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Research Article

Neuroendocrine and Developmental Impacts of Early Life Exposure to EDCs

Madeline Streifer, Lindsay M. Thompson, Skylar A. Mendez, and Andrea C. Gore

Division of Pharmacology & Toxicology, The University of Texas at Austin, Austin, TX 78712, USA

Correspondence: Andrea C. Gore, PhD, Division of Pharmacology and Toxicology, The University of Texas at Austin, 107 W. Dean Keeton St, Austin, TX 78712, USA. Email: andrea.gore@austin.utexas.edu.

Abstract

Polychlorinated biphenyls (PCBs) pose a global challenge to environmental and human health. Although toxic and carcinogenic at higher exposure levels, at lower concentrations they can act as endocrine-disrupting chemicals. Individuals are more vulnerable to endocrine-disrupting effects of PCB exposures during the perinatal period, when the neuroendocrine system is developing, although assessing the full impact of PCB exposure is difficult because of the often-latent onset of adverse effects. The goal of this study was to determine developmental effects of an estrogenic PCB mixture, Aroclor 1221 (A1221), on KNDy and kisspeptin neuron numbers in the hypothalamic arcuate nucleus and anteroventral periventricular nucleus (AVPV), together with measures of hypothalamic-pituitary-gonadal hormones and postnatal development. We conducted RNAscope of kisspeptin, prodynorphin, neurokinin B, and estrogen receptor alpha genes in the P30 hypothalamus. Early-life PCBs caused small but significant changes in development (body weight and anogenital index) but had no effect on puberty. We found sex-specific effects of treatment on serum LH, FSH, and estradiol in a sex- and developmental age-dependent manner. RNAscope results revealed increased prodynorphin in the AVPV of male rats, but no effects on kisspeptin or neurokinin B in AVPV or arcuate nucleus. An unexpected species difference was found: we were unable to detect prodynorphin coexpression with kisspeptin within KNDy neurons in rats, unlike mice, sheep, and primates. These data show that early-life PCBs can induce developmental and hormonal changes that together with other reports showing latent effects on behavior and the hypothalamic-pituitary-gonadal axis, indicate adverse endocrine and neurobehavioral outcomes.

Key Words: endocrine-disrupting chemicals (EDC), kisspeptin, prodynorphin, neurokinin B, polychlorinated biphenyls (PCB), hypothalamus

Abbreviations: AGI, anogenital index; ARC, arcuate nucleus; AVPV, anteroventral periventricular nucleus; E, embryonic; EDC, endocrine-disrupting chemical; FSH, follicle-stimulating hormone; GABA, gamma aminobutyric acid; GnRH, gonadotropin-releasing hormone; HPG, hypothalamic-pituitary-gonadal; KNDy, Kisspeptin—Neurokinin B—Dynorphin expressing; LH, luteinizing hormone; P, postnatal; PBS, phosphate-buffered saline; PCB, polychlorinated biphenyl; PFA, paraformaldehyde.

In recent years, the health risks associated with environmental pollutants have garnered considerable attention because of increasing evidence linking them to adverse health outcomes. Endocrine-disrupting chemicals (EDCs), which perturb the body's hormones, are detectable in almost every ecosystem [1] and are an increasing health concern, yet the mechanisms of their effects and long-term implications are not fully understood. Of relevance to the brain's neuroendocrine systems, which develop in a sexually dimorphic manner under the control of differential actions of steroid hormones such as testosterone and estradiol, exposures to EDCs during early periods of sexual differentiation can lead to perturbations in physiological functions and behaviors controlled by these regions. Although the mechanisms are complex, it is likely that developmental EDC exposures can cause changes in the neural circuitry that may not manifest until later in life when these circuits become activated [2].

Polychlorinated biphenyls (PCBs), a family of EDCs, have been banned for decades but were previously used in a variety of commercial and industrial applications. Their hydrophobic and lipophilic properties have led to their persistence in the environment. Our laboratory and that of others have shown that developmental exposure to environmentally relevant levels of Aroclor 1221 (A1221), a mixture of PCBs known to have weakly estrogenic effects, has effects on gene and protein expression in the hypothalamus [3-5], reproductive senescence [6], neurogenesis [7], and a number of social and anxiety-like behaviors [7-12].

Within the hypothalamus, kisspeptins are a family of peptides encoded in rodents by the gene *Kiss1*, and kisspeptin signaling is a key player in functions including but not limited to pubertal onset [13-15], pregnancy [16], and conveying information about stress [17] and nutritional status [18] on reproductive function. Hypothalamic kisspeptin neurons in the anteroventral periventricular nucleus (AVPV) and Kisspeptin—Neurokinin B—Dynorphin expressing (KNDy) neurons in the arcuate nucleus (ARC) play a crucial role in the secretion of gonadotropin-releasing hormone (GnRH), a major regulator of the hypothalamic-pituitary-gonadal (HPG) axis [19, 20]. Both ARC KNDy and AVPV kisspeptin neurons signal directly on GnRH neurons, KNDy neurons onto terminal regions and kisspeptin neurons onto somatic and terminal

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regions of GnRH neurons [21], thereby regulating the HPG axis. Once activated by kisspeptin, GnRH release from hypothalamus to anterior pituitary drives the release of the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These, in turn, signal the gonads to produce steroid hormones and gametogenesis. Neurokinin B and dynorphin are both regulators of kisspeptin signaling, stimulating or inhibiting kisspeptin release, respectively [13, 14, 22-32].

Kisspeptin neurons develop under the influence of gonadal hormones, notably estradiol, and limited evidence suggests that they are vulnerable to early exposure to estrogenic EDCs [4, 33-35]. Given the crucial role of kisspeptin in regulating reproductive function, a further understanding of the potential impact of PCB exposure on kisspeptin and KNDy neurons is needed.

Emerging evidence suggests that males and females may have differences in vulnerability to the effects of EDC exposure [8, 10, 36]. The influence of sex on brain development and function has long been recognized as a critical factor in understanding physiological and behavioral differences between males and females. The hypothalamus plays a pivotal role in regulating the processes involved in reproductive success and maturation. Investigating hypothalamic brain development and the unique susceptibility to EDC exposure between the sexes has implications for understanding the long-term consequences of environmental factors on sex-specific brain function and health. Our research aims to explore the relationship between prenatal exposure to PCBs and the sex-specific development of KNDy and kisspeptin neurons, key players in reproductive function, together with other measures of hormonal status and reproductive development.

Materials and Methods

Breeding, EDC Treatment, and Exposure Paradigm

All animal protocols were performed in accordance with National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee at the University of Texas at Austin. Adult Sprague-Dawley rats were purchased (Envigo, Indianapolis, IN), switched to a low phytoestrogen diet (Envigo-Teklad Global Diet) ad libitum, and housed in same-sex groups of 2 to 3 per cage on a 12:12 light cycle. Virgin dams were bred and the morning following a sperm-positive smear was deemed embryonic (E) day 1. Pregnant dams were randomly assigned to a treatment group, receiving either 1 mg/kg Aroclor 1221 (N = 19) or vehicle (3% DMSO in sesame oil; N = 22) from E8 to 18 and postnatal (P) days 1 to 21. Treatments were fed orally via a Nilla wafer at 0900 daily (2 hours before lights out) during the treatment period. Dosages and routes were selected to be environmentally relevant [37] and consistent with prior work in our laboratory [8]. These rats were part of a larger study on which pituitary and ovarian endpoints were previously reported [38, 39].

Developmental Endpoints and Rearing

At birth, litters were culled to 4 males and 4 females based on median anogenital index (AGI) (*Anogenital Index* $= \frac{Anogenital\ Distance}{\sqrt[3]{Bodyweight}}$). Body weight and anogenital distance were measured weekly; the date of eye opening and date of puberty onset (vaginal opening in females and preputial separation in males) were recorded as measures of developmental progression. In females, estrous cycles were tracked by vaginal cytology beginning at the date of vaginal opening (note: only in some cohorts because of COVID-19 restrictions) and continued until the date of euthanasia. Animals were weaned at P22 and double- or triple-housed with same-sex siblings. Littermates were distributed to different ages at euthanasia to minimize any contribution of litter to those endpoints.

