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# Effects of Perinatal Exposure to Polychlorinated Biphenyls on Development of Female Sexual Behavior

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Polychlorinated biphenyls (PCBs), a family of global environmental contaminants, have been shown to disrupt a wide array of physiological and behavioral systems, including reproduction and brain development. Some PCBs such as the commercial PCB mixture, Aroclor 1221 (A1221), act as endocrine disrupters because of their affinity for estrogen receptors (Bitman and Cecil 1970). Other PCBs, such as Aroclor 1254 (A1254), exhibit minimal binding to estrogen receptors, and some of their metabolites may even have antiestrogenic activity (Bitman and Cecil 1970; Moore et al 1997). Since estrogen has profound effects on sexual differentiation (MacLusky and Naftolin 1981) these two commercial PCB mixtures may have different effects on the development of sexual behavior.

PCBs also exhibit neurotoxic effects on dopaminergic neurons (Brouwer et al. 1995) which are important in controlling sexual behavior. Based on the position of their chlorine substitutes, PCBs can be categorized into coplanar (position 3, 4, 5 on the benzene ring) or ortho-substituted (position 2, 6 on the benzene ring) noncoplanar PCBs. With regard to their effects on dopaminergic systems, noncoplanar, ortho-substituted PCBs have been shown to reduce dopamine levels in the brain and in PC-12 cells, while the coplanar PCBs increase dopamine or have no effect (Seegal et al. 1990, 1997). Coplanar congeners, in addition to their ability to alter brain dopamine concentration, also exhibit estrogenic or antiestrogenic function (Gierthy et al. 1988; Spink et al. 1990). Since female sexual behavior can be disrupted by exposure to estrogen during perinatal development (MacLusky and Naftolin 1981) and since brain dopaminergic systems are involved in adult female sexual behavior (Carter and Davis 1977; Mani et al. 1994) the likelihood that developmental exposure to PCBs could disrupt feminine sexual behavior by disrupting dopaminergic systems seems high. In addition, lesions or dopamine administration into the medial preoptic area altered female paced sexual behavior (Whitney 1986) and A11 and A13 dopaminergic neurons appeared to be the major dopaminergic input into the medial preoptic area (Lindvall and Björklund 1983). In this report we compare the effect of two commercial PCB mixtures on female sexual behavior and All and A13 dopaminergic systems. A1221, an estrogenic PCB mixture composed of about 40% coplanar PCBs and 60% ortho-substituted PCBs (Webb and McCall 1972; Willis and Addison 1972) and A1254 a non-estrogenic, mainly ortho-substituted PCB mixture (Sissons and Welti 1971; Webb and McCall 1972) were used to examine the possible risks of PCB exposure during sexual differentiation on female sexual behavior and A11 and A13 dopaminergic systems.

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## MATERIALS AND METHODS

Sexually mature Long-Evans rats (*Rattus norvegicus*) were housed separately in plastic cages (57x25x20cm) in a vivarium with 48% humidity and temperature at 22°C (lights off 1100-1900). Rat chow (Harlan Teklad 22/5 rodent diet 8640, Madison, WI) and tap water were available *ad libitum.*

Forty time-mated pregnant rats (8/group) were give three intraperitoneal injections of 0.5ml sesame oil (Sigma Chemical Co., St. Louis, MO) or PCB solution (Fisher Scientific, Pittsburgh, PA) on gestation day 14, parturition day (day 1) and day 10 after birth. The PCB working solution for each injection was prepared by dissolving 5 mg or 15 mg A1221 (or A1254) in 1.5 ml sesame oil for each animal (3.33 mg/ml and 10 mg/ml, respectively). All the injections were administered at 0900 hr (2 hr before lights off) thus providing offspring with maternal exposure to a total PCB dosage of 0 mg (sesame oil group, control), 5 mg (about 14 mg/Kg) or 15 mg (about 42 mg/Kg) A1254 (or A1221). [Note: The actual dosage transferred to the pups is estimated to be about 10 µg (1.7 mg/Kg) and 30 µg (5 mg/Kg), respectively (according to Takagi et al. 1986)]. Pups were weaned at 28 days of age. Males and females were housed separately after weaning.

At 60 days of age, female offspring were ovariectomized and rehoused (3 rats/cage). Seven days (day 67 after parturition) after surgery, the ovariectomized rats were injected subcutaneously (sc) with estradiol benzoate (Sigma Chemical Co., St. Louis, MO) 0.5 µg/0.1 ml/rat (dissolved in sesame oil) for 3 consecutive days at 0900 hr then given progesterone (Sigma Chemical Co., St. Louis, MO), 0.5 mg/0.1 ml/rat sc (dissolved in sesame oil) on the fourth day at 0900 hr to bring them into sexual receptivity. Behavioral tests began four hours after progesterone treatment (at 1300 hr). Hormone-treated females were tested for sexual behavior once per week for five weeks from day 70 to day 105. The first behavior test was considered a pre-test and no data were collected.

