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Exposure to PCB 77 affects partner preference but not sexual behavior in the female rat

J.A. Cummings, L.G. Clemens, A.A. Nunez *

118 Linton Hall, The Graduate School, Michigan State University, East Lansing, MI 48824, United States

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ABSTRACT

In rats, exposure to the polychlorinated biphenyl congener 3, 4, 3′, 4′-tetrachlorobiphenyl (PCB 77) affects the brain and behavior of the offspring as well as the maternal behavior of the dams. In the present study, a cross-fostering design was used to examine the effects of pre- and/or postnatal exposure to PCB 77 on sexual behavior and partner preference in female rats, and to determine the role of altered maternal behavior in the mediation of these effects. Pregnant rats were treated with oil or PCB dissolved in oil (2 mg/kg b.w.) on gestation days 6–18 and then given pups that had been exposed to either the oil vehicle or PCB during gestation. As adults, the female offspring were tested for partner preference (that is, whether they preferred to spend time with a sexually receptive female or a sexually active male) and sexual behavior. None of the treatments affected female sexual behavior. However, both double exposure and postnatal exposure diminished the animals' preference for a male over a female stimulus, but partner preference was not affected by prenatal exposure alone. There were no significant correlations between the changes in partner preferences due to PCB exposure and the amount of maternal grooming and licking received by the treated litters. Thus, female partner preference is affected by early PCB exposure, and the effects depend upon whether the exposure is *in utero* or via lactation and may be independent of any effects of the PCB on maternal care.

1. Introduction

Polychlorinated biphenyls (PCBs) are industrial contaminants that persist in the environment because of their stable chemical structure and long half-life, even after their production was banned in the United States more than 30 years ago. While occupational exposure to PCBs has decreased dramatically, the risk for exposure via consumption of contaminated food remains significant. PCB exposure during development has been linked to a number of behavioral and physiological deficits in naturally-exposed humans [1–9] and experimentally-exposed animal models [10–21].

Work in our laboratory has shown that PCB treatment during development alters female rat sexual behavior [22–24]. However, those studies, as well as most of the developmental research using PCB exposure of pregnant animals, cannot differentiate between effects on the offspring due to actions of the contaminants on the developing fetus from those that result from postnatal exposure via lactation, because of the experimental design that is used. In addition to their ability to reach the developing fetus [25], PCBs accumulate in adipose tissue [26] and are found in maternal milk [27–30], indicating the likelihood of lactational transfer to the offspring in addition to *in utero* exposure. Unless these two routes of exposure are examined separately, it cannot be known whether effects seen in the offspring result from a combination of the pre- and postnatal exposures, or if the two types of exposure have differential impacts on various developmental outcomes.

Furthermore, rats that receive PCB treatment during pregnancy and/or rats that rear prenatally PCB-exposed offspring exhibit altered maternal behavior [31,32]. The importance of maternal care in several aspects of offspring development is well documented. For example, significantly decreasing the amount of time a dam licks and grooms her pups affects the sexual behavior of the male offspring [33], decreases the number of motor neurons in the sexually dimorphic spinal nucleus of the bulbocavernosus (SNB) [34] and significantly reduces total dendritic arbor in the SNB [35]. On the other hand, increasing the amount of licking and grooming (LG) the offspring receive decreases their level of fearfulness when confronted with a novel situation [36] or in response to stress [37]. Pup-directed LG has also been implicated in female reproductive success. Gomes et al. [38] demonstrated that females receiving more LG as a result of neonatal handling showed an increase in the number of anovulatory cycles compared to non-handled females. Thus, alterations in maternal care as a result of PCB exposure have the potential to affect the reproductive development of the offspring in important ways. It is therefore possible that the effects seen in the offspring after perinatal exposure to a contaminant may be partially due to toxin-induced

* Corresponding author. Tel.: +1 517 355 0301; fax: +1 517 353 3355.
E-mail address: nunez@gs.msu.edu (A.A. Nunez).

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changes in maternal behavior, particularly maternal grooming and licking of the pups, in addition to any direct effects of the toxin on the developing organism. Interestingly, pup-directed LG has an epigenetic effect [39], indicating the ease with which alterations in the behavior can be passed on to subsequent generations. Thus, changes in maternal LG brought about by exposure to the contaminant can potentially have effects on multiple generations.

In nature, exposure to PCBs most often results from ingesting contaminated food, and oral administration of PCBs in preferred food (i.e., a wafer) has been used in experiments with Sprague–Dawley rats [15]. However, in pilot studies with the Long–Evans strain used here, we found that after a few ingestive exposures, pregnant animals developed a salient taste aversion that interfered with the oral delivery of the PCB. Because of those pilot results, we adopted subcutaneous (sc) injections as the route of administration for PCB 77 here and in other experiments with pregnant Long–Evans rats [31,32]. A sc method of delivery has also been used successfully by others in a number of experiments to examine the effects of PCBs on various measures [11,18,20,40]. In addition, Hany et al. [11] have shown that PCB 77 accumulates in brain and adipose tissue of dams and offspring after sc maternal exposure. While PCBs are more often found as mixtures in the environment, it is also important to identify the specific risks associated with the most prominent congeners in order to help elucidate their cellular mechanisms of action.

