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The effects of prenatal PCBs on adult social behavior in rats

Michael P. Reilly [a], Connor D. Weeks [b], Viktoria Y. Topper [c], Lindsay M. Thompson [a], David Crews [d], Andrea C. Gore [a,*]

[a] Division of Pharmacology and Toxicology, College of Pharmacy, The University of Texas at Austin, Austin, TX 78712, USA
[b] College of Natural Sciences, The University of Texas at Austin, Austin, TX 78712, USA
[c] Institute for Cellular and Molecular Biology, The University of Texas at Austin, Austin, TX 78712, USA
[d] Section of Integrative Biology, The University of Texas at Austin, Austin, TX 78712, USA

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ABSTRACT

Endocrine disrupting chemical (EDC) exposures during critical periods of development may influence neuronal development and the manifestation of sexually dimorphic sociability and social novelty behaviors in adulthood. In this study, we assessed the effects of gestational exposure to PCBs on the social behavior of males and females later in adulthood. A weakly estrogenic PCB mixture, Aroclor 1221 (A1221, 0.5 or 1 mg/kg) was administered to pregnant Sprague–Dawley rat dams. Both a positive control (estradiol benzoate; EB, 50 μg/kg) and negative control (dimethylsulfoxide; DMSO in sesame oil vehicle) were similarly administered to separate sets of dams. The sexes responded differently in two tasks essential to sociality. Using a three-chamber apparatus that contained a caged, same-sex, gonadectomized stimulus animal and an empty stimulus cage, we found that both sexes showed a strong preference for affiliating with a stimulus animal (vs. an empty cage), an effect that was much more pronounced in the males. In the second task, a novel and a familiar stimulus animal were caged at opposite ends of the same apparatus. Females displayed a higher degree of novelty preference than the males. During both tests, females had significantly higher social approach behaviors while male engaged in significantly more interactive behaviors with the conspecific. Of particular interest, males born of dams that received prenatal A1221 (0.5 mg/kg) exhibited an overall decrease in nose-to-nose investigations. These behavioral data suggest that the males are more sensitive to A1221 treatment than are females. In addition to behavioral analysis, serum corticosterone was measured. Females born of dams treated with A1221 (0.5 mg/kg) had significantly higher concentrations of corticosterone than the DMSO female group; males were unaffected. Females also had significantly higher corticosterone concentrations than did males. Overall, our results suggest that the effects of gestational exposure to PCBs on adult social behavior are relatively limited within this particular paradigm.

Introduction

Prenatal exposure to endocrine disrupting chemicals (EDCs) can disrupt the neuroendocrine system, leading to alterations in adult social and sociosexual behaviors in a sexually-dimorphic manner. Most research has been conducted for bisphenol A (BPA), exposure to which causes a decrease in the territorial marking of male mice (Williams et al., 2013), as well as female-specific alteration of one-on-one social interactions in juvenile mice (Wolstenholme et al., 2011) and prairie voles (Sullivan et al., 2014). BPA also perturbs social recognition in mice (Wolstenholme et al., 2013). Exposure to other EDCs such as atrazine (mice: Belloni et al., 2011), PCBs (rats: Jolous-Jamshidi et al., 2010), and chlorpyrifos (mice: Venerosi et al., 2012) are associated with perturbations of normal social interactions. Polychlorinated biphenyls (PCBs) – including the Aroclor 1221 mixture (A1221) used in the current study – also disrupt sexual behavior in female rats (Chung and Clemens, 1999; Steinberg et al., 2007). However, beyond this work, studies of EDC effects on social affiliation (individual preference to associate with a conspecific) and social novelty (individual choice to affiliate with a strange versus a familiar conspecific) are limited. Research has shown sex differences in these behaviors, as male rats tend to spend more time interacting with an unfamiliar, same-sex conspecific than do females (Carrier and Kabbaj, 2012; Slamberová et al., 2011; Stack et al., 2010). However, to our knowledge there are no studies investigating the effects of gestational exposure to PCBs on this paradigm.

The purpose of this study was to provide a thorough characterization of the social behavioral phenotype caused by gestational EDC exposure. We assessed how treatment of a pregnant rat dam with A1221 during the third trimester of gestation affected the social behavior of male and female offspring later in adulthood. Two dosages of A1221 (0.5 and 1 mg/kg) were administered during the last trimester of gestation, during a critical period of sexual differentiation of the hypothalamus (Davis et al., 1996; Jacobson et al., 1980). Both positive control (estradiol

* Corresponding author.
E-mail address: andrea.gore@austin.utexas.edu (A.C. Gore).

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benzoate; EB) and negative control (DMSO vehicle) groups were used for comparison. Using this model, we were able to address hypotheses about sex differences in performance in two types of socially relevant tests, and to test the hypothesis that prenatal exposure to EDCs in these responses would have sex-specific effects. Because it is known that male and female rats differ in their basal concentrations of corticosterone (Kitay, 1961; Gillette et al., 2014) and, further, that circulating levels of corticosterone influence social behaviors in rats (Veenit et al., 2013), we also measured concentrations of this hormone in our experimental rats.

Materials and methods

Experimental design

Sprague–Dawley rats were purchased from Harlan Sprague–Dawley (Houston, TX), and all animal procedures were conducted in compliance with protocols approved by IACUC at the University of Texas at Austin. They were housed in a colony room with controlled temperature (22 °C) and light cycle (12:12 dark:light, lights on at 24:00). Virgin females were mated with sexually experienced males. The day following successful mating, as indicated by a sperm-positive vaginal smear, was termed embryonic day 1 (E1). Male and female stimulus rats were purchased as young adults from Harlan, and gonadectomized under isoflurane anesthesia. Stimulus animals were not treated with EDCs or vehicle.