Tissue Collection

Animals were euthanized 1 to 3 hours before lights out at 3 developmental time points: early life (P7-9), prepuberty (P29-31), and adulthood (P60-P63). Adult females were euthanized as close to P60 as possible while in proestrus to avoid effects of fluctuating hormones through the estrous cycle. Rats were anesthetized with 100 mg/kg ketamine and 40 mg/kg xylazine, a sample of cardiac blood was drawn, and rats were perfused with 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) following laboratory protocols [4]. Brains were removed and postfixed overnight in 4% PFA at 4 °C and then stored in sucrose cryoprotectant buffer at −20 °C for long-term storage. Tissues were blocked coronally and sectioned on a Leica VT1000 vibrating microtome at 30 µm from Bregma 0.36 mm to −4.44 mm. The blood sample was allowed to clot at room temperature and was centrifuged for 5 minutes at 1500*g*. Serum was stored at −80 °C.

RNAscope, Confocal Microscopy, and Cell Counting

RNAscope was performed on a subset of tissues from P30 rats, selected because this is a period when the HPG axis is becoming activated as indicated by a rise in pituitary and gonadal hormones [38-40] and GnRH receptor expression [41]. We used the RNAscope Multiplex Fluorescent Reagent Kit v2 (Cat. 323100; Advanced Cell Diagnostics, Newark, CA) according to the manufacturer's protocol with a modified pretreatment protocol to accommodate the fixed sections. Sections containing the AVPV (Bregma −0.12 mm) and ARC (Bregma −3.24 mm) were selected, washed in PBS, and mounted on Fisherbrand Superfrost Plus microscope slides (Fisher Scientific, Hampton, NH). The slides were dried at room temperature for 1 hour, rinsed in dH_2O to remove PBS residue, and baked at 60 °C for 30 minutes. The slides were fixed in room temperature 4% PFA to ensure tissue adherence and then dehydrated using an ethanol gradient (50%, 70%, 100%, 100%) for 5 minutes at each concentration. Slides were left covered to dry overnight. On day 2, the kit-provided H_2O_2 reagent was added directly onto tissue for 10 minutes at room temperature followed by a rinse in dH_2O. The slides were submerged in 1× target retrieval reagent at 95° for 5 minutes, rinsed in dH_2O, and dipped in 100% ethanol, and a hydrophobic barrier was drawn with an ImmEDGE pen (Vector Laboratories, Newark, CA). The slides were then placed in an enclosed slide holder with a wet towel to maintain humidity, Protease III reagent was added, and slides were baked for 40 minutes at 40 °C. The remainder of the assay was performed following manufacturers' specifications.

AVPV sections were labeled for *Kiss1*, *Esr1*, and *Pdyn*. ARC sections were labeled for *Kiss1*, *Tac3*, and *Pdyn*, with details on the probes shown in Table 1. A species-specific 3-plex positive control probe for housekeeping genes *Polr2a*, *Ppib*, and *Ubc*, and a negative control probe targeting the bacterial gene *Dapb* were included in each run to confirm triple-labeling. Opal fluorophore reagent packs (Akoya

Table 1. RNAscope probe details

Target	Gene ID	Cat. number (Advanced Cell Diagnostics)
Kisspeptin	*Kiss1*	Rn-Kiss1, cat. 503421
Estrogen receptor α	*Esr1*	Rn-Esr1-C2, cat. 317151-C2
Neurokinin B/ Tachykinin 3	*Tac3*	Rn-Tac3-C2, cat. 426121-C2
Prodynorphin	*Pdyn*	Rn-Pdyn-C3, cat. 417441-C3
3-plex positive control probe—Rn	*Polr2a, Ppib, Ubc*	Cat. 320891
3-plex negative control probe	*Dapb*	Cat. 320871

Biosciences, Marlborough, MA) were diluted 1:1500 in the kit provided TSA buffer. The following dyes were used for each target: Opal 520 reagent pack (cat. FP 1487001KT) for *Kiss1*, Opal 570 (cat. FP1488001KT) for *Esr1* and *Tac3*, Opal 650 (cat. FP1496001KT) for *Pdyn*. Slides were cover slipped using Vectashield Antifade Mounting Medium with DAPI (Vector Laboratories, Newark, CA), sealed with clear nail polish, and stored at 4° until imaging. All slides were imaged within 7 days of labeling. No more than 1 female and 1 male were selected from a litter to avoid the use of same-sex littermates.

To assess the effects of treatment on the number of KNDy (ARC) and kisspeptin (AVPV) neurons, mounted sections were imaged on a Nikon A1R Confocal Microscope in an 11-µm z-stack at 40× magnification (Plan Fluor DIC 40× (oil); NA-1.3; WD-0.24 mm). The AVPV and ARC boundaries were determined using the Rat Brain Atlas (Paxinos and Watson 6th Edition) and the number of DAPI-positive cells expressing *Kiss1, Tac3,* and *Pdyn* in the ARC and *Kiss1* and *Pdyn* in the AVPV and were counted on 1 hemisphere of the brain. A cell was counted as an expressor of a selected target if it contained >10 puncta and was detectable in more than 1 image in the z-stack. Because of the widespread labeling of *Esr1* in the AVPV, we were unable to distinguish expression in individual cells, so only its coexpression with *Kiss1* and *Pdyn* was counted. For each target, percent coexpression was calculated by $\frac{(\#\ of\ cells\ coexpressing\ target\ A\ and\ target\ B)}{Total\ \#\ of\ cells\ expressing\ target\ A} \times 100$.

Serum Hormones

Pituitary hormones LH and FSH were measured in duplicate serum samples using the Milliplex Rat Pituitary Magnetic Bead Assay (RPTMAG-86K; MilliporeSigma, Burlington, MA; RRID:AB_2716840) and were measured on a Luminex Magpix (Austin, TX). Serum hormone concentrations were calculated using Milliplex Analyst software. Intraassay variability was 3.4% for FSH and 6.4% for LH.

Serum estradiol concentration was measured in females in duplicate samples using an Ultra-Sensitive Estradiol RIA (DSL-4800; Beckman Coulter, Brea, CA; RRID:AB_3096131) with an intraassay variability of 1.4%. For estradiol in P8 females, samples had to be pooled within treatment groups due to the large volume (200 µL) of serum required for the assay. Serum testosterone concentration was measured in males in duplicate samples using the Testosterone Double Antibody RIA Kit (MP07189102; MP Biomedicals, Santa Ana, CA) with intraassay variability of 2.2%. All assays were run according to the manufacturers' protocols.

Statistical Analysis

For all data, significance was set at $P < .05$ after appropriate corrections for multiple comparisons. Detailed statistics are described next.

Development and serum hormones

Analysis of body weight and AGI were performed separately for each sex with a repeated-measure ANOVA with factors "Age" × "Treatment" followed by post hoc Šídák's multiple comparisons test. Date of puberty was analyzed with a 1-way ANOVA followed by post hoc Tukey test. Serum estradiol, testosterone, LH, and FSH were analyzed with a two-way ANOVA with factors "Age" and "Treatment" to determine main interactions, followed by post hoc Tukey's multiple comparison test. Because the concentrations of hormones varied vastly between the 3 age points, we conducted additional unpaired *t* tests at each age point to allow for detailed analysis of A1221 treatment at each age.

RNAscope

The percentage of neurons coexpressing multiple targets were calculated by $\frac{(\#\ of\ cells\ coexpressing\ target\ A\ and\ target\ B)}{Total\ \#\ of\ cells\ expressing\ target\ A} \times 100$. Two-way ANOVAs with factors "Sex" and "Treatment" were used to assess sex differences and effects of A1221 on the number of *Kiss1, Pdyn,* or *Tac3* expressing neurons in the ARC and AVPV.

Correlations of KNDy and kisspeptin gene expression, serum hormones, and physiology

To evaluate the systems-level response to EDCs, we calculated Pearson's *r* correlation between KNDy and kisspeptin cell numbers in the AVPV and ARC, serum hormone concentrations, and developmental measures for each treatment group independently to visualize any shifts in correlations because of A1221 treatment.