Thus, for the current experiment, pregnant rats were treated subcutaneously with PCB 77 during gestation and their female offspring were tested as adults to evaluate the effects of prenatal exposure (PCB received while the offspring were *in utero*) and/or postnatal exposure (PCB received via lactation) on partner preference and sexual behavior. The changes seen in the offspring were also evaluated in reference to the amount of maternal licking and grooming that the litters received during the first 6 days of life.

2. Methods

2.1. Animals and housing

The animals used in this study were born to Long–Evans dams that received subcutaneous injections of 3, 4, 3′, 4′-tetrachlorobiphenyl (PCB 77; Accustandard, New Haven, CT; >99% pure as determined by gas chromatography performed by the vendor) in corn oil or the oil vehicle alone in the dose of 2 mg/kg on gestation days (GD) 6–18. The volume of the dose ranged from 0.6–1.4 ml and was based upon the weight of the dam, which was determined immediately prior to injection. Parturition took place during the mid- to late-light phase of the animals' light/dark cycle on GD 22–23. On the day of birth [day of birth = postnatal day (PND) 0], the litters were culled to 8 animals (4 females and 4 males) and cross-fostered or left with their own mothers to create the following groups: (1) oil-treated dams with their own oil pups (oil–oil), (2) oil-treated dams with a new litter of oil pups (oil/oil), (3) PCB-treated dams with their own PCB pups (PCB–PCB), (4) PCB-treated dams with a different litter of PCB-exposed pups (PCB/PCB), (5) PCB-treated dams with a litter of oil pups (PCB/oil), and (6) oil-treated dams with a litter of PCB-exposed pups (oil/PCB). Each group contained six dams. Maternal behavior was recorded for 2 h/day on PND 1, 2, 4 and 6 (see [32] for details of the maternal behavior analysis). Briefly, any time during those observation periods that the dam was licking one or more pups, she was scored as being engaged in pup-directed licking and grooming (LG). Two females were weaned from each dam on PND 23 and housed in pairs for the duration of the study in plastic cages (45×22×21 cm) with wood shavings as bedding. Animals were maintained on a 14:10-h light:dark cycle with lights off at 1100 h and at an ambient temperature of 21 °C, in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. The Michigan State University Animal Care and Use Committee approved all experimental procedures.

2.2. Hormone treatments

At 60 days of age, the two female offspring from each dam were ovariectomized (anesthetized with a ketamine/xylazine cocktail containing 44 mg/kg ketamine, 10 mg/kg xylazine) and implanted subcutaneously with an estradiol benzoate-filled Silastic capsule. Capsules were made from Silastic brand laboratory tubing (Dow Corning, cat. No. 508-006), with an inner and outer diameter of 0.058 in. and 0.077 in., respectively. Estradiol benzoate (EB, Sigma) was dissolved in ethanol and allowed to sit in a Petri dish for 48 h or until all of the liquid evaporated from the dish. The crystalline EB was then packed in the pre-cut Silastic tubing to fill a length of 5 mm. The ends of the capsules were sealed with medical-grade Silastic adhesive (Dow Corning), and implants were incubated in PBS for 24 h prior to implantation. After one week of recovery and 4 h prior to the start of the sexual behavior tests, females were injected with 0.5 mg/0.1 ml progesterone (Sigma). Stimulus females were at least 60 days of age at the start of testing. They were not ovariectomized but were treated with estrogen and progesterone in a manner similar to experimental females, and were checked for receptivity prior to their use. The stimulus males were intact and were sexually active.

2.3. Testing procedure

The experimental animals began their 5-week testing paradigm at 67 days of age. The first week consisted of a partner preference test during which animals were sexually naïve, followed by sexual behavior tests once a week for three weeks, with behavioral data collected only on the third and final test. The testing paradigm ended with a partner preference test during the fifth week. All tests took place under dim red illumination during the dark phase of the light–dark cycle. It is important to note that although we refer to our choice paradigm as a "partner preference" we do not imply that we tested the animals for their "mate choice", although the animals occasionally displayed sexual behavior during those tests.

2.4. Partner preference tests

Animals were tested in a three-compartment apparatus made of Plexiglas. The middle compartment was connected to the two outer compartments (30×59×39 cm each chamber, 90×59×39 cm total) by openings in the back of the apparatus. One of the outer compartments contained a sexually active male and the other a sexually receptive, hormone-treated female. Both stimulus animals were tethered to the front of the apparatus using a Velcro harness and stainless steel wires with swivels. Stimulus animals were able to display sexual behavior but were not able to leave their respective chambers. Experimental females were able to traverse the chambers freely through the openings in the back of the apparatus. The experimental female was placed in the center chamber and allowed to acclimate for 5 min prior to the start of the test. After 5 min, the clear plastic divider that closes the middle compartment off from the rest of the apparatus was removed. The test lasted 20 min and for each test, the amount of time the experimental female spent in each chamber was recorded. A preference score was calculated in which the amount of time the experimental female spent with the stimulus male was subtracted from the amount of time spent with the stimulus female. Thus, a positive preference score indicates that an animal prefers to spend time with the stimulus female rather than the stimulus male. No data on sexual behavior were collected during the partner preference tests.

Three investigators scored the partner preference tests from the video recordings. Because of procedural demands, it was impossible to keep one of these three investigators unaware of the treatment received by each offspring. A subset of the recordings was scored independently by all investigators and a correlational analysis was used to evaluate the inter-observer reliability. The positive correlation