_{(1,29)}$ = 2.90	1.04 ± 0.05	1.23 ± 0.05	** $F_{(1,29)}$ = 8.16
Nfkb1	0.97 ± 0.05	1.18 ± 0.08	* $F_{(1,29)}$ = 4.49	1.11 ± 0.05	1.33 ± 0.08	* $F_{(1,29)}$ = 5.41
Oprk1	0.97 ± 0.06	1.3 ± 0.1	* $F_{(1,29)}$ = 5.31	1.04 ± 0.05	1.17 ± 0.06	ns, $F_{(1,29)}$ = 2.42
Oprm1	1.05 ± 0.06	1.14 ± 0.05	ns, $F_{(1,29)}$ = 1.36	0.97 ± 0.04	1.09 ± 0.04	* $F_{(1,29)}$ = 4.36
Ptgs2	1.2 ± 0.1	3.7 ± 0.4	** MW	1.04 ± 0.06	3.9 ± 0.4	** MW
Ptges	1.0 ± 0.1	7.1 ± 0.8	** MW	1.3 ± 0.1	10 ± 1	** MW
Rela	1.14 ± 0.09	1.22 ± 0.07	$F_{(1,29)}$ = 0.58	1.12 ± 0.06	1.37 ± 0.07	** $F_{(1,29)}$ = 7.74
Tnf	1.0 ± 0.1	1.8 ± 0.2	ns, MW	1.4 ± 0.2	2.6 ± 0.3	** $F_{(1,29)}$ = 8.54

Table 3
Summary of effects of PCB exposure and/or LPS challenge on adolescent mesocorticolimbic gene expression. Significant effects (*p < 0.05) were observed only in the prefrontal cortex, and only in males.

Brain region	Gene		Effect of	Females		Males	
				PCB	LPS	PCB	LPSs
Prefrontal cortex							
Neuroimmune signaling	*Ikbkb*	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta				LPS X PCB*	
	Nfkb1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1					
	Rela	v-rel reticuloendotheliosis viral oncogene homolog A (avian)				Sal < LPS*, LPS X PCB*	
	Tlr4	Toll-like receptor 4				LPS X PCB*	
Dopamine receptors	*Drd1a*	Dopamine receptor D1A					
	Drd2	Dopamine receptor D2					
Striatum: no significant effets of PCB exposure or LPS challenge on *Tlr4*, *Drdd1a*, or *Drd2*							
Midbrain: no significant effets of PCB exposure or LPS challenge on *Tlr4*, *Drdd1a*, *Drd2*, or *Th*							

hypothalamus, PCB exposure increased gene expression of *Nfkb1* and *Rela*. These genes code for the p50 and p65 subunits of the NF-κB transcription factor, respectively, that drives production of cytokines and other proinflammatory proteins, including itself (Karin, 2011). This PCB-induced increase in *Nfkb1* and *Rela* expression is additive with an LPS-induced increase; therefore, PCBs can enhance proinflammatory signaling at both basal and immune-activated states in the hypothalamus. The only gene that was affected by PCBs in females was in this complex—*Ikbkb*, the protein product of which phosphorylates IκB for degradation and frees the NF-κB complex to translocate to nucleus. We previously reported this same effect of PCBs in P1 female but not male hypothalamus (Bell et al., 2018). Thus, both males and females show a generally proinflammatory response to PCBs, independent of LPS activation, but via different gene targets.

Overall, adolescent males appear to be substantially more responsive to effects of PCBs on neuroimmune signaling than females. In males, PCB exposure increased expression of *Itgam*, which codes for the integrin subunit CD11b and is important in reactive oxygen species production and phagocytosis in microglia, the resident immune cells of the brain (Brown and Neher, 2014; Linnartz and Neumann, 2013;

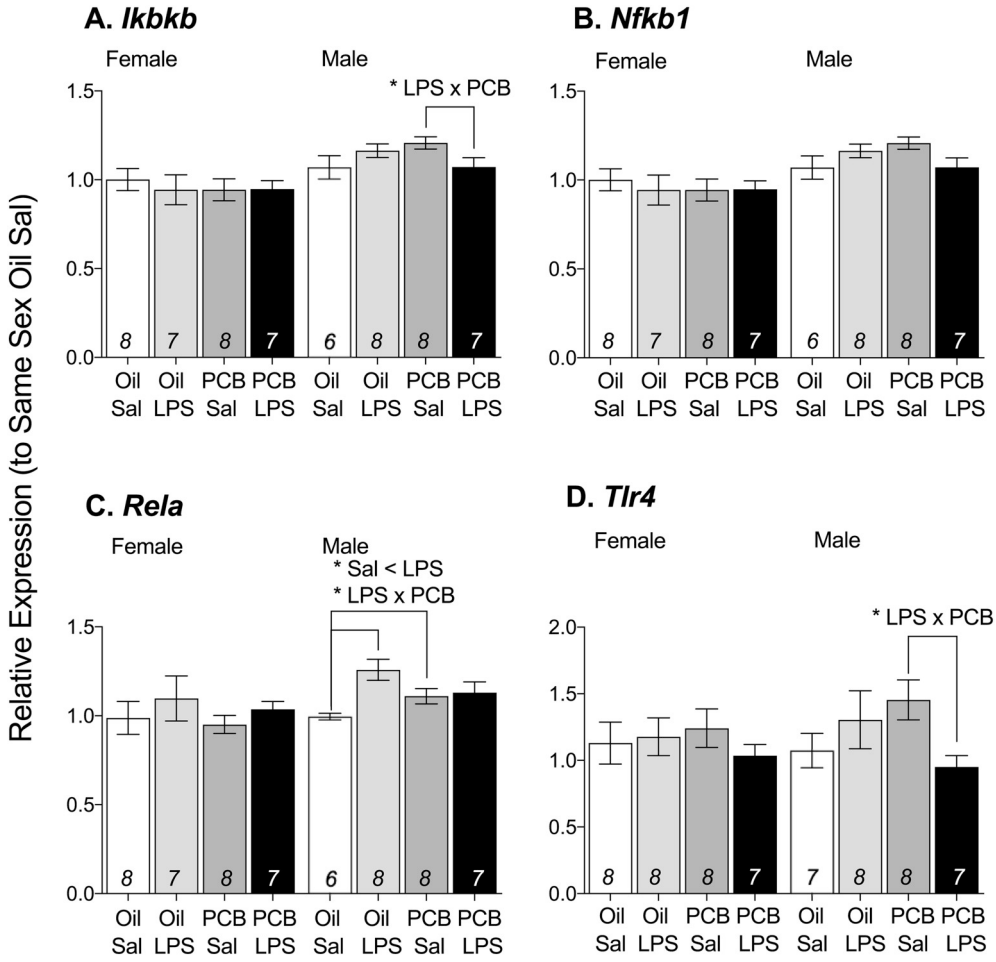

Fig. 5. PCB exposure altered responses to LPS in male prefrontal cortex. In response to PCB and LPS exposure, males showed a change in relative expression of *Ikbkb* (A), *Rela* (C), and *Tlr4* (D) but not *Nfkb1* (B). Data are presented as mean values ± SEM with final n per group, after removing outliers and samples that failed to amplify, shown within bars. Significant effects (*p < 0.05) are noted within sex.

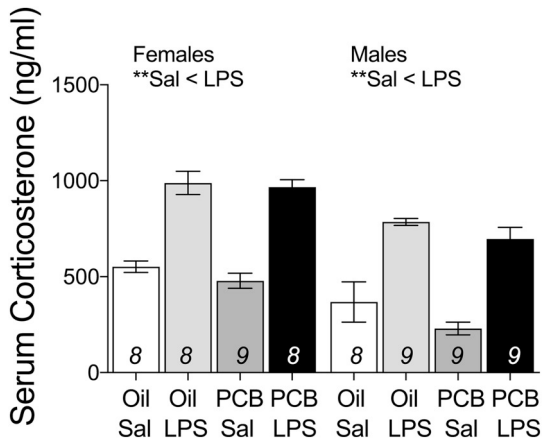

Fig. 6. LPS increased concentrations of serum corticosterone in both males and females, independent of PCB exposure. Data are presented as mean values ± SEM with final n per group, after outlier removal, shown within bars. Significant effects (**p < 0.01) are noted within sex.

Schafer et al., 2012). PCB exposure, independent of LPS challenge, also increased *Tgfb2* expression in the male hypothalamus. TGFβ2 is a pleiotropic growth factor and cytokine that is generally anti-inflammatory in adulthood and regulates development (Bottner et al., 2000; Flanders et al., 1998; Sanyal et al., 2004). TGFβ1 appears to provide some auto-regulation of microglial activation (Dobolyi et al., 2012), and perhaps TGFβ2 is playing a similar role in the current study. While the NF-κB subunits studied herein are expressed in neurons, astrocytes, and microglia (Dresselhaus and Meffert, 2019; Kawai and Akira, 2007), together, these findings raise microglia as a novel target of potential PCB action. Moreover, male microglia are more prevalent during neonatal development (Lenz and McCarthy, 2015; Schwarz et al., 2012), contain more active NF-κB (Guneykaya et al., 2018; Villa et al., 2019; Villa et al., 2018), and are more vulnerable to immune or toxin challenge (Hanamsagar et al., 2017; Rebuli et al., 2016; Villa et al., 2018), than female microglia. Indeed, in the current study, adolescent males showed a broader response to LPS exposure, including an increase in the expression of *Tnf*, a cytokine predominately, but not exclusively, produced by microglia (Bennett et al., 2016; Chung and Benveniste, 1990; Welser-Alves and Milner, 2013; Zhang et al., 2014). This *Tnf* effect was also present in neonatal males, but not females (Bell et al., 2018). Thus, effects of PCBs on microglia could explain why almost all of the effects of PCBs are observed only in males in this study. Differences in microglial activity between sexes could also induce sex differences in neurons and immuno-competent astrocytes (Dong and Benveniste, 2001; Kopec et al., 2018; Mallya et al., 2018; Nelson and Lenz, 2017a; Siracusa et al., 2019; VanRyzin et al., 2019).