Results

Developmental Endpoints

Development was monitored on the entire population of rats (19 litters of Aroclor 1221 and 22 litters of vehicle; 4 males and 4 females per litter). Body weight was measured weekly beginning at P1 until the date of euthanasia. Data are presented separately for the early postnatal, adolescent, and adult periods to account for different growth curves during those stages. In females, analysis detected an effect of both age ($F_{(2,451)} = 6069$, $P < .0001$) and treatment ($F_{(1,451)} = 12.83$, $P = .0004$) in the postnatal period (P1-P14). Post hoc analysis showed that A1221 treatment significantly decreased body weight in P14 females ($P = .0016$; Fig. 1A). In both the adolescent (Fig. 1B) and adult group (Fig. 1C), there was a main effect of age ($F_{(2,305)} = 1988$, $P < .0001$; $F_{(2,210)} = 287.6$, $P < .0001$) but not treatment on female body weight. Female AGI significantly increased with age ($F_{(2,474)} = 2368$, $P < .0001$), but was unaffected by treatment (Fig. 1D).

Similar to females, a main effect of both age ($F_{(2,322)} = 5689$, $P < .0001$) and treatment ($F_{(1,322)} = 16.61$, $P < .0001$) was found in the postnatal period in males. A1221 significantly decreased body weight at both P7 ($P = .0036$) and P14 ($P = .021$) (Fig. 1E). Main effects of age were found in adolescent ($F_{(2,125)} = 1384$, $P < .0001$; Fig. 1F) and adult ($F_{(2,44)} = 1640.4$,

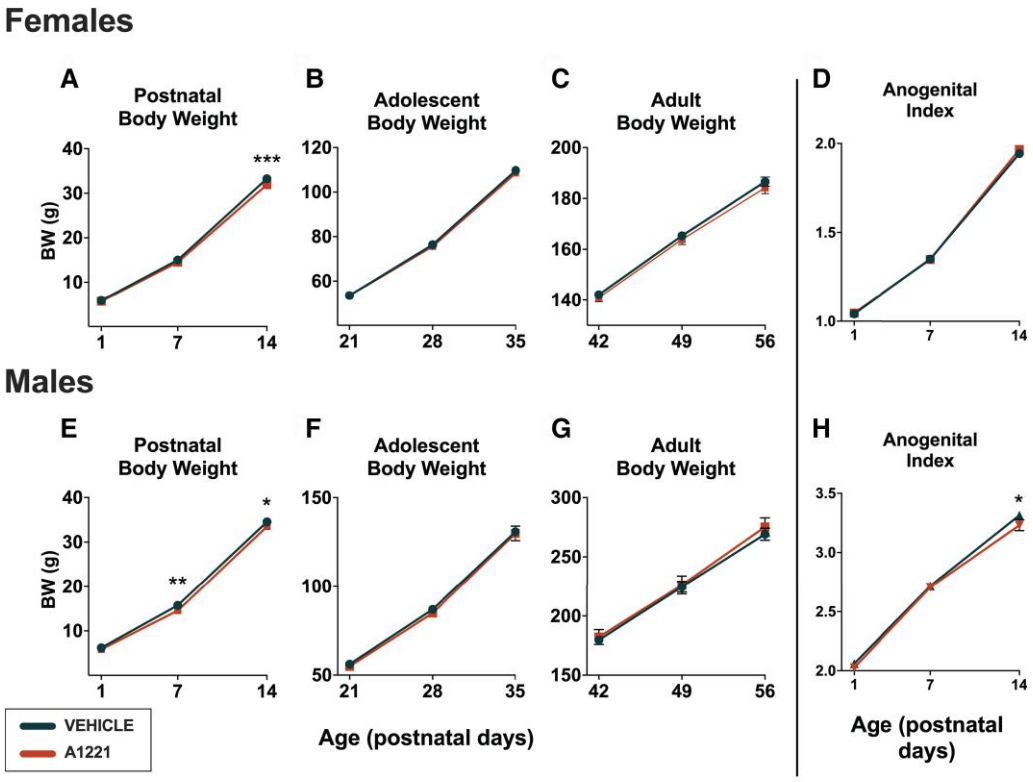

Figure 1. Effects of A1221 on postnatal, adolescent, and adult body weight and anogenital index in female and male rats. Mean ± SEM body weight and anogenital index (AGI) are shown. (A) Vehicle females had a significantly higher body weight (BW) than A1221 females at P14 ($P = .00016$). Female BW was otherwise unaffected by A1221 treatment (B, C). A1221 had no effect on AGI in females (D) or males (H). (E) A1221 treatment significantly decreased BW in males at P7 ($P = .0036$) and P14 ($P = .021$). Male BW was unaffected by A1221 treatment at other ages (F, G). (H) AGI was decreased by A1221 treatment in males at P14 ($P = .025$). Female and male body weight and AGI significantly increased across development ($P < .0001$). Postnatal females: n = 71 vehicle, n = 61 A1221; postnatal males: n = 56 vehicle, n = 42 A1221; adolescent females: n = 38 vehicle, n = 34 A1221; adolescent males: n = 18 vehicle, n = 13 A1221; adult females: n = 38 vehicle, n = 34 A1221; adult males: n = 10 vehicle, n = 7 A1221. $*P \leq .05$, $**P \leq .01$, $***P \leq .001$.

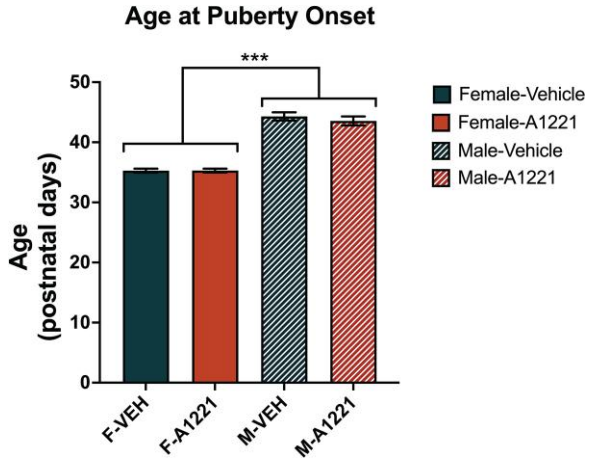

Figure 2. No effects of A1221 on the age of puberty onset in females and males. Mean ± SEM of age at puberty onset are shown. Females underwent puberty at a significantly earlier age than males ($P < .0001$). Females: n = 38 vehicle (F-VEH), n = 34 A1221 (F-A1221); males: n = 10 vehicle (M-VEH), n = 9 A1221 (M-A1221). $***P \leq .001$.

$P < .0001$) males (Fig. 1G), but there were no additional treatment effects. There was a main effect of both age ($F_{(2,322)} = 1175$, $P < .0001$) and treatment ($F_{(1,322)} = 4.424$, $P = .0362$) on AGI in males. A1221 significantly decreased AGI in males at P14 ($P = .025$; Fig. 1H).

Age at puberty was determined using vaginal opening in females and preputial separation in males. There were no effects of A1221 treatment on the timing of puberty in either sex. As expected, males underwent puberty at a significantly later age than females ($P < .0001$; Fig. 2).