Pregnant rats were exposed to one of four treatments, administered via intraperitoneal injections, on E16 and E18, the beginning of the period of brain sexual differentiation (Davis et al., 1996; Jacobson et al., 1980). The dosages used were based on prior work conducted in the Gore lab that showed physiological, behavioral, and neuroendocrine effects (Steinberg et al., 2007, 2008; Dickerson et al., 2011a; Walker et al., 2013a, 2013b): (1) Vehicle (3% DMSO/sesame oil mix), (2) Estradiol benzoate (EB; 50 μg/kg), (3) Aroclor 1221 (A1221, 0.5 mg/kg), or (4) A1221 (1 mg/kg). The number of litters per treatment was 11, 11, 10, and 10, respectively. Although we did not measure body burden or tissue content in the exposed offspring, the literature suggests that maternal–fetal transfer results in an exposure to approximately 1–2 μg/kg A1221, and 100 ng/kg EB, in the fetuses (Takagi et al., 1986).

The day of parturition was called postnatal day 0 (P0). At P1, the newborn pups were weighed and their anogenital distance measured; litters were culled to 4 males and 4 females. The pups were monitored daily for eye opening, while body weights and anogenital distance were taken weekly following birth. The pups were weaned at P21 and rehoused in same-sex groups where they were monitored daily for signs of pubertal development: vaginal opening in females and preputial separation in males (Steinberg et al., 2007; Walker et al., 2012). Following vaginal opening, daily vaginal smears were taken and cell cytology was examined as a measure of estrous cyclicity in the females. Beginning at P60 animals were subjected to a battery of the following tests in random order: sociability and social novelty, mate preference, open field and elevated plus maze; fear conditioning always was the last test. The total number of behaviorally characterized animals was 82 females and 80 males. Order of testing had no effect on behavioral outcomes. Experimental rats were weighed and euthanized 30 days after testing was completed, and bloods centrifuged and frozen for hormone assay, and adrenals and gonads removed and weighed.

Hormone radioimmunoassay

Around P90, animals were euthanized by rapid decapitation and trunk blood was collected; females were euthanized in proestrus. In addition, animals from the same litters that were not behaviorally tested were used to increase sample size; this resulted in a total number of 158 females and 153 males. 10 μl of sample from each individual was used to measure serum corticosterone concentration in a single non-human radioimmunoassay (MP Biomedicals; Corticosterone ^{125}I RIA — 07120103). Assay sensitivity was 7.7 ng/ml, and intra-assay variability was 4.1%.

Behavioral paradigm

A three-chamber social apparatus (100 cm × 100 cm; Stoelting, Fig. 1) was used as the testing arena (Crews et al., 2012; Moy et al., 2004). Testing was conducted under dim red light during the dark period of their light–dark cycle, approximately 2 following lights out. The experimental animal was placed in the middle chamber of the apparatus, with doors to the two side chambers closed. For females, estrous cycle status on the day of testing was recorded to identify any potential differences relating to the behaviors examined. Same-sex gonadectomized stimulus animals were placed in a 7 cm × 15 cm cylindrical stimulus cage located in a corner of the lateral chambers; bars allowed for nose-to-nose investigation but did not permit further contact.

Sociability and social novelty

A five minute habituation period was used to allow the experimental rats access to the center chamber only. The doors were then opened and the experimental animal allowed to freely move around the entire apparatus for the two 10 min periods. All behaviors were video recorded throughout the testing. The entire apparatus was dismantled and all surfaces wiped clean with a 70% ethanol solution between each test.

During the first Sociability test, one of the stimulus cages, randomly selected, held a novel same-sex (untreated by EDCs, and gonadectomized in adulthood) rat while the other stimulus cage remained empty (Fig. 1A). At the test's conclusion, the experimental animal was removed from the apparatus and temporarily placed in a holding cage.

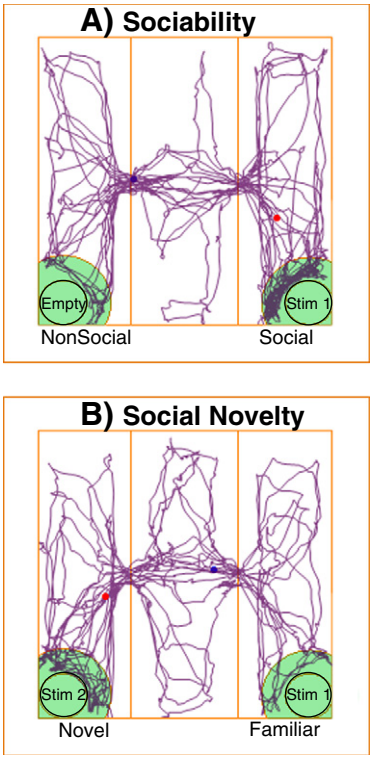

Fig. 1. A diagram of the 3-chamber apparatus, with a representative tracking profile from ANY-maze for an individual rat, is shown for the Sociability (A) and Social novelty (B) tests. In Sociability (A), the stimulus rat (Stim 1) was a same-sex, gonadectomized rat, and the other cage was empty. In Social novelty (B), the same animal (Stim 1) was used again as the familiar rat, together with an unfamiliar same-sex, gonadectomized rat in the other cage (Stim 2).