The mechanisms by which PCBs could exert these effects are numerous and not mutually exclusive. Dioxin-like PCBs can bind aryl hydrocarbon receptors to increase oxidative stress and induce NF-κB activity and cytokine production (Gourronc et al., 2018; Hennig et al., 2002), while non-dioxin-like congeners may do so by activating NAD(P)H oxidase or by directly damaging DNA (Abliz et al., 2016; Choi et al., 2003; Choi et al., 2010; Kwon et al., 2002; Lu et al., 2004; Marabini et al., 2011; Phillips et al., 2018; Sipka et al., 2008). In addition,

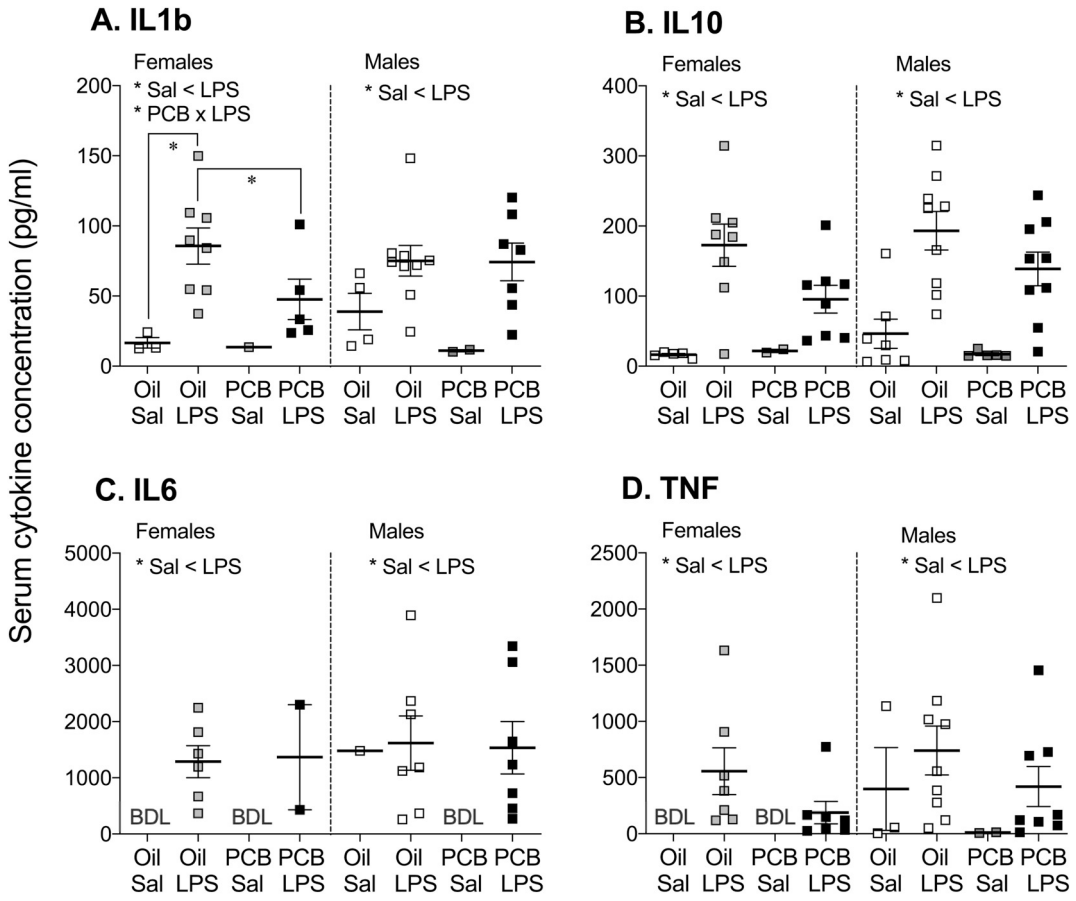

Fig. 7. LPS challenge increased concentrations of serum cytokines, IL1b (A), IL10 (B), IL6 (C), and TNF (D), in both males and females independent of PCB exposure. In females exposed to PCBs, the increase in IL1b is blunted. Each group included 7–9 samples, but many were below detection limits (BDL). Because of this, and the high variability within groups, data are presented as mean values ± SEM, with detectable samples shown as overlaid data points. Significant effects (*p < 0.05) are noted within sex.

different PCB congeners can both agonize or antagonize estrogen receptor activity (Hamers et al., 2011; Pliskova et al., 2005; Warner et al., 2012), which is known to modulate both aryl hydrocarbon receptor and NF-κB activity (Frasor et al., 2015; Maggi et al., 2004). Microglia are altered depending on circulating estradiol (Lenz et al., 2013; Loram et al., 2012; Saijo et al., 2011; Sierra et al., 2008; Vegeto et al., 2006; Vegeto et al., 2001) and their activational patterns are programmed early in life (Crain et al., 2013; Villa et al., 2018), making estrogenic mechanisms an interesting possibility. Finally, some PCB congeners are known to interact with ryanodine receptors and alter thyroid hormone action (Pessah et al., 2019; Sethi et al., 2019), both of which could potentially affect neuroimmune processes (Hopp et al., 2015; Klegeris et al., 2007; Lima et al., 2001; Mancini et al., 2016). However, any effects observed in the current study are a result of both early life organizational and adolescent acute effects of a mix of PCB congeners, the metabolites of which likely shift over time; as such, continued research is required.

4.2. Perinatal PCB exposure altered responses to immune challenge in the adolescent male prefrontal cortex, but not striatum or midbrain of either sex

Of the four regions analyzed, the prefrontal cortex was the only one affected by PCBs and LPS interactions, and again, the effects were limited to males. Specifically, interactions between PCB exposure and subsequent LPS challenge decreased or prevented expression of the NF-κB complex components *Rela* and *Ikbkb*, as well as *Tlr4*, a toll like receptor expressed by microglia and other cells (Aurelian et al., 2016; Shen et al., 2016). TLR4 normally binds pathogen associated molecular patterns (PAMPs) to stimulate an immune reaction upstream of NF-κB activation, and *Tlr4* upregulation occurs concurrent with immune and microglial activation (Doyle et al., 2017; Hoogland et al., 2015), but see (Loram et al., 2012). Thus, PCBs appear to be blunting a typical neuroimmune response in the prefrontal cortex. These results are in agreement with other studies showing that mixtures of non-dioxin-like and ortho-substituted PCBs inhibit LPS-stimulated *Tlr4* expression, NF-κB activity, and cytokine production in primary mouse peritoneal macrophages (Santoro et al., 2015) and proliferation of primary mouse splenocytes (Smithwick et al., 2003).

Some remaining questions are why the effects of PCBs are so different between the hypothalamus and prefrontal cortex, and why striatum and midbrain were unaffected. One possibility could be differential sensitivity to oxidative stress, as is found in different populations of dopaminergic cells (Benskey et al., 2013; Wang and Michaelis, 2010). Another potential reason for regional differences is sensitivity to hormones, with the hypothalamus expressing significantly more receptors for estradiol and androgen than mesocorticolimbic regions (Simerly et al., 1990; Zuloaga et al., 2014). Glia in the hypothalamus are extremely responsive to estradiol perinatally (Lenz et al., 2013), but cortical glia may be differentially sensitive during adolescence. PCBs have also been shown to alter estradiol production and metabolism by disrupting activity of aromatase and estrone sulfotransferase (Hamers et al., 2011). As aromatase is known to be locally regulated within brain regions (Amateau et al., 2004; Konkle and McCarthy, 2011), and males express more aromatase than females in the hypothalamus (Wu et al., 2009), this is another possible rationale for region- and sex-specific effects of PCBs. Microglia also show different phenotypes across brain regions (Crain and Watters, 2015; De Biase et al., 2017; Pintado et al., 2011) and so may react differently to these challenges.

4.3. Perinatal PCB exposure up-regulates expression of receptors for estradiol receptor beta, dopamine, and serotonin in the hypothalamus

In the hypothalamus, expression of *Esr2*, the gene that codes for ERβ, is increased by both LPS and PCB exposure in males. However this effect is most noticeable as a response to LPS in PCB-exposed animals, in agreement with effects of immune activation to increase *Esr2* expression in estradiol-treated microglia and macrophages (Liu et al., 2005; Villa et al., 2015). *Esr2* was affected by PCBs and LPS in neonatal siblings in a similar pattern, but in females instead of males (Bell et al., 2018). This emphasizes the dynamic nature of PCB effects across development that could be dependent on time since acute PCB exposure, metabolism of the original congeners, current developmental processes, and/or circulating gonadal hormones.

PCBs increased baseline *Drd1a* and *Drd2* gene expression in male hypothalamus, a result consistent with the effect of Aroclor 1221 in adult male hypothalamus (Bell, 2014), independent of LPS challenge. We have previously reported that prenatal PCB exposure caused a reduction in *Th*, the gene that codes for the rate limiting enzyme in catecholamine production, and *Slc6a3*, the gene that codes for the dopamine transporter, in neonatal siblings of the animals in this study (Bell et al., 2018). As such, the greater expression of dopamine receptors in the adolescent hypothalamus may be compensating for this reduced dopamine production and content early in life. Serotonin signaling was also altered by PCB exposure, and in a similar pattern developmentally as with dopamine: *Slc6a4*, the gene that codes for the serotonin transporter, was decreased by PCBs in neonates while *Htr2a*, a gene that codes for a serotonin receptor, was increased by PCBs in adolescents. While effects of PCBs on serotonergic endpoints have been varied, the developing hypothalamus appears to be sensitive (Boix and Cauli, 2012; Dervola et al., 2015; Elnar et al., 2012; Mariussen and Fonnum, 2001). Of note is that hypothalamic dopamine and serotonin both regulate food intake (Legrand et al., 2015; Voigt and Fink, 2015). As such, these results could be contributing factors to the greater body weight found in PCB-exposed animals in the current study and is an area of future interest.