Serum Hormones

Concentrations of serum hormones varied across age and were affected by A1221 treatment in a sex- and age-dependent manner. In females, analysis found a main effect of age ($F_{(2,41)} = 57.17$, $P < .0001$), treatment ($F_{(1,41)} = 14.68$, $P = .0004$), and an interaction between age and treatment ($F_{(2,41)} = 9.644$, $P = .0004$). Estradiol was highest at P8 when compared to both P30 and P60 females ($P < .0001$). A1221 significantly increased serum estradiol at P8 ($P < .0001$), but there were no treatment effects at P30 and P60 (Fig. 3A). Analysis of LH concentrations in females showed a main effect of age ($F_{(2,47)} = 6.035$, $P = .0046$), treatment ($F_{(1,47)} = 4.346$, $P = .0425$), and an interaction between age and treatment ($F_{(2,47)} = 4.959$, $P = .0111$). Serum LH peaked at P8, and levels significantly dropped at the P30 ($P < .0001$) and P60 ($P = .016$) time points. A1221 treatment significantly decreased LH at P8 ($P = .0002$), though there were no additional treatment effects at P30 and P60 (Fig. 3). In females, analysis of FSH concentrations showed a main effects in age ($F_{(2,52)} = 137.0$, $P < .0001$), treatment ($F_{(1,52)} = 4.064$, $P = .049$), and an interaction between age and treatment ($F_{(2,52)} = 6.58$, $P = .0028$). FSH was highest

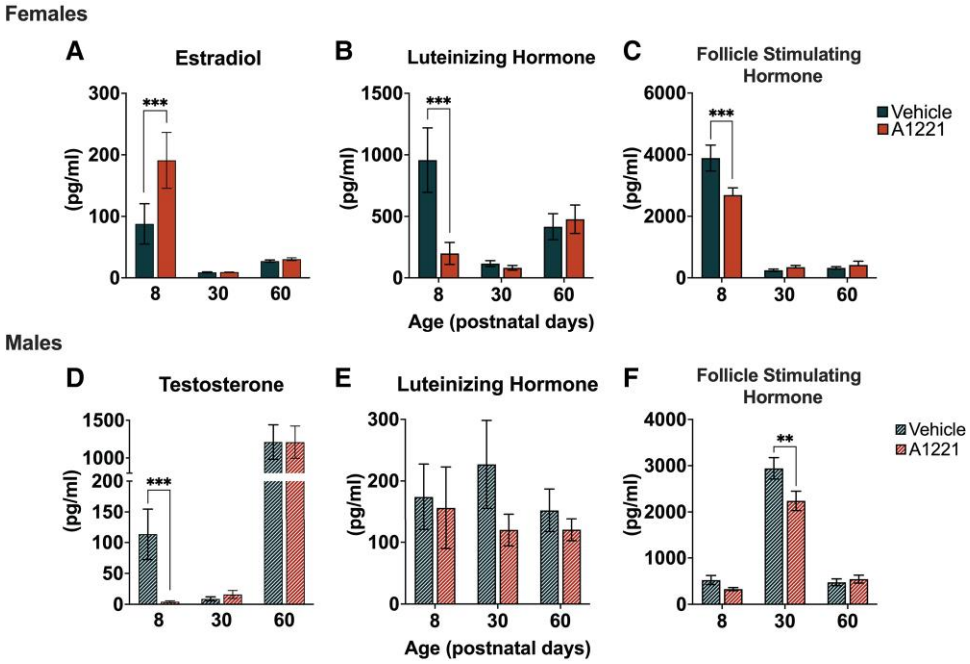

Figure 3. Effects of A1221 exposure on serum hormones in female (top) and male (bottom) rats. Mean ± SEM of serum hormones in females (A-C) and males (D-F) are shown at postnatal (*P*) days 8, 30, and 60. Statistics for significant treatment effects are indicated with asterisks; significant age effects are described herein. (A) In females, A1221 significantly increased serum estradiol at P8 ($P < .0001$) when compared to vehicle females. Serum concentrations of estradiol were significantly higher at P8 when compared to both P30 and P60 females (both $P < .0001$). (B) Serum LH was significantly decreased by A1221 treatment in P8 females ($P = .0004$). LH was highest in vehicle P8 females when compared to P30 ($P < .0001$) and P60 ($P = .016$) vehicle females. (C) FSH was significantly decreased by A1221 in P8 females ($P = .0002$). FSH was significantly higher in P8 females than P30 and P60 females ($P < .0001$). (D) In males, testosterone was significantly decreased by A1221 at P8 ($P < .0001$), but P30 and P60 males were unaffected by treatment. Concentrations of testosterone were significantly higher in P60 males than in P8 and P30 males (all $P < .0001$). (E) In males, LH serum concentrations were unaffected by both age and treatment. (F) A1221 treatment significantly decreased FSH in males at P30 ($P = .0023$). FSH concentrations were highest in P30 males when compared to P8 and P60 males (all $P < .0001$). Female estradiol at P8, P30, and P60: n = 4, 12, and 11 vehicle, n = 4, 9, and 8 A1221; female LH: n = 10, 11, and 10 vehicle, n = 8, 7, and 7 A1221; female FSH: n = 10, 13, and 10 vehicle, n = 8, 9, and 8 A1221; male testosterone: n = 9, 8, and 10 vehicle, n = 5, 6, and 9 A1221; male LH: *n* = 9, 9, and 10 vehicle, n = 5, 5, and 9 A1221; male FSH: n = 9, 9, and 10 vehicle, n = 5, 6, and 10 A1221. $^{**}P < .01$, $^{***}P < .0001$.

at P8 when compared to P30 and P60 (both $P < .0001$). A1221 treatment significantly decreased concentrations of FSH at P8 ($P = .0002$), but levels at P30 and P60 were unaffected (Fig. 3C).

In males, a 2-way ANOVA on the concentrations of testosterone detected a main effect of age ($F_{(2,40)} = 36.8$, $P < .0001$), and post hoc analysis showed that levels were significantly higher at P60 than at P8 and P30 (both $P < .0001$). The *t*-tests found a significant decrease in testosterone in A1221-treated P8 males ($P < .0001$), but there were no differences in testosterone concentrations between vehicle and treated males at P30 and P60 (Fig. 3D). LH concentration in males was unaffected by both age and treatment (Fig. 3E). Analysis on FSH concentrations found a main effect of age ($F_{(2,41)} = 130.1$, $P < .0001$), treatment ($F_{(1,41)} = 4.846$, $P = .033$), and an interaction between age and treatment ($F_{(2,41)} = 3.659$, $P = .0354$). FSH was significantly higher at P30 than P8 and P60 (both $P < .0001$). A1221 treatment significantly decreased FSH in males at P30 ($P = .0023$; Fig. 3F).

RNAscope

The number of DAPI-positive cells coexpressing kisspeptin (*Kiss1)*, neurokinin B *(Tac3)*, or prodynorphin (*Pdyn)* in the ARC were counted on one hemisphere of each rat (Fig. 4). In the ARC, there were no effects of sex or A1221 treatment on the number of kisspeptin-, neurokinin B-, or prodynorphin-expressing neurons (Fig. 4B). In both females and males, nearly all kisspeptin cells coexpressed *Tac3* (95.5% in females, 89.5% in males; Fig. 4C). There were no effects of treatment on the percentage of kisspeptin neurons coexpressing neurokinin B in females or males. Analysis uncovered a main effect of sex ($F_{(1,22)} = 13.07$, $P = .0015$) on the percentage of neurokinin B neurons coexpressing kisspeptin. A significantly higher percentage of neurokinin B neurons also expressed kisspeptin in females (84.7%) than in both vehicle ($P = .018$) and treated ($P = .027$) males (65.9%; Fig. 4D). Surprisingly, we did not see any prodynorphin expression in kisspeptin- or neurokinin B-labeled neurons in the ARC, although they were detected lateral of the ARC (Fig. 4A, Fig. 5).

In the AVPV, the number of cells expressing kisspeptin (*Kiss1)* or prodynorphin (*Pdyn)*, as well as the number of kisspeptin or prodynorphin labeled cells coexpressing ERα, were counted on 1 hemisphere for each rat (Fig. 6). Coexpression of kisspeptin and dynorphin in the AVPV has been reported in mice [42] but was not detected in our samples. We were unable to count the total number of cells expressing ERα (*Esr1*) because the labeling was too dense to resolve individual neurons. There was a main effect of sex ($F_{(1,23)} = 10.94$, $P = .0031$) but not treatment on the number of kisspeptin neurons in the AVPV (Fig. 6B). A1221 did not affect the number of prodynorphin neurons in females but significantly increased it in males ($P = .028$; Fig. 6B). There was no change