Female sexual behavior was evaluated with two separate test paradigms: a standard lordosis test and a pacing test. The two tests were performed on the same day but the sequence was reversed in the subsequent week of the test period. Sexually receptive females respond to mounts by a male by exhibiting a pronounced arching of the back, lordosis. In a standard test, sexual receptivity was measured by counting the number of times the female responded to the male with lordosis (lordosis quotient, LQ). The male rat was placed in the testing arena, a Plexiglas cage (56x44x49cm) for 5 min after which the female rat was placed in the test chamber for the start of the test. The test ended when the male had mounted 10 times.

The pacing test was conducted in a two-compartment cage. For this test, the chamber (56x44x49cm) was divided into two, a main chamber (34x44x49cm) and an escape chamber (22x44x49cm). During copulatory tests, females could escape from the male through holes in the dividing partition which the larger male was unable to pass through. The female rat was introduced into the escape chamber 5 min after the male was placed in the main chamber. Approach latency was the time from the introduction of the female into the escape chamber to her crossing the partition to approach the male. Mount return latency, intromission return latency and postejeculatory refractory period were the measures of time that the female spent in the escape chamber after each male copulatory event (mount, intromission or ejaculation, respectively). The

percentage of times that the female entered the escape chamber after different copulatory events, i.e., percentages of mount leave and intromission leave, were also used to assess female sexual behavior.

Tyrosine hydroxylase immunoreactivity (TH-IR) was used to identify dopaminergic neurons in the caudal incertohypothalamic region. Twenty-five female rats were randomly selected (from different litter in each treatment group), sacrificed right after the last behavior test and simultaneously processed for immunohistochemistry (modified from the procedure of Arbogast and Voogt 1994). The antibody for TH was purchased from Sigma Chemical Co., St. Louis, MO (TH-2, monoclonal mouse-anti-TH, 1:10,000).

The mean of each behavioral parameter in each test was calculated and the mean of the four behavior tests was then used to calculate the mean for each litter and for further statistical analyses (StatView). Approach latency, mount return latency, intromission return latency and postejaculatory refractory period were analyzed by one-way ANOVA. Fisher's LSD post hoc test was performed when there was a significant difference among treatments ($p<0.05$). Percentages of mount leave, intromission leave and LQ were analyzed by Kruskal-Wallis Test. The post hoc tests were performed when there was a significant difference among treatments ($p<0.05$). The number of TH-IR neurons in A11 or A13 region was analyzed by both one-way ANOVA and Kruskal-Wallis Test. To compare the measurements of two sides of A11 or A13, two-way ANOVA (two sides as repeated measurements) was used.

## RESULTS AND DISCUSSION

**Table 1.** The effect of perinatal A1221 treatment on female sexual behavior.

| | Control | A1221 (5 mg) | A1221 (15 mg) |
|---|---|---|---|
| Intromission Return Latency (sec) | 18.950±2.670 (n=8) | 36.938±8.772[a] (n=8) | 11.133±1.602[c] (n=6) |
| Percentage of Intromission Leave | 55.040±7.129 (n=8) | 73.104±2.754[a] (n=8) | 49.466±4.554[c] (n=6) |
| Lordosis Quotient | 83.125±5.083 (n=8) | 48.125±8.014[b] (n=8) | 44.167±15.352[b] (n=6) |

Data were represented as mean±S.E.
[a]: significantly different from control ($p<0.05$)
[b]: significantly different from control ($p<0.01$)
[c]: significantly different from 5 mg group ($p<0.01$)

**Table 2.** The effect of perinatal A1254 treatment on female sexual behavior.

| | Control | A1254 (5 mg) | A1254 (15 mg) |
|---|---|---|---|
| Percentage of Mount Leave | 36.676±4.824 (n=8) | 57.250±3.880[a] (n=6) | 55.763±8.437[a] (n=5) |
| Percentage of Intromission Leave | 55.040±7.129 (n=8) | 77.873±4.771[b] (n=6) | 77.701±4.990[b] (n=5) |

Data were represented as mean±S.E.
[a]: significantly different from control ($p<0.05$)
[b]: significantly different from control ($p<0.01$)

Perinatal exposure to A1221 decreased sexual receptivity in ovariectomized, hormone treated Long-Evans rats as measured by LQ (Table 1), but treatment with A1254 did not (Table 2). These results are consistent with the idea of an estrogen-induced defeminizing effect of A1221 (MacLusky and Naftolin 1981). A1221 has been reported to have estrogenic activity in other assays (Bitman and Cecil 1970). Estrogen treatment during gestation or shortly after birth results in a pattern of anovulatory sterility in adulthood and lower receptivity as measured by LQ (Gorski 1971) and Gellert (1978) has reported induction of precocious puberty, persistent vaginal estrus, and anovulation following developmental treatment with A1221.