In contrast to effects in the hypothalamus, neither PCBs nor LPS altered dopaminergic gene expression throughout the mesocorticolimbic system in the current study. This regional specificity could be explained by different sensitivities of these dopaminergic populations to injury (Matzuk and Saper, 1985). While previous work has shown effects of PCBs on dopamine systems in the frontal cortex, striatum, and midbrain, these studies differ slightly in their endpoints (dopamine transporter activity or metabolism, for example) and experimental design (PCB congeners and dose, age at exposure or tissue collection, and sexes studied) which could cause these different observations (Caudle et al., 2006; Choksi et al., 1997; Dervola et al., 2015; Enayah et al., 2018; Fielding et al., 2013; Lee et al., 2012; Lesmana et al., 2014; Mariussen et al., 1999; Seegal, 1994; Seegal et al., 2005; Tian et al., 2011).

4.4. Peripheral LPS altered expression of neuroimmune genes

As a strong activator of the immune system, LPS challenge altered expression of cytokine- and prostaglandin-related genes in the current study, thereby validating our experimental design. LPS also increased some of the same immune signaling genes in this study that were identified in the neonatal siblings of these animals in a companion study: *Cxcl19*, *IL1b*, *Ptgs2*, *Ptges*, and *TNFa* (Bell et al., 2018). The current study also identified additional genes affected in adolescent but not neonatal animals: *Ccl22*, *Il7r*, and *Myd88*. Interestingly, an opioid receptor response to LPS was reversed over development, as expression of *Oprk1* and *Oprm1* was decreased by LPS in neonates but increased by LPS in adolescents. While it is well established that neonatal immune function is immature and continues to develop during puberty (Goble et al., 2011; Holsapple et al., 2004; Van Loveren and Piersma, 2004), this opposite response to immune challenge is notable and merits further study.

The mechanism by which peripheral LPS can alter neuroimmune signaling is still an area of active study. Intraperitoneal LPS could activate neuroimmune cells via vagal nerve activity, transport of proinflammatory products like cytokines across the blood brain barrier, or signaling of epithelial cells intrinsic in the barrier itself (Hoogland

et al., 2018; Nakano et al., 2015). LPS challenge did increase production of IL1b, IL6, IL10, and TNF peripherally which could have all relayed that signal to the brain. PCBs are also known to alter blood brain barrier permeability, perhaps differentially increasing relay of peripheral cytokine LPS response as a mechanism of the observed PCB x LPS interactions on *Tlr4* and NF-κB complex genes in the prefrontal cortex (Choi et al., 2012). In our study, PCB exposure blunted the serum cytokine IL1b response to LPS in females; however, due to the wide spread data and low sample size, further research is needed to confirm this effect. While it is likely not a cause for the differential brain responses to PCBs or LPS, it may indicate that other immune-active peripheral tissues are being altered by PCBs, and are therefore responding to LPS differently.

4.5. Limitations

While this study raises neuroimmune processes as potential targets and effectors of PCBs' neurotoxic effects, it also leaves several questions for future study. Ongoing work is actively investigating whether the relatively small effect sizes in gene expression translate into biologically meaningful shifts in neuroimmune system function. In addition, we used a single dose of a PCB mixture because of logistical considerations associated with eight experimental groups. We estimated this dose to be within the range of human infant exposure, but using multiple doses and confirming animal body burdens is an appropriate next step. It would also be interesting to investigate effects of non-Aroclor congeners that now constitute 10% of the maternal human serum burden, on average (Koh et al., 2015). While this Aroclor mixture does contain many of the most common congeners recently detected in maternal serum, non-legacy PCB 11 is not represented (Frame et al., 1996; Sethi et al., 2019).

4.6. Relevance to human health

Overall, this study demonstrates that exposure to an environmentally relevant dose and mixture of PCBs early in life can lead to later adolescent disruption in neuroimmune activity. Importantly, these effects are observed predominately in males and in both basal and LPS-challenged states in the hypothalamus and prefrontal cortex, respectively. Thus, this work emphasizes the importance of assessing effects of any possible immunotoxin on both male and female subjects, as to not miss sex-specific effects that may provide insights into mechanisms of action. Chronic neuroinflammation, oxidative stress, or other alterations in neuroimmune signaling are linked to neurodegenerative disorders (Ghosh et al., 2013; Hickman et al., 2018; V.M. Miller et al., 2009), mental illness (Dowlati et al., 2010; Howren et al., 2009; Hung et al., 2014), and altered reward seeking behaviors (Cheng et al., 2016; Hutchinson and Watkins, 2014; Kwon et al., 2017; Northcutt et al., 2015). In addition to genes related to neuroimmune function, genes related to both serotoninergic and dopaminergic systems were affected in the current study. Importantly, depressive-like behavior is modulated by dynamic cross-talk between microglia and serotonergic signaling (Ledo et al., 2016), while adolescent social reward is dependent on microglia and dopaminergic cell interactions (Kopec et al., 2018). The development of neuroimmune systems seems particularly important to later behavioral health (Nelson and Lenz, 2017b), as NF-κB has been implicated in adolescence bipolar and major depressive disorder (Miklowitz et al., 2016), and the sex-specific development of microglia is linked to Alzheimer's disease and Autism Spectrum Disorder (Hanamsagar et al., 2017). As such, neuroimmune dysfunction may be one mechanism behind increased depressive-like symptomology in humans exposed to PCBs (Fitzgerald et al., 2008), and an important area for continued research.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abdelrahim, M., Ariazi, E., Kim, K., Khan, S., Barhoumi, R., Burghardt, R., Liu, S.X., Hill, D., Finnell, R., Wlodarczyk, B., Jordan, V.C., Safe, S., 2006. 3-Methylcholanthrene and other aryl hydrocarbon receptor agonists directly activate estrogen receptor alpha. Cancer Res. 66 (4), 2459–2467.

Abliz, A., Chen, C., Deng, W., Wang, W., Sun, R., 2016. NADPH oxidase inhibitor apocynin attenuates PCB153-induced thyroid injury in rats. Int. J. Endocrinol. 2016, 8354745.

Amateau, S.K., Alt, J.J., Stamps, C.L., McCarthy, M.M., 2004. Brain estradiol content in newborn rats: sex differences, regional heterogeneity, and possible de novo synthesis by the female telencephalon. Endocrinology 145 (6), 2906–2917.

Aurelian, L., Warnock, K.T., Balan, I., Puche, A., June, H., 2016. TLR4 signaling in VTA dopaminergic neurons regulates impulsivity through tyrosine hydroxylase modulation. Transl. Psychiatry 6, e815.

Bell, M.R., 2014. Endocrine-disrupting actions of PCBs on brain development and social and reproductive behaviors. Curr. Opin. Pharmacol. 19, 134–144.

Bell, M.R., Hart, B.G., Gore, A.C., 2016a. Two-hit exposure to polychlorinated biphenyls at gestational and juvenile life stages: 2. Sex-specific neuromolecular effects in the brain. Mol. Cell. Endocrinol. 420, 125–137.

Bell, M.R., Thompson, L.M., Rodriguez, K., Gore, A.C., 2016b. Two-hit exposure to polychlorinated biphenyls at gestational and juvenile life stages: 1. Sexually dimorphic effects on social and anxiety-like behaviors. Horm. Behav. 78, 168–177.

Bell, M.R., Dryden, A., Will, R., Gore, A.C., 2018. Sex differences in effects of gestational polychlorinated biphenyl exposure on hypothalamic neuroimmune and neuromodulator systems in neonatal rats. Toxicol. Appl. Pharmacol. 353, 55–66.

Bellavance, M.A., Rivest, S., 2012. The neuroendocrine control of the innate immune system in health and brain diseases. Immunol. Rev. 248 (1), 36–55.

Bennett, M.L., Bennett, F.C., Liddelow, S.A., Ajami, B., Zamanian, J.L., Fernhoff, N.B., Mulinyawe, S.B., Bohlen, C.J., Adil, A., Tucker, A., Weissman, I.L., Chang, E.F., Li, G., Grant, G.A., Hayden Gephart, M.G., Barres, B.A., 2016. New tools for studying microglia in the mouse and human CNS. Proc. Natl. Acad. Sci. U. S. A. 113 (12), E1738–E1746.

Benskey, M., Lee, K.Y., Parikh, K., Lookingland, K.J., Goudreau, J.L., 2013. Sustained resistance to acute MPTP toxicity by hypothalamic dopamine neurons following chronic neurotoxicant exposure is associated with sustained up-regulation of parkin protein. Neurotoxicology 37, 144–153.

Bergeron, J.M., Crews, D., Mclachlan, J.A., 1994. PCBs as environmental estrogens-turtle sex determination as a biomarker of environmental contamination. Environ Health Persp 102 (9), 780–781.

Boix, J., Cauli, O., 2012. Alteration of serotonin system by polychlorinated biphenyls exposure. Neurochem. Int. 60 (8), 809–816.

Borja, J., Taleon, D.M., Auresenia, J., Gallardo, S., 2005. Polychlorinated biphenyls and their biodegradation. Process Biochem. 40 (6), 1999–2013.

Bottner, M., Krieglstein, K., Unsicker, K., 2000. The transforming growth factor-betas: structure, signaling, and roles in nervous system development and functions. J. Neurochem. 75 (6), 2227–2240.

Braun, J.M., Muckle, G., Arbuckle, T., Bouchard, M.F., Fraser, W.D., Ouellet, E., Séguin, J.R., Oulhote, Y., Webster, G.M., Lanphear, B.P., 2017. Associations of prenatal urinary bisphenol A concentrations with child behaviors and cognitive abilities. Environ. Health Perspect. 125 (6), 067008.

Brenhouse, H.C., Schwarz, J.M., 2016. Immunoadolescence: neuroimmune development and adolescent behavior. Neurosci. Biobehav. Rev. 70, 288–299.

Brown, G.C., Neher, J.J., 2014. Microglial phagocytosis of live neurons. Nat. Rev. Neurosci. 15 (4), 209–216.