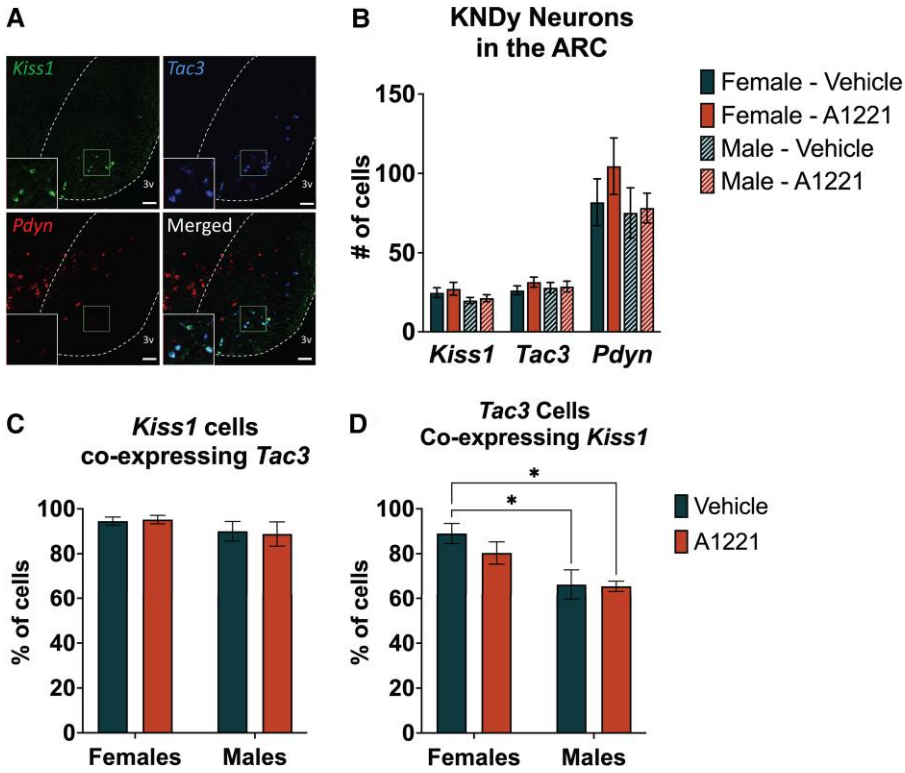

Figure 4. Results of RNAscope of kisspeptin (*Kiss1*), neurokinin B (*Tac3*), and prodynorphin (*Pdyn*) in the ARC. (A) RNAscope in situ hybridization in a female P30 rat ARC was imaged using confocal microscopy (Nikon A1R, Plan Fluor DIC 40× [oil]). Expression of kisspeptin (green), neurokinin B (blue), prodynorphin (red), and a merged image are shown. Boundaries for the ARC are indicated by dashed lines, insets are a 4× enlargement of the indicated area to show coexpression. Scale bars = 50 μm. (B) The mean ± SEM of the number of cells expressing kisspeptin, neurokinin B, and dynorphin in the ARC are presented. There were no effects of sex or treatment on any of these cell types. (C) The mean ± SEM of the percentage of kisspeptin cells coexpressing neurokinin B is shown. There were no effects of sex or treatment. Nearly all kisspeptin cells in the ARC coexpressed neurokinin B (95.5% in females, 89.5% in males). (D) The mean ± SEM of the percentage of neurokinin B cells expressing kisspeptin is shown. Vehicle females had significantly more neurokinin B cells coexpressing kisspeptin than both vehicle males ($P = .018$) and A1221 males ($P = .027$), but there were no effects of treatment. Females: n = 7 vehicle, n = 7 A1221; males: n = 7 vehicle, n = 5 A1221. *$P < .05$.

resulting from sex or treatment on the percentage of kisspeptin cells coexpressing ERα in females (99.2%) or males (100%; Fig. 6C). The percentage of dynorphin cells coexpressing ERα was also unaffected by sex or treatment (overall 12.9% in females, 22.8% in males; Fig. 6D).

Correlations Between KNDy and Kisspeptin Expression, Serum Hormones, and Physiology

To identify any systems-level changes resulting from A1221 exposure, we analyzed correlations across ARC *Kiss1, Pdyn*, and *Tac3* cell numbers, AVPV *Kiss1* and *Pdyn* cell numbers, serum hormone concentrations (all of the previous on P30), and body weight on P28. In the vehicle females, the number of neurons in the ARC expressing *Kiss1* and *Tac3* were positively correlated ($P = .001$). In A1221 females, similar to the vehicle females, numbers of ARC *Kiss1* and ARC *Tac3* cells were significantly positively correlated ($P = .03$). Unlike the vehicle females, in A1221 females, a positive correlation was identified between the number of ARC *Pdyn* neurons and serum estradiol concentrations ($P = .05$). Additionally, P28 body weight and serum FSH were negatively correlated ($P = .036$).

In vehicle males, positive correlations were found between the number of ARC *Kiss1* and *Tac3* neurons ($P = .04$); between serum LH and the number of *Kiss1* neurons in the AVPV ($P = .022$); and between LH and serum testosterone ($P = .036$). This group exhibited a negative correlation between the number of ARC *Pdyn* neurons and serum FSH ($P = .05$). A1221-exposed males also exhibited this positive correlation between LH and testosterone ($P = .0002$), but unlike the vehicle males, did not have a correlation between LH and the number of AVPV *Kiss1* neurons (Table 2). The correlation between ARC *Kiss1* and *Pdyn*, although positive, was nonsignificant ($P = .2$).

Discussion

In this study, we determined the effects of perinatal exposure to Aroclor 1221 (A1221), a weakly estrogenic PCB mixture and known EDC, on the developing HPG axis, focusing on hypothalamic KNDy and kisspeptin neurons because of their importance in the hypothalamic control of reproduction. Previous work on kisspeptin signaling in rats has been limited by the availability of antibodies that reliably detect kisspeptin in the brain. We were able to overcome this in our established EDC exposure model in rats by using RNAscope to evaluate kisspeptin expression and its coexpression with other genes involved in the hypothalamic neuroendocrine circuitry in the ARC and AVPV. Work was done in female and male rats because developmental EDC effects are often sex-specific and because our exposure period overlapped with the critical period of brain sexual differentiation in rats [43].