5 mg A1221 treatment increased the percentage of times the female left the male and increased her latency to return to him following an intromission (Table 1). A1254 treatment increased the percentage of time the female left following either an intromission or a mount, but did not affect her return latencies (Table 2). Other temporal measures of female sexual behavior, approach latency, mount return latency and postejaculatory refractory period, were not affected by the perinatal treatments (data not shown).

The explanation for different effects of A1254 and A1221 is unclear. Comparing the results from A1221 and A1254 treatments, it appears that the lordosis reflex may have been altered by the estrogenic effects of coplanar PCBs, while the increase in leaving after a copulatory event may be due to the effect of the ortho-substituted PCBs. Since estrogen interacts with brain dopaminergic systems (Mani et al. 1994) and since coplanar and ortho-substituted PCBs have opposite effects on dopamine levels (Seegal et al. 1990, 1997) the differential effect in the A1221 5mg and 15 mg groups may be attributed to the antagonism between two types of PCBs in the mixture.

Changes in the female's approach and withdrawal from the male may reflect effects on the female's sexual motivation. However, the temporal pattern of female copulation also depends on peripheral sensory factors (Erskine 1989). Consequently, we cannot exclude the possibility that PCB treatments had peripheral effects which influenced the female's sensitivity to vaginocervical stimulation and thereby altered temporal measures of her copulatory behavior.

**Table 3.** Perinatal PCB treatments had no effect on A11 or A13 dopaminergic neurons.

| | Control (sesame oil) | A1221 (5 mg) | A1221 (15 mg) | A1254 (15 mg) |
|---|---|---|---|---|
| A11 TH-IR Neurons (major) | 545.4±52.2 (n=5) | 686.0±16.5 (n=3) | 464.4±58.0 (n=5) | 451.5±121.8 (n=4) |
| A11 TH-IR Neurons (minor) | 475.2±52.6 (n=5) | 588.0±74.8 (n=3) | 411.6±48.9 (n=5) | 411.6±48.9 (n=4) |
| A13 TH-IR Neurons (major) | 1609.2±233.6 (n=5) | 1302.0±211.5 (n=3) | 1302.6±132.3 (n=5) | 1353.8±330.3 (n=4) |
| A13 TH-IR Neurons (minor) | 1288.8±189.0 (n=5) | 1019.0±224.0 (n=3) | 1107.0±76.7 (n=5) | 1134.8±241.3 (n=4) |

Data were represented as mean±S.E.
Note: The brain samples for 5 mg A1254 group were accidentally lost.

Alternatively, since some PCBs have a dioxin-like structure, it is possible that they also

act through a cytosolic protein, the aryl hydrocarbon (Ah) receptor (Birnbaum 1994). It is well known that coplanar PCBs can mimic the structure of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin and, therefore, bind to the Ah receptor. However, none of the coplanar components of A1221 or A1254 fulfill the structural requirements for induction of Ah receptor activity (Poland and Glover 1977). It is therefore unlikely that A1221 or A1254 acts through Ah receptors.

Perinatal A1221 or A1254 treatment did not change the number of TH-IR neurons at A11 or A13 regions. However, we found an asymmetry in A11 and A13 TH-IR cell number on two sides of the brain (pooled data from all groups; $p<0.001$ and $p=0.001$, respectively). One side of the brain (major) had more neurons than the other (minor) (Table 3). Asymmetry of dopaminergic systems in the brain is well documented (Afonso et al. 1993). This report also supports our previous finding of A13 TH-IR asymmetry (Chung and Clemens 1998).

There is no relation between the effect of perinatal PCB treatment on lordosis and the dopaminergic neurons in the A11 or A13 regions of the brain. The behavior-al effect of A1221 thus resulted from some other action on the brain or peripheral nervous system. Several dopaminergic systems in the hypothalamus and midbrain have been implicated in the control of lordosis in female rats, including the substantia nigra, central tegmental area (Herndon 1976) and the arcuate nucleus (Lofstrom 1977). Indeed, PCBs have been shown to decrease dopamine contents in the substantia nigra and other hypothalamic nuclei (Seegal et al. 1990).

The timing of the PCB treatment is also an important factor. In contrast to the present finding, neonatal treatment alone with A1254 (day 1 to day 7) disrupted female sexual behavior and the asymmetry of A11 and A13 dopaminergic neurons while neonatal treatment of A1221 did not alter sexual behavior (Chung and Clemens 1996, 1998). These results suggest that A1221 is able to disrupt the development of sexual behavior when given prenatally while A1254 is more active neonatally. The discrepancy between the perinatal and neonatal A1254 treatments may be due to the different treatment duration in these two experiments. The timing of differential PCB action may be linked with the ontogeny and the susceptibility of various dopaminergic system to different PCB congeners.

Exposure to PCBs during development disrupted development of female sexual behavior in the adult rat. Perinatal exposure to estrogenic coplanar PCBs was more disruptive than exposure to noncoplanar compounds. This effect on female sexual behavior was not associated with a change in hypothalamic dopaminergic systems.

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