Bruce-Keller, A.J., Keeling, J.L., Keller, J.N., Huang, F.F., Camondola, S., Mattson, M.P., 2000. Antiinflammatory effects of estrogen on microglial activation. Endocrinology 141 (10), 3646–3656.

Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R.,

Nolan, T., Pfaffl, M.W., Shipley, G.L., Vandesompele, J., Wittwer, C.T., 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin. Chem. 55 (4), 611–622.
Capuron, L., Pagnoni, G., Drake, D.F., Woolwine, B.J., Spivey, J.R., Crowe, R.J., Votaw, J.R., Goodman, M.M., Miller, A.H., 2012. Dopaminergic mechanisms of reduced basal ganglia responses to hedonic reward during interferon alfa administration. Arch. Gen. Psychiatry 69 (10), 1044–1053.
Caudle, W.M., Richardson, J.R., Delea, K.C., Guillot, T.S., Wang, M., Pennell, K.D., Miller, G.W., 2006. Polychlorinated biphenyl-induced reduction of dopamine transporter expression as a precursor to Parkinson's disease-associated dopamine toxicity. Toxicol. Sci. 92 (2), 490–499.
Cheng, W., Rolls, E.T., Qiu, J., Liu, W., Tang, Y., Huang, C.C., Wang, X., Zhang, J., Lin, W., Zheng, L., Pu, J., Tsai, S.J., Yang, A.C., Lin, C.P., Wang, F., Xie, P., Feng, J., 2016. Medial reward and lateral non-reward orbitofrontal cortex circuits change in opposite directions in depression. Brain 139 (12), 3296–3309.
Choi, W., Eum, S.Y., Lee, Y.W., Hennig, B., Robertson, L.W., Toborek, M., 2003. PCB 104-induced proinflammatory reactions in human vascular endothelial cells: relationship to cancer metastasis and atherogenesis. Toxicol. Sci. 75 (1), 47–56.
Choi, Y.J., Seelbach, M.J., Pu, H., Eum, S.Y., Chen, L., Zhang, B., Hennig, B., Toborek, M., 2010. Polychlorinated biphenyls disrupt intestinal integrity via NADPH oxidase-induced alterations of tight junction protein expression. Environ. Health Perspect. 118 (7), 976–981.
Choi, J.J., Choi, Y.J., Chen, L., Zhang, B., Eum, S.Y., Abreu, M.T., Toborek, M., 2012. Lipopolysaccharide potentiates polychlorinated biphenyl-induced disruption of the blood-brain barrier via TLR4/IRF-3 signaling. Toxicology 302 (2–3), 212–220.
Choksi, N.Y., Kodavanti, P.R., Tilson, H.A., Booth, R.G., 1997. Effects of polychlorinated biphenyls (PCBs) on brain tyrosine hydroxylase activity and dopamine synthesis in rats. Fundam. Appl. Toxicol. 39 (1), 76–80.
Chung, I.Y., Benveniste, E.N., 1990. Tumor necrosis factor-alpha production by astrocytes. Induction by lipopolysaccharide, IFN-gamma, and IL-1 beta. J. Immunol. 144 (8), 2999–3007.
Crain, J.M., Watters, J.J., 2015. Microglial P2 purinergic receptor and immunomodulatory gene transcripts vary by region, sex, and age in the healthy mouse CNS. Transcr Open Access 3 (2).
Crain, J.M., Nikodemova, M., Watters, J.J., 2013. Microglia express distinct M1 and M2 phenotypic markers in the postnatal and adult central nervous system in male and female mice. J. Neurosci. Res. 91 (9), 1143–1151.
Dantzer, R., 2018. Neuroimmune interactions: from the brain to the immune system and vice versa. Physiol. Rev. 98 (1), 477–504.
De Biase, L.M., Schuebel, K.E., Fusfeld, Z.H., Jair, K., Hawes, I.A., Cimbro, R., Zhang, H.Y., Liu, Q.R., Shen, H., Xi, Z.X., Goldman, D., Bonci, A., 2017. Local cues establish and maintain region-specific phenotypes of basal ganglia microglia. Neuron 95 (2), 341–356 e346.
DeKoning, E.P., Karmaus, W., 2000. PCB exposure in utero and via breast milk. A review. J. Expo. Anal. Environ. Epidemiol. 10 (3), 285–293.
Dervola, K.S., Johansen, E.B., Walaas, S.I., Fonnum, F., 2015. Gender-dependent and genotype-sensitive monoaminergic changes induced by polychlorinated biphenyl 153 in the rat brain. Neurotoxicology 50, 38–45.
Desaulniers, D., Xiao, G.H., Cummings-Lorbetskie, C., 2013. Effects of lactational and/or in utero exposure to environmental contaminants on the glucocorticoid stress-response and DNA methylation of the glucocorticoid receptor promoter in male rats. Toxicology 308, 20–33.
Dewailly, E., Mulvad, G., Pedersen, H.S., Ayotte, P., Demers, A., Weber, J.P., Hansen, J.C., 1999. Concentration of organochlorines in human brain, liver, and adipose tissue autopsy samples from Greenland. Environ. Health Perspect. 107 (10), 823–828.
Dewailly, E., Ayotte, P., Bruneau, S., Gingras, S., Belles-Isles, M., Roy, R., 2000. Susceptibility to infections and immune status in Inuit infants exposed to organochlorines. Environ. Health Perspect. 108 (3), 205–211.
Dobolyi, A., Vincze, C., Pal, G., Lovas, G., 2012. The neuroprotective functions of transforming growth factor beta proteins. Int. J. Mol. Sci. 13 (7), 8219–8258.
Dong, Y., Benveniste, E.N., 2001. Immune function of astrocytes. Glia 36 (2), 180–190.
Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E.K., Lanctot, K.L., 2010. A meta-analysis of cytokines in major depression. Biol. Psychiatry 67 (5), 446–457.
Doyle, H.H., Eidson, L.N., Sinkiewicz, D.M., Murphy, A.Z., 2017. Sex differences in microglia activity within the periaqueductal gray of the rat: a potential mechanism driving the dimorphic effects of morphine. J. Neurosci. Off. J. Soc. Neurosci. 37 (12), 3202–3214.
Dresselhaus, E.C., Meffert, M.K., 2019. Cellular specificity of NF-κB function in the nervous system. Front. Immunol. 10, 1043.
Elnar, A.A., Diesel, B., Desor, F., Feidt, C., Bouayed, J., Kiemer, A.K., Soulimani, R., 2012. Neurodevelopmental and behavioral toxicity via lactational exposure to the sum of six indicator non-dioxin-like-polychlorinated biphenyls (summation operator6 NDL-PCBs) in mice. Toxicology 299 (1), 44–54.
Enayah, S.H., Vanle, B.C., Fuortes, L.J., Doorn, J.A., Ludewig, G., 2018. PCB95 and PCB153 change dopamine levels and turn-over in PC12 cells. Toxicology 394, 93–101.
Felger, J.C., Alagbe, O., Hu, F., Mook, D., Freeman, A.A., Sanchez, M.M., Kalin, N.H., Ratti, E., Nemeroff, C.B., Miller, A.H., 2007. Effects of interferon-alpha on rhesus monkeys: a nonhuman primate model of cytokine-induced depression. Biol. Psychiatry 62 (11), 1324–1333.
Felger, J.C., Mun, J., Kimmel, H.L., Nye, J.A., Drake, D.F., Hernandez, C.R., Freeman, A.A., Rye, D.B., Goodman, M.M., Howell, L.L., Miller, A.H., 2013. Chronic interferon-alpha decreases dopamine 2 receptor binding and striatal dopamine release in association with anhedonia-like behavior in nonhuman primates. Neuropsychopharmacology 38 (11), 2179–2187.