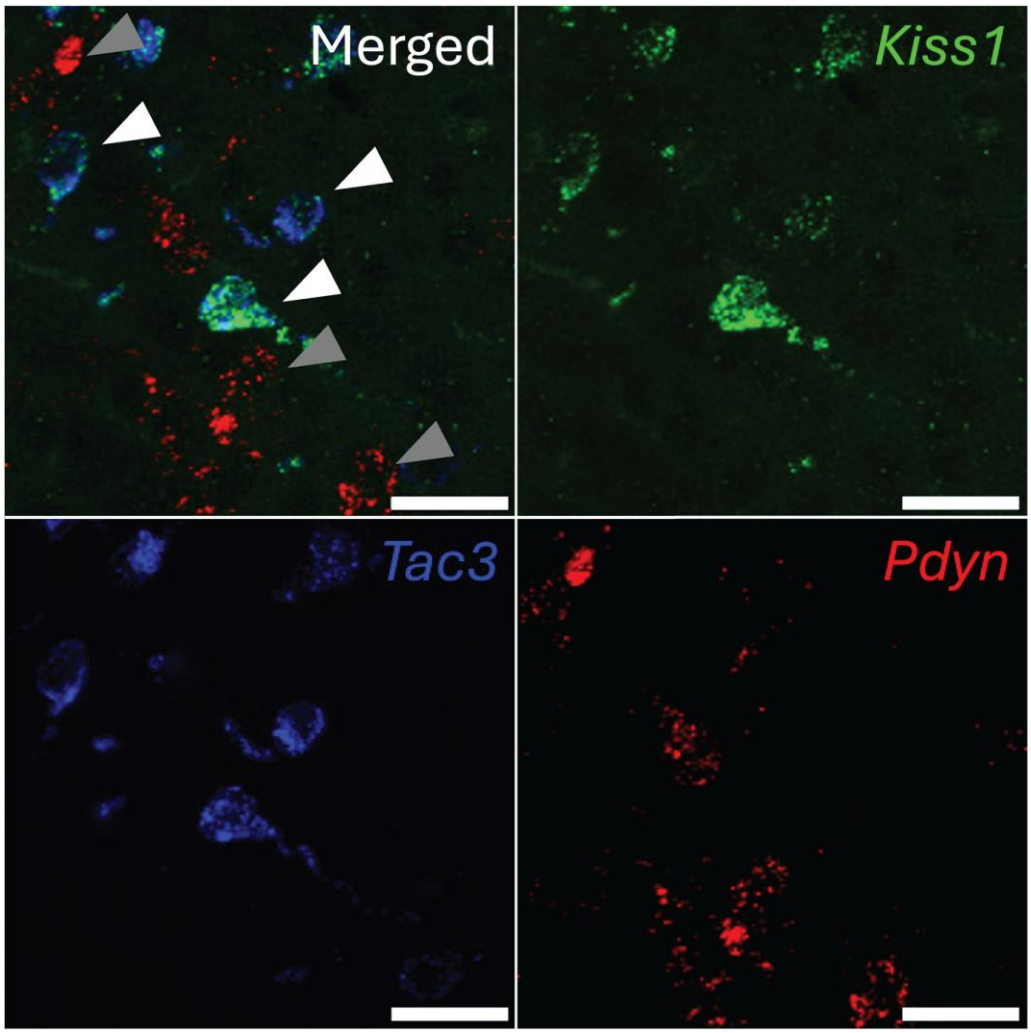

Figure 5. Micrograph showing the absence of kisspeptin and prodynorphin coexpression in cells of the ARC in a P30 vehicle female rat. The section was imaged (Plan Fluor DIC 40× [oil]) for triple-labeling of kisspeptin (green), neurokinin B (blue), and prodynorphin (red). White arrows indicate neurons co-expressing kisspeptin and neurokinin, and gray arrows indicate a separate population of neurons expressing prodynorphin. Scale bar, 25 μm.

Developmental Effects of A1221

Low-dose A1221 exposure had several effects on our measured developmental outcomes. Anogenital index, an indicator of prenatal androgen exposure with AGI greater in males than females [44], was decreased in A1221 males only at P14. Because we did not measure AGI after P14, we do not know if that difference persisted with age. It is notable that although traditionally cited for its estrogenic properties, A1221 can also have antiandrogenic actions [45]. The antiandrogenic actions would be consistent with a decreased AGI in male rats, and because androgens are low in females, unlikely to affect AGI. Previous work in our laboratory has found that other modes of exposure to A1221 (different timing and/or route) or a PCB mixture did not affect AGI in females or males [46], though exposure to a mix of EDCs decreased AGI in males [47]. Other EDCs with antiandrogenic properties, such as phthalates [48], have been found to decrease AGI in males.

We measured body weight weekly because some EDCs are associated with metabolic dysfunctions [49]. Body weight was slightly lower in treated animals in the postnatal period, but this effect was no longer observed in pubertal and adult rats. It is notable that rat dams were fed a cookie with A1221 or vehicle when pups were aged P1 to P21, which presumably delivered continuous exposure to the pups via lactation. This might explain why body weight was only affected during this period and, as discussed later, some hormones were also only affected at the P8 age. Other work has shown that exposure to PCBs at different doses and routes of exposure has varying effects on body weight [46, 50, 51], and there is little consistency between the affected ages. The current study did not reveal any effect of treatment on the age of puberty onset in either sex. Similar to body weight, the effects of A1221 on puberty have varied depending on the exposure paradigm [4, 7]. Further studies examining different dosages or treatment paradigms could provide additional insight. As a whole, these results suggest small developmental effects of perinatal A1221 exposure on postnatal development in rats in a sex-specific manner.

Serum Hormones Were Affected in an age- and sex-dependent Manner

We further determined effects of A1221 on the HPG axis through measures of the gonadotropins, LH and FSH, and

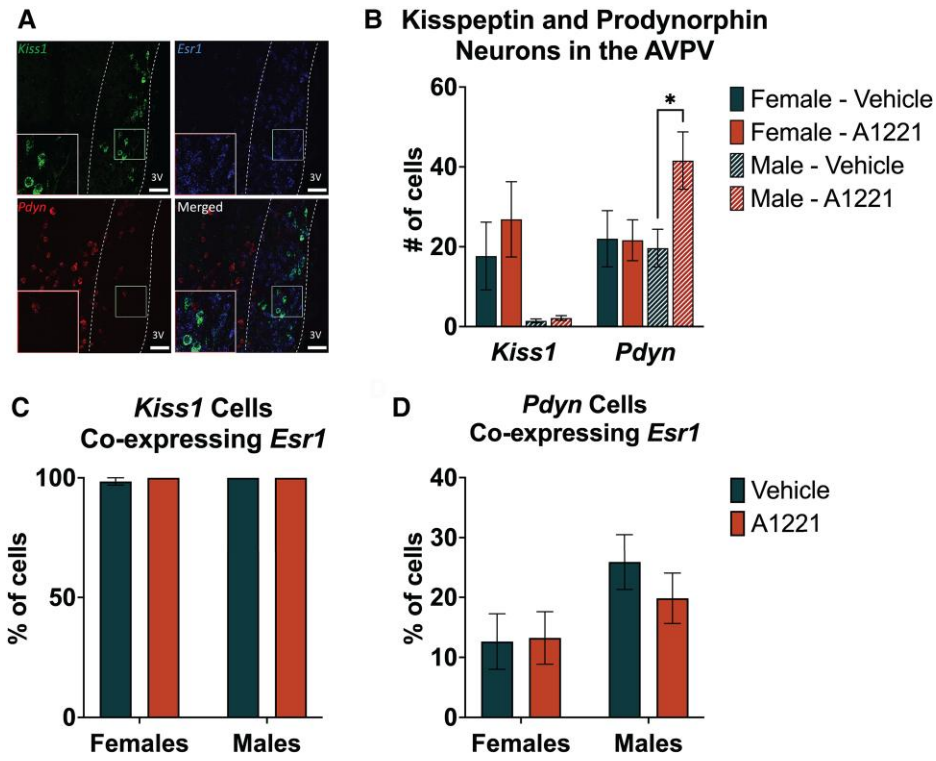

Figure 6. Results of RNAscope of kisspeptin (*Kiss1*), ERα (*Esr1*), and dynorphin (*Pdyn*) in the AVPV. (A) RNAscope in situ hybridization in a female P30 rat AVPV was imaged using confocal microscopy (Nikon A1R, Plan Fluor DIC 40× [oil]). Expression of kisspeptin (green), ERα (blue), prodynorphin (red), and a merged image are shown. Boundaries for the AVPV are indicated by dashed lines, insets are a 4× enlargement of the indicated area to show coexpression. Scale bars = 50 μm. (B) The mean ± SEM of the number of cells in the AVPV expressing kisspeptin or prodynorphin are presented. Females overall had more kisspeptin neurons than males ($P = .0031$). There were no effects of treatment on the number of kisspeptin cells in the AVPV. For prodynorphin, females were unaffected by treatment, but A1221 significantly increased the number of prodynorphin neurons in males ($P = .028$). (C) The mean ± SEM of the percentage of kisspeptin cells coexpressing ERα is shown. The percentage of kisspeptin cells coexpressing ERα was unaffected by sex and treatment (99.2% in females and 100% in males). (D) The mean ± SEM of the percentage of dynorphin cells coexpressing ERα is shown. The percentage of dynorphin cells coexpressing ERα (12.9% in females and 22.8% in males) was unaffected by sex and treatment. Females: n = 6 vehicle, n = 8 A1221; males: n = 7 vehicle, n = 7 A1221. *$P < .05$.

ovarian (estradiol) and testicular (testosterone) hormones through development. The most interesting results were found at P8: in females, A1221 animals had significantly lower LH and FSH concentrations and significantly higher estradiol concentrations. It is plausible that the higher estradiol exerted negative feedback on LH and FSH release, explaining why A1221 treated animals had lower concentrations of LH and FSH at P8. No differences in estradiol, LH, or FSH were found at P30 or P60, possibly because the A1221 treatment was no longer "on board" when rats were weaned. Males had substantially and significantly lower testosterone at P8 in A1221 compared to vehicle rats. These hormonal changes in P8 females (higher estradiol in the A1221 group) and males (lower testosterone in the A1221 group) may influence brain development because P8 is the end of the critical period of brain sexual differentiation in rats.

The design of this study enabled us to compare estradiol and the gonadotropins as they changed through postnatal development in females. Consistent with previous findings [40, 52], we observed that estradiol concentrations were lowest at P30 compared to earlier and adult stages. Estradiol has been reported to have a peak from P9 to P23 in female rats, followed by a significant drop until the onset of puberty, when concentrations increase once more [52]. Similarly, LH levels exhibit a spike from P9 to P21 before declining at P30, remaining relatively stable thereafter. We observed a spike in FSH at P8 that dropped at P30 and P60, consistent with prior findings reporting high levels of FSH from birth into the postnatal period followed by a decrease that lasts into adulthood [52]. LH and FSH levels are reported to be notably high during the postnatal period because of gamma aminobutyric acid (GABA) exerting an excitatory effect onto GnRH neurons, stimulating LH and FSH production. Embryonic GnRH neurons exhibit a depolarizing $GABA_A$ receptor-mediated response to GABA [53], and this continues after birth until the time of puberty when $GABA_A$ receptor activation switches to elicit hyperpolarization [54]. These high LH/FSH levels in the postnatal and infantile period are consistent with normal development of the ovary [55, 56].

In males, A1221 significantly decreased testosterone at P8 when compared to the vehicle, but no differences were seen at P30 or P60. Other research has showed that maternal exposure to PCBs decreases testosterone in males at 3 weeks of age, and depending on the PCB, this effect is lost at 6 and 15 weeks [57]. Similar to the females, this decrease in testosterone only at P8 could be due to the concurrent A1221 exposure; treatment had ceased by the P30 and P60 ages. The expected nadir of testosterone levels at P30 recapitulates previous data from our laboratory [40, 58]. LH concentrations, which were unaffected at any age by A1221, did not exhibit a developmental change, although variability within groups was very high, presumably because of the pulsatile nature of

Table 2. Pearson correlations between endpoints in P30 rats

Variable	ARC *Kiss1*	ARC *Pdyn*	ARC *Tac3*	AVPV *Kiss1*	AVPV *Pdyn*	Estradiol	LH	FSH
A. Female—vehicle								
ARC *Pdyn*	0.72							
ARC *Tac3*	**0.98**	0.69						
AVPV *Kiss1*	−0.42	0.39	−0.15					
AVPV *Pdyn*	−0.28	−0.17	−0.18	0.53				
Estradiol	−0.16	−0.15	−0.09	−0.27	−0.17			
LH	0.59	0.13	0.58	−0.48	−0.35	0.59		
FSH	−0.11	−0.39	−0.29	−0.18	0.52	−0.20	−0.02	
P28 BW	0.36	0.49	0.22	−0.63	−0.39	0.52	0.67	−0.16
B. Female—A1221								
ARC *Pdyn*	0.23							
ARC *Tac3*	**0.85**	0.58						
AVPV *Kiss1*	−0.26	−0.31	−0.57					
AVPV *Pdyn*	0.19	−0.54	0.23	−0.19				
Estradiol	0.18	**0.75**	0.62	−0.67	−0.07			
LH	−0.26	0.57	−0.08	−0.11	−0.55	0.62		
FSH	0.12	0.26	−0.08	−0.41	−0.46	0.03	0.21	
P28 BW	−0.42	−0.70	−0.36	0.50	0.12	−0.28	−0.36	**−0.84**
Variable	**ARC *Kiss1***	**ARC *Pdyn***	**ARC *Tac3***	**AVPV *Kiss1***	**AVPV *Pdyn***	**Testosterone**	**LH**	**FSH**
C. Male—vehicle								
ARC *Pdyn*	−0.20							
ARC *Tac3*	**0.78**	0.23						
AVPV *Kiss1*	−0.37	−0.01	−0.17					
AVPV *Pdyn*	−0.16	−0.20	−0.34	−0.45				
Testosterone	−0.40	0.57	0.15	0.62	−0.68			
LH	−0.63	0.33	−0.28	**0.83**	−0.48	**0.74**		
FSH	−0.07	**−0.82**	−0.24	0.52	−0.02	−0.19	0.32	
P28 BW	0.55	−0.83	0.49	−0.03	0.48	−0.41	−0.23	0.62
D. Male—A1221								
ARC *Pdyn*	−0.10							
ARC *Tac3*	0.80	0.39						
AVPV *Kiss1*	−0.73	−0.47	−0.41					
AVPV *Pdyn*	0.52	−0.49	**−0.99**	−0.11				
Testosterone	−0.50	0.14	0.83	−0.13	−0.66			
LH	−0.02	0.12	0.80	−0.19	−0.77	**0.99**		
FSH	0.48	−0.87	−0.76	0.22	0.73	−0.53	−0.49	
P28 BW	0.60	−0.44	−0.56	0.10	0.60	0.01	0.04	0.54

Pearson correlations were calculated among ARC *Kiss1*, *Pdyn,* and *Tac3* neuron numbers; AVPV *Kiss1* and *Pdyn* neuron numbers; serum hormones (all of the previous on P30); and body weight on P28, for vehicle and A1221 females (A, B) and males (C, D). (A) In vehicle females there was a significant positive correlation between ARC *Kiss1* and ARC *Tac3* ($P = .001$). (B) In A1221 females, there was a significant positive correlation between ARC *Kiss1* and ARC *Tac3* ($P = .03$); ARC *Pdyn* and estradiol ($P = .05$), and a negative correlation between P28 body weight and FSH ($P = .036$). (C) In vehicle males, ARC *Kiss1* and ARC *Tac3* were positively correlated ($P = .04$). Serum concentrations of LH were positively correlated with the number of AVPV *Kiss1* cells ($P = .022$) and with testosterone ($P = .036$). ARC *Pdyn* was negatively correlated with FSH ($P = .046$). (D) A1221 treated males had a strong negative correlation between ARC *Tac3* and AVPV *Pdyn* ($P < .001$), and a strong positive correlation between serum LH and testosterone ($P = .0002$). Significant correlations are indicated in bold text.
Abbreviation: BW, body weight.

LH release. Serum FSH concentrations showed a dramatic spike at P30, characteristic of the maturation of the reproductive system in males. During prepuberty, male rats undergo a phase of sexual maturation characterized by the initiation of testicular growth and the development of spermatozoa [59]. FSH is essential for the proliferation and maturation of Sertoli cells within the testes, which provide structural and nutritional support necessary for development of sperm cells. The prepubertal spike in FSH is linked to activation of the HPG axis, marking the beginning of the preparatory phase of spermatogenesis [60, 61]. FSH in males was significantly decreased by A1221 exposure at P30, which could point to a shift in the timing of the spike in FSH. Altered timing of the prepubertal increase of FSH could change the timeline of the maturation of the testes and may be a partial mechanism for the shift in puberty in males seen from exposure to some EDCs [6, 47, 62].

Overall, our findings demonstrate that A1221 impacts serum hormones in an age- and sex-dependent manner, and further research needs to be done to determine how these changes may manifest in other developmental pathways. It is possible that the early life differences in hormones may contribute to the decreased AGI that emerged in the A1221-exposed males at P14, and perhaps also relates to the body weight differences seen at P7 and P14 but not at P1.

The rat KNDy and Kisspeptin Networks Show Limited Effects of A1221

Our RNAscope analysis revealed few effects of perinatal A1221 exposure on the population of kisspeptin, neurokinin B, and/or dynorphin neurons in the ARC or AVPV. These results were surprising because kisspeptin pathways have previously been found to be significantly affected by other endocrine disruptors such as phthalates [35] and bisphenols [34]. Using a different exposure model (different timing and route), we previously reported that kisspeptin immunoreactivity was significantly decreased by A1221 in the female but not male rat AVPV [4]. Other studies measuring *Kiss1* mRNA have reported decreases in *Kiss1* mRNA in the hypothalamus in response to PCBs, phthalates, and BPA [34, 35, 58, 63]. Further consideration of these results, and of sex differences, follow.