Fielding, J.R., Rogers, T.D., Meyer, A.E., Miller, M.M., Nelms, J.L., Mittleman, G., Blaha, C.D., Sable, H.J., 2013. Stimulation-evoked dopamine release in the nucleus accumbens following cocaine administration in rats perinatally exposed to polychlorinated biphenyls. Toxicol. Sci. 136 (1), 144–153.
Fitzgerald, E.F., Belanger, E.E., Gomez, M.I., Cayo, M., McCaffrey, R.J., Seegal, R.F., Jansing, R.L., Hwang, S.A., Hicks, H.E., 2008. Polychlorinated biphenyl exposure and neuropsychological status among older residents of upper Hudson River communities. Environ. Health Perspect. 116 (2), 209–215.
Flanders, K.C., Ren, R.F., Lippa, C.F., 1998. Transforming growth factor-betas in neurodegenerative disease. Prog. Neurobiol. 54 (1), 71–85.
Frame, G.M., Wagner, R.E., Carnahan, J.C., Brown, J.J.F., May, R.J., Smullen, L.A., Bedard, D.L., 1996. Comprehensive, quantitative, congener-specific analyses of eight aroclors and complete PCB congener assignments on DB-1 capillary GC columns. Chemosphere 33 (4), 603–623.
Frasor, J., El-Shennawy, L., Stender, J.D., Kastrati, I., 2015. NF kappa B affects estrogen receptor expression and activity in breast cancer through multiple mechanisms. Mol. Cell. Endocrinol. 418, 235–239.
Ghosh, S., Wu, M.D., Shaftel, S.S., Kyrkanides, S., LaFerla, F.M., Olschowka, J.A., O'Banion, M.K., 2013. Sustained interleukin-1beta overexpression exacerbates tau pathology despite reduced amyloid burden in an Alzheimer's mouse model. J. Neurosci. 33 (11), 5053–5064.
Glebov, K., Löchner, M., Jabs, R., Lau, T., Merkel, O., Schloss, P., Steinhäuser, C., Walter, J., 2015. Serotonin stimulates secretion of exosomes from microglia cells. Glia 63 (4), 626–634.
Goble, K.H., Bain, Z.A., Padow, V.A., Lui, P., Klein, Z.A., Romeo, R.D., 2011. Pubertal-related changes in hypothalamic-pituitary-adrenal axis reactivity and cytokine secretion in response to an immunological stressor. J. Neuroendocrinol. 23 (2), 129–135.
Gore, A.C., Chappell, V.A., Fenton, S.E., Flaws, J.A., Nadal, A., Prins, G.S., Toppari, J., Zoeller, R.T., 2015. EDC-2: the Endocrine Society's second scientific statement on endocrine-disrupting chemicals. Endocr. Rev. 36 (6), E1–E150.
Gore, A.C., Krishnan, K., Reilly, M.P., 2019. Endocrine-disrupting chemicals: effects on neuroendocrine systems and the neurobiology of social behavior. Horm. Behav. 111, 7–22.
Gourronc, F.A., Robertson, L.W., Klingelhutz, A.J., 2018. A delayed proinflammatory response of human preadipocytes to PCB126 is dependent on the aryl hydrocarbon receptor. Environ. Sci. Pollut. Res. Int. 25 (17), 16481–16492.
Grandjean, P., Weihe, P., Needham, L.L., Burse, V.W., Patterson Jr., D.G., Sampson, E.J., Jorgensen, P.J., Vahter, M., 1995. Relation of a seafood diet to mercury, selenium, arsenic, and polychlorinated biphenyl and other organochlorine concentrations in human milk. Environ. Res. 71 (1), 29–38.
Greene, R.K., Walsh, E., Mosner, M.G., Dichter, G.S., 2019. A potential mechanistic role for neuroinflammation in reward processing impairments in autism spectrum disorder. Biol. Psychol. 142, 1–12.
Grimm, F.A., Hu, D., Kania-Korwel, I., Lehmler, H.J., Ludewig, G., Hornbuckle, K.C., Duffel, M.W., Bergman, A., Robertson, L.W., 2015. Metabolism and metabolites of polychlorinated biphenyls. Crit. Rev. Toxicol. 45 (3), 245–272.
Guneykaya, D., Ivanov, A., Hernandez, D.P., Haage, V., Wojtas, B., Meyer, N., Maricos, M., Jordan, P., Buonfiglioli, A., Gielniewski, B., Ochocka, N., Comert, C., Friedrich, C., Artiles, L.S., Kaminska, B., Mertins, P., Beule, D., Kettenmann, H., Wolf, S.A., 2018. Transcriptional and translational differences of microglia from male and female brains. Cell Rep. 24 (10), 2773–2783 e2776.
Hamers, T., Kamstra, J.H., Cenijn, P.H., Pencikova, K., Palkova, L., Simeckova, P., Vondracek, J., Andersson, P.L., Stenberg, M., Machala, M., 2011. In vitro toxicity profiling of ultrapure non-dioxin-like polychlorinated biphenyl congeners and their relative toxic contribution to PCB mixtures in humans. Toxicol. Sci. 121 (1), 88–100.
Hanamsagar, R., Alter, M.D., Block, C.S., Sullivan, H., Bolton, J.L., Bilbo, S.D., 2017. Generation of a microglial developmental index in mice and in humans reveals a sex difference in maturation and immune reactivity. Glia 65 (9), 1504–1520.
Hany, J., Lilienthal, H., Sarasin, A., Roth-Härer, A., Fastabend, A., Dunemann, L., Lichtensteiger, W., Winneke, G., 1999. Developmental exposure of rats to a reconstituted PCB mixture or Aroclor 1254: effects on organ weights, aromatase activity, sex hormone levels, and sweet preference behavior. Toxicol. Appl. Pharmacol. 158 (3), 231–243.
Hayley, S., Mangano, E., Crowe, G., Li, N., Bowers, W.J., 2011. An in vivo animal study assessing long-term changes in hypothalamic cytokines following perinatal exposure to a chemical mixture based on Arctic maternal body burden. Environ. Health 10, 65.
Heilmann, C., Grandjean, P., Weihe, P., Nielsen, F., Budtz-Jorgensen, E., 2006. Reduced antibody responses to vaccinations in children exposed to polychlorinated biphenyls. PLoS Med. 3 (8), e311.
Heilmann, C., Budtz-Jorgensen, E., Nielsen, F., Heinzow, B., Weihe, P., Grandjean, P., 2010. Serum concentrations of antibodies against vaccine toxoids in children exposed perinatally to immunotoxicants. Environ. Health Perspect. 118 (10), 1434–1438.
Hennig, B., Hammock, B.D., Slim, R., Toborek, M., Saraswathi, V., Robertson, L.W., 2002. PCB-induced oxidative stress in endothelial cells: modulation by nutrients. Int. J. Hyg. Environ. Health 205 (1–2), 95–102.
Hickman, S., Izzy, S., Sen, P., Morsett, L., El Khoury, J., 2018. Microglia in neurodegeneration. Nat. Neurosci. 21 (10), 1359–1369.
Hitchcock, D.J., Andersen, T., Varpe, Ø., Borgå, K., 2019. Effects of maternal reproductive investment on sex-specific pollutant accumulation in seabirds: a meta-analysis. Environ. Sci. Technol. 53 (13), 7821–7829.
Hites, R.A., Foran, J.A., Carpenter, D.O., Hamilton, M.C., Knuth, B.A., Schwager, S.J., 2004. Global assessment of organic contaminants in farmed salmon. Science 303 (5655), 226–229.
Hochstenbach, K., van Leeuwen, D.M., Gmuender, H., Gottschalk, R.W., Stolevik, S.B., Nygaard, U.C., Lovik, M., Granum, B., Namork, E., Meltzer, H.M., Kleinjans, J.C., van

Delft, J.H., van Loveren, H., 2012. Toxicogenomic profiles in relation to maternal immunotoxic exposure and immune functionality in newborns. Toxicol. Sci. 129 (2), 315–324.
Holsapple, M.P., Paustenbach, D.J., Charnley, G., West, L.J., Luster, M.I., Dietert, R.R., Burns-Naas, L.A., 2004. Symposium summary: children's health risk—what's so special about the developing immune system? Toxicol. Appl. Pharmacol. 199 (1), 61–70.
Hoogland, I.C., Houbolt, C., van Westerloo, D.J., van Gool, W.A., van de Beek, D., 2015. Systemic inflammation and microglial activation: systematic review of animal experiments. J. Neuroinflammation 12, 114.
Hoogland, I.C.M., Westhoff, D., Engelen-Lee, J.Y., Melief, J., Valls Seron, M., Houben-Weerts, J., Huitinga, I., van Westerloo, D.J., van der Poll, T., van Gool, W.A., van de Beek, D., 2018. Microglial activation after systemic stimulation with lipopolysaccharide and Escherichia coli. Front. Cell. Neurosci. 12, 110.
Hoops, D., Flores, C., 2017. Making dopamine connections in adolescence. Trends Neurosci. 40 (12), 709–719.
Hopp, S.C., Royer, S.E., D'Angelo, H.M., Kaercher, R.M., Fisher, D.A., Wenk, G.L., 2015. Differential neuroprotective and anti-inflammatory effects of L-type voltage dependent calcium channel and ryanodine receptor antagonists in the substantia nigra and locus coeruleus. J. NeuroImmune Pharmacol. 10 (1), 35–44.
Howren, M.B., Lamkin, D.M., Suls, J., 2009. Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. Psychosom. Med. 71 (2), 171–186.
Hung, Y.Y., Kang, H.Y., Huang, K.W., Huang, T.L., 2014. Association between toll-like receptors expression and major depressive disorder. Psychiatry Res. 220 (1–2), 283–286.
Hutchinson, M.R., Watkins, L.R., 2014. Why is neuroimmunopharmacology crucial for the future of addiction research? Neuropharmacology 76 (Pt B), 218–227.
Jackson, E.N., Thatcher, S.E., Larian, N., English, V., Soman, S., Morris, A.J., Weng, J., Stromberg, A., Swanson, H.I., Pearson, K., Cassis, L.A., 2019. Effects of aryl hydrocarbon receptor deficiency on PCB-77-induced impairment of glucose homeostasis during weight loss in male and female obese mice. Environ. Health Perspect. 127 (7), 077004.
Karin, M., 2011. NF-kB in Health and Disease. Springer Verlag, Heidelberg.
Kasten-Jolly, J., Lawrence, D.A., 2017. Sex-specific effects of developmental lead exposure on the immune-neuroendocrine network. Toxicol. Appl. Pharmacol. 334, 142–157.
Kawai, T., Akira, S., 2007. Signaling to NF-kappa B by toll-like receptors. Trends Mol. Med. 13 (11), 460–469.
Keil, K.P., Sethi, S., Lein, P.J., 2018. Sex-dependent effects of 2,2′,3,5′,6-pentachlorobiphenyl on dendritic arborization of primary mouse neurons. Toxicol. Sci. 168 (1), 95–109.
Kentner, A.C., Miguelez, M., James, J.S., Bielajew, C., 2006. Behavioral and physiological effects of a single injection of rat interferon-alpha on male Sprague-Dawley rats: a long-term evaluation. Brain Res. 1095 (1), 96–106.
Keogh, M.J., Taras, B., Beckmen, K.B., Burek-Huntington, K.A., Ylitalo, G.M., Fadely, B.S., Rea, L.D., Pitcher, K.W., 2020. Organochlorine contaminant concentrations in blubber of young Steller sea lion (Eumetopias jubatus) are influenced by region, age, sex, and lipid stores. Sci. Total Environ. 698, 134183.
Kilkenny, C., Browne, W.J., Cuthill, I.C., Emerson, M., Altman, D.G., 2010. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biol. 8 (6), e1000412.
Klegeris, A., Choi, H.B., McLarnon, J.G., McGeer, P.L., 2007. Functional ryanodine receptors are expressed by human microglia and THP-1 cells: their possible involvement in modulation of neurotoxicity. J. Neurosci. Res. 85 (10), 2207–2215.
Klein, S.L., Flanagan, K.L., 2016. Sex differences in immune responses. Nat Rev Immunol 16 (10), 626–638.
Kodavanti, P.R., 2006. Neurotoxicity of persistent organic pollutants: possible mode(s) of action and further considerations. Dose Response 3 (3), 273–305.
Kodavanti, P.R.S., Ward, T.R., Derr-Yellin, E.C., Mundy, W.R., Casey, A.C., Bush, B., Tilson, H.A., 1998. Congener-specific distribution of polychlorinated biphenyls in brain regions, blood, liver, and fat of adult rats following repeated exposure to Aroclor 1254. Toxicol. Appl. Pharmacol. 153 (2), 199–210.
Koh, W.X., Hornbuckle, K.C., Thorne, P.S., 2015. Human serum from urban and rural adolescents and their mothers shows exposure to polychlorinated biphenyls not found in commercial mixtures. Environ. Sci. Technol. 49 (13), 8105–8112.
Konkle, A.T., McCarthy, M.M., 2011. Developmental time course of estradiol, testosterone, and dihydrotestosterone levels in discrete regions of male and female rat brain. Endocrinology 152 (1), 223–235.
Kopec, A.M., Smith, C.J., Ayre, N.R., Sweat, S.C., Bilbo, S.D., 2018. Microglial dopamine receptor elimination defines sex-specific nucleus accumbens development and social behavior in adolescent rats. Nat. Commun. 9 (1), 3769.
Kostyniak, P.J., Hansen, L.G., Widholm, J.J., Fitzpatrick, R.D., Olson, J.R., Helferich, J.L., Kim, K.H., Sable, H.J.K., Seegal, R.F., Pessah, I.N., Schantz, S.L., 2005. Formulation and characterization of an experimental PCB mixture designed to mimic human exposure from contaminated fish. Toxicol. Sci. 88 (2), 400–411.
Kwon, O., Lee, E., Moon, T.C., Jung, H., Lin, C.X., Nam, K.S., Baek, S.H., Min, H.K., Chang, H.W., 2002. Expression of cyclooxygenase-2 and pro-inflammatory cytokines induced by 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153) in human mast cells requires NF-kappa B activation. Biol. Pharm. Bull. 25 (9), 1165–1168.
Kwon, S.H., Han, J.K., Choi, M., Kwon, Y.J., Kim, S.J., Yi, E.H., Shin, J.C., Cho, I.H., Kim, B.H., Jeong Kim, S., Ye, S.K., 2017. Dysfunction of microglial STAT3 alleviates depressive behavior via neuron-microglia interactions. Neuropsychopharmacology 42 (10), 2072–2086.
Lanting, C.I., Huisman, M., Muskiet, F.A., van der Paauw, C.G., Essed, C.E., Boersma, E.R., 1998. Polychlorinated biphenyls in adipose tissue, liver, and brain from nine stillborns of varying gestational ages. Pediatr. Res. 44 (2), 222–225.
Lawrence, T., 2009. The nuclear factor NF-kappaB pathway in inflammation. Cold Spring Harb. Perspect. Biol. 1 (6), a001651.
Layton, A.C., Sanseverino, J., Gregory, B.W., Easter, J.P., Sayler, G.S., Schultz, T.W., 2002. In vitro estrogen receptor binding of PCBs: measured activity and detection of hydroxylated metabolites in a recombinant yeast assay. Toxicol. Appl. Pharmacol. 180 (3), 157–163.
Ledo, J.H., Azevedo, E.P., Beckman, D., Ribeiro, F.C., Santos, L.E., Razolli, D.S., Kincheski, G.C., Melo, H.M., Bellio, M., Teixeira, A.L., Velloso, L.A., Foguel, D., De Felice, F.G., Ferreira, S.T., 2016. Cross talk between brain innate immunity and serotonin signaling underlies depressive-like behavior induced by Alzheimer's amyloid-β oligomers in mice. J. Neurosci. 36 (48), 12106–12116.
Lee, G.S., Subramanian, N., Kim, A.I., Aksentijevich, I., Goldbach-Mansky, R., Sacks, D.B., Germain, R.N., Kastner, D.L., Chae, J.J., 2012. The calcium-sensing receptor regulates the NLRP3 inflammasome through Ca2+ and cAMP. Nature 492 (7427), 123–127.
Legrand, R., Lucas, N., Breton, J., Dechelotte, P., Fetissov, S.O., 2015. Dopamine release in the lateral hypothalamus is stimulated by alpha-MSH in both the anticipatory and consummatory phases of feeding. Psychoneuroendocrinology 56, 79–87.
Lenz, K.M., McCarthy, M.M., 2015. A starring role for microglia in brain sex differences. Neuroscientist 21 (3), 306–321.
Lenz, K.M., Nugent, B.M., Haliyur, R., McCarthy, M.M., 2013. Microglia are essential to masculinization of brain and behavior. J. Neurosci. 33 (7), 2761–2772.
Lesmana, R., Shimokawa, N., Takatsuru, Y., Iwasaki, T., Koibuchi, N., 2014. Lactational exposure to hydroxylated polychlorinated biphenyl (OH-PCB 106) causes hyperactivity in male rat pups by aberrant increase in dopamine and its receptor. Environ. Toxicol. 29 (8), 876–883.
Lima, F.R., Gervais, A., Colin, C., Izembart, M., Neto, V.M., Mallat, M., 2001. Regulation of microglial development: a novel role for thyroid hormone. J. Neurosci. 21 (6), 2028–2038.
Linnartz, B., Neumann, H., 2013. Microglial activatory (immunoreceptor tyrosine-based activation motif)- and inhibitory (immunoreceptor tyrosine-based inhibition motif)-signaling receptors for recognition of the neuronal glycocalyx. Glia 61 (1), 37–46.
Liu, X., Fan, X.L., Zhao, Y., Luo, G.R., Li, X.P., Li, R., Le, W.D., 2005. Estrogen provides neuroprotection against activated microglia-induced dopaminergic neuronal injury through both estrogen receptor-alpha and estrogen receptor-beta in microglia. J. Neurosci. Res. 81 (5), 653–665.
Loram, L.C., Sholar, P.W., Taylor, F.R., Wiesler, J.L., Babb, J.A., Strand, K.A., Berkelhammer, D., Day, H.E., Maier, S.F., Watkins, L.R., 2012. Sex and estradiol influence glial pro-inflammatory responses to lipopolysaccharide in rats. Psychoneuroendocrinology 37 (10), 1688–1699.
Lu, Z., Lee, E.Y., Robertson, L.W., Glauert, H.P., Spear, B.T., 2004. Effect of 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB-153) on hepatocyte proliferation and apoptosis in mice deficient in the p50 subunit of the transcription factor NF-kappaB. Toxicol. Sci. 81 (1), 35–42.
Maggi, A., Ciana, P., Belcredito, S., Vegeto, E., 2004. Estrogens in the nervous system: mechanisms and nonreproductive functions. Annu. Rev. Physiol. 66, 291–313.
Mallya, A.P., Wang, H.-D., Lee, H.N.R., Deutch, A.Y., 2018. Microglial pruning of synapses in the prefrontal cortex during adolescence. Cereb. Cortex 29 (4), 1634–1643.
Mancini, A., Di Segni, C., Raimondo, S., Olivieri, G., Silvestrini, A., Meucci, E., Curro, D., 2016. Thyroid hormones, oxidative stress, and inflammation. Mediat. Inflamm. 2016, 6757154.
Marabini, L., Calo, R., Fucile, S., 2011. Genotoxic effects of polychlorinated biphenyls (PCB 153, 138, 101, 118) in a fish cell line (RTG-2). Toxicol. in Vitro 25 (5), 1045–1052.
Marín, O., 2016. Developmental timing and critical windows for the treatment of psychiatric disorders. Nat. Med. 22 (11), 1229–1238.
Mariussen, E., Fonnum, F., 2001. The effect of polychlorinated biphenyls on the high affinity uptake of the neurotransmitters, dopamine, serotonin, glutamate and GABA, into rat brain synaptosomes. Toxicology 159 (1–2), 11–21.
Mariussen, E., Morch Andersen, J., Fonnum, F., 1999. The effect of polychlorinated biphenyls on the uptake of dopamine and other neurotransmitters into rat brain synaptic vesicles. Toxicol. Appl. Pharmacol. 161 (3), 274–282.
Matthews, J., Wihlen, B., Heldring, N., MacPherson, L., Helguero, L., Treuter, E., Haldosen, L.A., Gustafsson, J.A., 2007. Co-planar 3,3′,4,4′,5-pentachlorinated biphenyl and non-co-planar 2,2′,4,6,6′-pentachlorinated biphenyl differentially induce recruitment of oestrogen receptor alpha to aryl hydrocarbon receptor target genes. Biochem. J. 406 (2), 343–353.
Matzuk, M.M., Saper, C.B., 1985. Preservation of hypothalamic dopaminergic neurons in Parkinson's disease. Ann. Neurol. 18 (5), 552–555.
Miklowitz, D.J., Portnoff, L.C., Armstrong, C.C., Keenan-Miller, D., Breen, E.C., Muscatell, K.A., Eisenberger, N.I., Irwin, M.R., 2016. Inflammatory cytokines and nuclear factor-kappa B activation in adolescents with bipolar and major depressive disorders. Psychiatry Res. 241, 315–322.
Miller, V.M., Lawrence, D.A., Mondal, T.K., Seegal, R.F., 2009a. Reduced glutathione is highly expressed in white matter and neurons in the unperturbed mouse brain—implications for oxidative stress associated with neurodegeneration. Brain Res. 1276, 22–30.
Miller, A.H., Maletic, V., Raison, C.L., 2009b. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biol. Psychiatry 65 (9), 732–741.
Nakano, Y., Furube, E., Morita, S., Wanaka, A., Nakashima, T., Miyata, S., 2015. Astrocytic TLR4 expression and LPS-induced nuclear translocation of STAT3 in the sensory circumventricular organs of adult mouse brain. J. Neuroimmunol. 278, 144–158.
Nelson, L.H., Lenz, K.M., 2017a. The immune system as a novel regulator of sex differences in brain and behavioral development. J. Neurosci. Res. 95 (1–2), 447–461.
Nelson, L.H., Lenz, K.M., 2017b. Microglia depletion in early life programs persistent