KNDy neurons in the ARC

Our study revealed no sex differences in KNDy neurons in the ARC, which is consistent with other reports that neither the number of kisspeptin-immunoreactive cells or levels of *Kiss1* mRNA differ between the sexes in the ARC of adult rodents [64-66]. The percentage of kisspeptin cells coexpressing neurokinin B in the ARC was also not dimorphic. We did find an interesting sex difference with males having a significantly lower percentage of neurokinin B cells coexpressing kisspeptin than females. Neurokinin B stimulates the release of kisspeptin in the ARC and is, at least in part, regulated by estradiol and testosterone [67, 68]. This sex difference in neurokinin B/kisspeptin coexpression could be influenced by several factors. Females undergo puberty at an earlier age in rats; therefore, the P30 time point is likely representative of mid-puberty in females and pre- or early puberty in males. The gonadal hormones estradiol and testosterone play significant roles in the regulation of neurokinin B and kisspeptin, so it is possible the increased coexpression of neurokinin B and kisspeptin in females is related to differences in the hormonal milieu. This could be a transient part of the normal developmental processes that occur as different regulatory mechanisms come into play, and further research is required to determine if this sex difference persists into adulthood.

Kisspeptin and dynorphin in the AVPV

In the AVPV, kisspeptin cell numbers were far higher in females than males, consistent with previous studies [65, 69, 70]. Developmentally, kisspeptin-immunoreactive neurons are first detectable in this region at postnatal day 15 [20] and females have more neurons expressing kisspeptin than males [70], possibly because of its key role in driving GnRH neurons to initiate the preovulatory LH surge. This sex difference appears to be a result of prenatal exposure to estradiol [69, 71, 72] and in the current study was unaffected by A1221. Coexpression of ERα with kisspeptin in the AVPV, which was close to 100%, was similarly unaffected by treatment in either sex.

In females, the number of dynorphin neurons was unaffected by A1221; however, A1221-treated males had significantly more dynorphin neurons than untreated males. Dynorphin inhibits the kisspeptin signaling pathway [27, 28]. Given that our data did not show changes in the number of kisspeptin neurons at this time point and considering that GnRH neurons express the kappa opioid receptor in rats [73], it is possible that this increase in dynorphin neurons directly affects GnRH neurons and could be the mechanism behind the decrease in serum concentration of FSH found in males at the same age (P30). As described previously, this prepubertal spike in FSH is a marker of prepubertal activation of the HPG axis [60, 61]. Additional research into the development of the AVPV in males could uncover how A1221 is driving this increase in dynorphin neurons and what the long-term health effects of this change may be.

RNAscope in rat Revealed Unexpected Species Differences in the ARC KNDy Population

A notable finding of this study was the lack of dynorphin expression in both KNDy neurons in the ARC (Fig. 4, Fig. 5) and kisspeptin neurons in the AVPV (Fig. 6). Regarding the former, coexpression of kisspeptin-neurokinin B-dynorphin in KNDy neurons of the ARC has been well established in mammals such as ewes and mice [33, 62, 74-76]. In addition, AVPV kisspeptin neurons were reported to coexpress dynorphin in mice [42], differing from current results in rats. A recent study using spatial transcriptomic sequencing in the rat ARC supports the possibility that dynorphin is not expressed in the same neurons as kisspeptin and neurokinin B [77], consistent with our current RNAscope data. However, other reports using double-label immunofluorescence reported coexpression of kisspeptin and neurokinin B [78], and neurokinin B and dynorphin in the rat ARC [79]. Both studies used absorption controls, which can lack specificity. This method involves preincubating the primary antibody with an excess of its corresponding antigen to block specific binding sites, but it does not account for nonspecific binding that may still occur [80]. As a result, the staining may not accurately represent the presence of neurokinin B and dynorphin, potentially leading to false-positive results or an overestimation of co-expression.

Dynorphin expression has been reported in the medial basal hypothalamus during the prepubertal period in female rats [81], but it has been primarily localized in the ventromedial nucleus up to P45 [77]. It is possible dynorphin expression is quiescent in KNDy and kisspeptin neurons during our observed P30 time point; however, the presence of some dynorphin cells in both the ARC and AVPV, as well as the transcriptomic analyses suggesting that dynorphin is expressed in a separate population of cells than kisspeptin and neurokinin B, supports our hypothesis that these are 2 discrete populations of neurons. Furthermore, and importantly, in the rat, dynorphin cannot be used as an accurate biomarker for kisspeptin neurons in the ARC and AVPV. Although further classification needs to be done, the discovery of this species difference is intriguing.

Relationships Among Physiological Endpoints and Hormones are Shifted in A1221-treated Rats

Several interesting correlations were identified in the analysis of body weight, serum hormones, and RNAscope results. In

both males and females, ARC *Kiss1* and ARC *Tac3* were positively correlated, significantly in vehicle females, A1221 females, and vehicle males, although this did not reach significance in male A1221 rats. These relationships are consistent with the co-localization of *Kiss1* and *Tac3* in KNDy neurons. Notably, ARC *Pdyn* was not significantly correlated with ARC *Kiss1* or *Tac3*, mirroring our RNAscope finding that rat "KNDy" neurons do not contain *Pdyn*.

Some correlations emerged in A1221-exposed females that were not seen in vehicle females. A1221 females exhibited a significant negative correlation between body weight and FSH levels. These results are consistent with studies in humans indicating that, in the prepubertal period, FSH and body mass index are negatively correlated [82]. In addition, ARC *Pdyn* was positively correlated with serum estradiol in A1221 but not vehicle females. These results suggest that early-life A1221 exposure changes the developmental trajectory of both individual endpoints, as well as their relationships.

In males, we found strong positive correlations between serum levels of testosterone and LH in both vehicle and A1221 groups, likely representing the fact that LH stimulates the release of testosterone from the testes [83, 84]. Vehicle males showed a positive relationship between LH and the number of kisspeptin neurons in the AVPV, which is consistent with findings that kisspeptin is a potent stimulator of LH secretion [85]. However, in the A1221 males, there was no correlation found between AVPV kisspeptin and LH, a phenotype more similar to the females than to the vehicle male, suggesting that A1221 may influence LH secretion in males. The strong negative correlation between ARC *Pdyn* and FSH in vehicle males was not significant in A1221 males, although the latter had a trend ($P = .56$) for this relationship. Finally, a negative correlation between ARC *Tac3* and AVPV *Pdyn* was found in A1221 but not vehicle males.

As a whole, these correlations suggest several changes to the developmental trajectory of neuroendocrine development in both female and male rats by early-life exposure to A1221 and changes in relationships among aspects of HPG function.

Conclusion

This study provides insights into the effects of developmental PCB exposure on a range of neurodevelopmental and reproductive outcomes. We explored how perinatal exposure to A1221 influences hormone levels, anogenital distance, body weight, and puberty onset in rats, emphasizing the importance of considering sex-specific responses and temporal dynamics in developmental outcomes. The brain's kisspeptin systems were relatively resilient to exposure, at least at P30, and only prodynorphin in the AVPV of male rats was changed (increased) by A1221 treatment. It is important to note that whereas early-life A1221 exposure did not induce across-the-board changes to the neuroendocrine system, it induced a number of small but significant alterations in individual endpoints that, together with shifts in the relationships among those endpoints, suggests a change in the reproductive neuroendocrine phenotype.

Furthermore, the early developmental changes in hormones induced by A1221 observed here may affect brain organization and contribute to the changes in sexually dimorphic behaviors that are induced by exposure. We reported that A1221 led to alterations in sociosexual and mate preference [7, 10], and social and anxiety-like behaviors [8, 9]. In addition, evidence of brain reorganization caused by early life PCBs is provided by reported changes in gene expression and neurogenesis in sexually differentiated brain regions such as the preoptic area and ventromedial nucleus [4, 58, 86].

These and other studies on effects of EDCs at environmentally relevant doses commonly reveal latent effects at multiple levels of analysis. Research focused on low level exposures is critical because it reflects the actual risk faced by wildlife and human populations. Additionally, our findings regarding the absence of prodynorphin expression in KNDy neurons stands out as a significant departure from findings in other species, highlighting the unique neuroendocrine landscape in rats. This insight helps further our understanding of the diversity and complexity of reproductive physiology across mammalian species, laying the groundwork for future investigations and advancements in reproductive health research.

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Disclosures

A.C.G. is a consultant on a legal case related to PCBs. The other authors have nothing to disclose.

Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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