changes in social, mood-related, and locomotor behavior in male and female rats. Behav. Brain Res. 316, 279–293.
Nissen, J.C., 2017. Microglial function across the spectrum of age and gender. Int. J. Mol. Sci. 18 (3), e561.
Northcutt, A.L., Hutchinson, M.R., Wang, X., Baratta, M.V., Hiranita, T., Cochran, T.A., Pomrenze, M.B., Galer, E.L., Kopajtic, T.A., Li, C.M., Amat, J., Larson, G., Cooper, D.C., Huang, Y., O'Neill, C.E., Yin, H., Zahniser, N.R., Katz, J.L., Rice, K.C., Maier, S.F., Bachtell, R.K., Watkins, L.R., 2015. DAT isn't all that: cocaine reward and reinforcement require toll-like receptor 4 signaling. Mol. Psychiatry 20 (12), 1525–1537.
O'Neill, L.A., Kaltschmidt, C., 1997. NF-kappa B: a crucial transcription factor for glial and neuronal cell function. Trends Neurosci. 20 (6), 252–258.
Perkins, J.T., Petriello, M.C., Newsome, B.J., Hennig, B., 2016. Polychlorinated biphenyls and links to cardiovascular disease. Environ. Sci. Pollut. Res. Int. 23 (3), 2160–2172.
Pessah, I.N., Cherednichenko, G., Lein, P.J., 2010. Minding the calcium store: ryanodine receptor activation as a convergent mechanism of PCB toxicity. Pharmacol. Ther. 125 (2), 260–285.
Pessah, I.N., Lein, P.J., Seegal, R.F., Sagiv, S.K., 2019. Neurotoxicity of polychlorinated biphenyls and related organohalogens. Acta Neuropathol. 138 (3), 363–387.
Phillips, M.C., Dheer, R., Santaolalla, R., Davies, J.M., Burgueno, J., Lang, J.K., Toborek, M., Abreu, M.T., 2018. Intestinal exposure to PCB 153 induces inflammation via the ATM/NEMO pathway. Toxicol. Appl. Pharmacol. 339, 24–33.
Pintado, C., Revilla, E., Vizuete, M.L., Jiménez, S., García-Cuervo, L., Vitorica, J., Ruano, D., Castaño, A., 2011. Regional difference in inflammatory response to LPS-injection in the brain: role of microglia cell density. J. Neuroimmunol. 238 (1), 44–51.
Pliskova, M., Vondracek, J., Canton, R.F., Nera, J., Kocan, A., Petrik, J., Trnovec, T., Sanderson, T., van den Berg, M., Machala, M., 2005. Impact of polychlorinated biphenyls contamination on estrogenic activity in human male serum. Environ. Health Perspect. 113 (10), 1277–1284.
Qin, L., Wu, X., Block, M.L., Liu, Y., Breese, G.R., Hong, J.S., Knapp, D.J., Crews, F.T., 2007. Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. Glia 55 (5), 453–462.
Rebuli, M.E., Patisaul, H.B., 2016. Assessment of sex specific endocrine disrupting effects in the prenatal and pre-pubertal rodent brain. J. Steroid Biochem. Mol. Biol. 160, 148–159.
Rebuli, M.E., Gibson, P., Rhodes, C.L., Cushing, B.S., Patisaul, H.B., 2016. Sex differences in microglial colonization and vulnerabilities to endocrine disruption in the social brain. Gen. Comp. Endocrinol. 238, 39–46.
Robson, M.J., Quinlan, M.A., Blakely, R.D., 2017. Immune system activation and depression: roles of serotonin in the central nervous system and periphery. ACS Chem. Neurosci. 8 (5), 932–942.
Ruszkiewicz, J.A., Bowman, A.B., Farina, M., Rocha, J.B.T., Aschner, M., 2016. Sex- and structure-specific differences in antioxidant responses to methylmercury during early development. NeuroToxicology 56, 118–126.
Saijo, K., Collier, J.G., Li, A.C., Katzenellenbogen, J.A., Glass, C.K., 2011. An ADIOL-ERbeta-CtBP transrepression pathway negatively regulates microglia-mediated inflammation. Cell 145 (4), 584–595.
Santoro, A., Ferrante, M.C., Di Guida, F., Pirozzi, C., Lama, A., Simeoli, R., Clausi, M.T., Monnolo, A., Mollica, M.P., Mattace Raso, G., Meli, R., 2015. Polychlorinated biphenyls (PCB 101, 153, and 180) impair murine macrophage responsiveness to lipopolysaccharide: involvement of NF-kappaB pathway. Toxicol. Sci. 147 (1), 255–269.
Sanyal, S., Kim, S.M., Ramaswami, M., 2004. Retrograde regulation in the CNS; neuron-specific interpretations of TGF-beta signaling. Neuron 41 (6), 845–848.
Sarkar, C., Basu, B., Chakroborty, D., Dasgupta, P.S., Basu, S., 2010. The immunoregulatory role of dopamine: an update. Brain Behav. Immun. 24 (4), 525–528.
Schafer, D.P., Lehrman, E.K., Kautzman, A.G., Koyama, R., Mardinly, A.R., Yamasaki, R., Ransohoff, R.M., Greenberg, M.E., Barres, B.A., Stevens, B., 2012. Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. Neuron 74 (4), 691–705.
Schwarz, J.M., Sholar, P.W., Bilbo, S.D., 2012. Sex differences in microglial colonization of the developing rat brain. J. Neurochem. 120 (6), 948–963.
Seegal, R.F., 1994. The neurochemical effects of PCB exposure are age-dependent. Arch. Toxicol. Suppl. 16, 128–137.
Seegal, R.F., 2000. The neurotoxicological consequences of developmental exposure to PCBs. Toxicological sciences: an official journal of the Society of Toxicology 57 (1), 1–3.
Seegal, R.F., Brosch, K.O., Okoniewski, R., 1988. The degree of PCB chlorination determines whether the rise in urinary homovanillic acid production in rats is peripheral or central in origin. Toxicol. Appl. Pharmacol. 96 (3), 560–564.
Seegal, R.F., Okoniewski, R.J., Brosch, K.O., Bemis, J.C., 2002. Polychlorinated biphenyls alter extraneuronal but not tissue dopamine concentrations in adult rat striatum: an in vivo microdialysis study. Environ. Health Perspect. 110 (11), 1113–1117.
Seegal, R.F., Brosch, K.O., Okoniewski, R.J., 2005. Coplanar PCB congeners increase uterine weight and frontal cortical dopamine in the developing rat: implications for developmental neurotoxicity. Toxicol. Sci. 86 (1), 125–131.
Seegal, R.F., Fitzgerald, E.F., Hills, E.A., Wolff, M.S., Haase, R.F., Todd, A.C., Parsons, P., Molho, E.S., Higgins, D.S., Factor, S.A., Marek, K.L., Seibyl, J.P., Jennings, D.L., McCaffrey, R.J., 2011. Estimating the half-lives of PCB congeners in former capacitor workers measured over a 28-year interval. J Expo Sci Environ Epidemiol 21 (3), 234–246.
Sethi, S., Keil, K.P., Lein, P.J., 2018. Species and sex differences in the morphogenic response of primary rodent neurons to 3,3′-dichlorobiphenyl (PCB 11). Toxics 6 (1), 4.
Sethi, S., Morgan, R.K., Feng, W., Lin, Y., Li, X., Luna, C., Koch, M., Bansal, R., Duffel, M.W., Puschner, B., Zoeller, R.T., Lehmler, H.-J., Pessah, I.N., Lein, P.J., 2019. Comparative analyses of the 12 most abundant PCB congeners detected in human maternal serum for activity at the thyroid hormone receptor and ryanodine receptor. Environ. Sci. Technol. 53 (7), 3948–3958.
Shain, W., Bush, B., Seegal, R., 1991. Neurotoxicity of polychlorinated biphenyls: structure-activity relationship of individual congeners. Toxicol. Appl. Pharmacol. 111 (1), 33–42.
Shao, W., Zhang, S.Z., Tang, M., Zhang, X.H., Zhou, Z., Yin, Y.Q., Zhou, Q.B., Huang, Y.Y., Liu, Y.J., Wawrousek, E., Chen, T., Li, S.B., Xu, M., Zhou, J.N., Hu, G., Zhou, J.W., 2013. Suppression of neuroinflammation by astrocytic dopamine D2 receptors via alpha B-crystallin. Nature 494 (7435), 90–94.
Shen, Y., Qin, H., Chen, J., Mou, L., He, Y., Yan, Y., Zhou, H., Lv, Y., Chen, Z., Wang, J., Zhou, Y.D., 2016. Postnatal activation of TLR4 in astrocytes promotes excitatory synaptogenesis in hippocampal neurons. J. Cell Biol. 215 (5), 719–734.
Sierra, A., Gottfried-Blackmore, A., Milner, T.A., McEwen, B.S., Bulloch, K., 2008. Steroid hormone receptor expression and function in microglia. Glia 56 (6), 659–674.
Simerly, R.B., Chang, C., Muramatsu, M., Swanson, L.W., 1990. Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridization study. J. Comp. Neurol. 294 (1), 76–95.
Sipka, S., Eum, S.Y., Son, K.W., Xu, S., Gavalas, V.G., Hennig, B., Toborek, M., 2008. Oral administration of PCBs induces proinflammatory and prometastic responses. Environ. Toxicol. Pharmacol. 25 (2), 251–259.
Siracusa, R., Fusco, R., Cuzzocrea, S., 2019. Astrocytes: role and functions in brain pathologies. Front. Pharmacol. 10 (1114).
Smithwick, L.A., Smith, A., Quensen III, J.F., Stack, A., London, L., Morris, P.J., 2003. Inhibition of LPS-induced splenocyte proliferation by ortho-substituted polychlorinated biphenyl congeners. Toxicology 188 (2–3), 319–333.
Stellman, S.D., Djordjevic, M.V., Muscat, J.E., Gong, L., Bernstein, D., Citron, M.L., White, A., Kemeny, M., Busch, E., Nafziger, A.N., 1998. Relative abundance of organochlorine pesticides and polychlorinated biphenyls in adipose tissue and serum of women in Long Island, New York. Cancer Epidemiol. Biomark. Prev. 7 (6), 489–496.
Stolevik, S.B., Nygaard, U.C., Namork, E., Haugen, M., Meltzer, H.M., Alexander, J., Knutsen, H.K., Aaberge, I., Vainio, K., van Loveren, H., Lovik, M., Granum, B., 2013. Prenatal exposure to polychlorinated biphenyls and dioxins from the maternal diet may be associated with immunosuppressive effects that persist into early childhood. Food Chem. Toxicol. 51, 165–172.
Takagi, Y., Otake, T., Kataoka, M., Murata, Y., Aburada, S., 1976. Studies of the transfer and distribution of [14C]polychlorinated biphenyls from maternal to fetal and suckling rats. Toxicol. Appl. Pharmacol. 38 (3), 549–558.
Takagi, Y., Aburada, S., Hashimoto, K., Kitaura, T., 1986. Transfer and distribution of accumulated (14C)polychlorinated biphenyls from maternal to fetal and suckling rats. Arch. Environ. Contam. Toxicol. 15 (6), 709–715.
Takeuchi, S., Anezaki, K., Kojima, H., 2017. Effects of unintentional PCBs in pigments and chemical products on transcriptional activity via aryl hydrocarbon and nuclear hormone receptors. Environ. Pollut. 227, 306–313.
Tavolari, S., Bucci, L., Tomasi, V., Guarnieri, T., 2006. Selected polychlorobiphenyls congeners bind to estrogen receptor alpha in human umbilical vascular endothelial (HUVE) cells modulating angiogenesis. Toxicology 218 (1), 67–74.
Tian, Y.H., Hwan Kim, S., Lee, S.Y., Jang, C.G., 2011. Lactational and postnatal exposure to polychlorinated biphenyls induces sex-specific anxiolytic behavior and cognitive deficit in mice offspring. Synapse 65 (10), 1032–1041.
Tilson, H.A., Jacobson, J.L., Rogan, W.J., 1990. Polychlorinated biphenyls and the developing nervous system: cross-species comparisons. Neurotoxicol. Teratol. 12 (3), 239–248.
Tilson, H.A., Kodavanti, P.R., Mundy, W.R., Bushnell, P.J., 1998. Neurotoxicity of environmental chemicals and their mechanism of action. Toxicol. Lett. 102–103, 631–635.
Turunen, A.W., Jula, A., Suominen, A.L., Mannisto, S., Marniemi, J., Kiviranta, H., Tiittanen, P., Karanko, H., Moilanen, L., Nieminen, M.S., Kesaniemi, Y.A., Kahonen, M., Verkasalo, P.K., 2013. Fish consumption, omega-3 fatty acids, and environmental contaminants in relation to low-grade inflammation and early atherosclerosis. Environ. Res. 120, 43–54.
Van Loveren, H., Piersma, A., 2004. Immunotoxicological consequences of perinatal chemical exposures. Toxicol. Lett. 149 (1–3), 141–145.
VanRyzin, J.W., Marquardt, A.E., Argue, K.J., Vecchiarelli, H.A., Ashton, S.E., Arambula, S.E., Hill, M.N., McCarthy, M.M., 2019. Microglial phagocytosis of newborn cells is induced by endocannabinoids and sculpts sex differences in juvenile rat social play. Neuron 102 (2), 435–449 (e436).
Vegeto, E., Bonincontro, C., Pollio, G., Sala, A., Viappiani, S., Nardi, F., Brusadelli, A., Viviani, B., Ciana, P., Maggi, A., 2001. Estrogen prevents the lipopolysaccharide-induced inflammatory response in microglia. J. Neurosci. 21 (6), 1809–1818.
Vegeto, E., Belcredito, S., Ghisletti, S., Meda, C., Etteri, S., Maggi, A., 2006. The endogenous estrogen status regulates microglia reactivity in animal models of neuroinflammation. Endocrinology 147 (5), 2263–2272.
Villa, A., Rizzi, N., Vegeto, E., Ciana, P., Maggi, A., 2015. Estrogen accelerates the resolution of inflammation in macrophagic cells. Sci. Rep. 5, 15224.
Villa, A., Vegeto, E., Poletti, A., Maggi, A., 2016. Estrogens, Neuroinflammation, and Neurodegeneration. Endocr. Rev. 37 (4), 372–402.
Villa, A., Gelosa, P., Castiglioni, L., Cimino, M., Rizzi, N., Pepe, G., Lolli, F., Marcello, E., Sironi, L., Vegeto, E., Maggi, A., 2018. Sex-specific features of microglia from adult mice. Cell Rep. 23 (12), 3501–3511.
Villa, A., Della Torre, S., Maggi, A., 2019. Sexual differentiation of microglia. Front. Neuroendocrinol. 52, 156–164.
Viluksela, M., Heikkinen, P., van der Ven, L.T.M., Rendel, F., Roos, R., Esteban, J., Korkalainen, M., Lensu, S., Miettinen, H.M., Savolainen, K., Sankari, S., Lilienthal, H., Adamsson, A., Toppari, J., Herlin, M., Finnilä, M., Tuukkanen, J., Leslie, H.A., Hamers, T., Hamscher, G., Al-Anati, L., Stenius, U., Dervola, K.-S., Bogen, I.-L.,

Fonnum, F., Andersson, P.L., Schrenk, D., Halldin, K., Håkansson, H., 2014. Toxicological profile of ultrapure 2,2′,3,4,4′,5,5′-heptachlorbiphenyl (PCB 180) in adult rats. PLoS One 9 (8), e104639.
Voigt, J.P., Fink, H., 2015. Serotonin controlling feeding and satiety. Behav. Brain Res. 277, 14–31.
Walker, D.M., Bell, M.R., Flores, C., Gulley, J.M., Willing, J., Paul, M.J., 2017. Adolescence and reward: making sense of neural and behavioral changes amid the chaos. J. Neurosci. 37 (45), 10855.
Wang, X., Michaelis, E.K., 2010. Selective neuronal vulnerability to oxidative stress in the brain. Front. Aging Neurosci. 2, 12.
Wang, Q., Lam, J.C.-W., Man, Y.-C., Lai, N.L.-S., Kwok, K.Y., Guo, Y.y., Lam, P.K.-S., Zhou, B., 2015. Bioconcentration, metabolism and neurotoxicity of the organophorous flame retardant 1,3-dichloro 2-propyl phosphate (TDCPP) to zebrafish. Aquat. Toxicol. 158, 108–115.
Wang, C., Petriello, M.C., Zhu, B., Hennig, B., 2019. PCB 126 induces monocyte/macrophage polarization and inflammation through AhR and NF-kappaB pathways. Toxicol. Appl. Pharmacol. 367, 71–81.
Warner, J., Osuch, J.R., Karmaus, W., Landgraf, J.R., Taffe, B., O'Keefe, M., Mikucki, D., Haan, P., 2012. Common classification schemes for PCB congeners and the gene expression of CYP17, CYP19, ESR1 and ESR2. Sci. Total Environ. 414, 81–89.
Waugh, C.A., Arukwe, A., Jaspers, V.L.B., 2018. Deregulation of microRNA-155 and its transcription factor NF-kB by polychlorinated biphenyls during viral infections. APMIS 126 (3), 234–240.
Weisglas-Kuperus, N., 1998. Neurodevelopmental, immunological and endocrinological indices of perinatal human exposure to PCBs and dioxins. Chemosphere 37 (9–12), 1845–1853.
Weisglas-Kuperus, N., Patandin, S., Berbers, G.A., Sas, T.C., Mulder, P.G., Sauer, P.J., Hooijkaas, H., 2000. Immunologic effects of background exposure to polychlorinated biphenyls and dioxins in Dutch preschool children. Environ. Health Perspect. 108 (12), 1203–1207.
Welser-Alves, J.V., Milner, R., 2013. Microglia are the major source of TNF-α and TGF-β1 in postnatal glial cultures; regulation by cytokines, lipopolysaccharide, and vitronectin. Neurochem. Int. 63 (1), 47–53.
Wu, M.V., Manoli, D.S., Fraser, E.J., Coats, J.K., Tollkuhn, J., Honda, S., Harada, N., Shah, N.M., 2009. Estrogen masculinizes neural pathways and sex-specific behaviors. Cell 139 (1), 61–72.
Yan, Y.Q., Jiang, W., Liu, L., Wang, X.Q., Ding, C., Tian, Z.G., Zhou, R.B., 2015. Dopamine controls systemic inflammation through inhibition of NLRP3 inflammasome. Cell 160 (1–2), 62–73.
Yang, E., Pavuk, M., Sjödin, A., Lewin, M., Jones, R., Olson, J., Birnbaum, L., 2018. Exposure of dioxin-like chemicals in participants of the Anniston community health survey follow-up. Sci. Total Environ. 637-638, 881–891.
Zhang, Y., Chen, K., Sloan, S.A., Bennett, M.L., Scholze, A.R., O'Keeffe, S., Phatnani, H.P., Guarnieri, P., Caneda, C., Ruderisch, N., Deng, S., Liddelow, S.A., Zhang, C., Daneman, R., Maniatis, T., Barres, B.A., Wu, J.Q., 2014. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J. Neurosci. 34 (36), 11929–11947.
Zuloaga, D.G., Zuloaga, K.L., Hinds, L.R., Carbone, D.L., Handa, R.J., 2014. Estrogen receptor beta expression in the mouse forebrain: age and sex differences. J. Comp. Neurol. 522 (2), 358–371.